# Utilization of NIR Spectroscopy To Estimate the Proximate Composition of Trout Muscle with Minimal Sample Pretreatment

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Reflectance spectroscopy in the near-infrared (NIR) region (900–1800 nm) was used to predict the proximate composition of either fresh or frozen rainbow trout muscle. The standard error of prediction (RMSEP) for moisture was 1.1% (over the range 71.5–76.7% moisture), for crude lipid, 3.1% [range 8.8–17.0% dry weight basis (dwb)], and for protein nitrogen (Kjeldahl, % N × 6.25), 5.4% [over the range 69.3–87.4% protein (dwb)]. Two calibration methods, partial least squares (PLS) and multiple linear regression (MLR), were used to develop models that related spectral absorbances to moisture, lipid, and protein concentrations. This NIR reflectance spectroscopic method could be useful for making a rough estimate of the moisture and protein content of fish muscle for quality control or animal husbandry purposes. Sample preparation is minimal, and the method does not require that the muscle sample be homogenized, dried, or extracted prior to analysis.

## INTRODUCTION

The application of NIR spectroscopy to estimate the distribution of macronutrients in fish products provides a rapid method for predicting yield and fish quality without resorting to conventional techniques of proximate analysis which are lengthy, involved, tedious, dangerous, and expensive. Standard methods for proximate analysis provide only a limited amount of information, such as the quantity of solvent-extractable fat or total nitrogen, and take from 1 to 3 days to complete. Furthermore, these methods do not determine the concentrations of analytes in situ, which are more meaningful quantities. Conventional methods are also destructive and require that a significant number of animals be sacrificed to obtain a representative sample for analysis.

In contrast, near-infrared (NIR) spectroscopy can be used to rapidly analyze materials with little or no sample preparation. Because the absorptivities of NIR bands are very small, highly absorbing samples (which are very thick and poorly scattering) can be analyzed directly. NIR has been widely used to determine the proximate composition of food commodity items, including cereals and meat products (Norris, 1983; Osborne and Fearn, 1986). The use of multivariate calibration methods in conjunction with NIR to predict the proximate analysis of agricultural materials is widely used (Naes and Martens, 1987; Williams and Norris, 1987). NIR spectroscopic methods for composition determination of wild caught or aquacultured fish would provide a rapid means for monitoring husbandry and feeding practices and could be used to predict product yield without resorting to labor-intensive conventional techniques.

Little research has been conducted on the application of NIR spectroscopy to the proximate analysis of fish. The major limitation of the NIR method is the interference of water bands with important spectral bands of other analytes. The work of Gjerde and Martens (1987) indicated that selected NIR absorbances from the spectra of freeze-dried fish and chemical constituents in the fish are highly correlated; $r = 0.95$ for moisture and fat content and 0.91 for protein nitrogen. The primary difficulty with their method is that 36–72 h must be added to the sampling time to allow for freeze-drying. Computerized (X-ray) tomography has also been employed to estimate carcass composition of rainbow trout by using fresh tissue and anesthetized fish. However, the capital cost involved with this system is prohibitive (Gjerde, 1987). Recent work with mid-infrared transmission spectroscopy, a method commonly used for milk and for the analysis of fish tissue homogenates, indicated that results within 1% of the standard chemical method can be obtained (Darwish et al., 1989). Mid-infrared transmission spectroscopy, too, involves extensive sample preparation prior to analysis.

The use of multivariate calibration methods with NIR spectroscopy is common because NIR bands from individual analytes are usually highly overlapped. In this work, two methods of calibration of NIR spectra to constituent data were used: (1) multiple linear regression (MLR) and (2) partial least squares (PLS). In the MLR method (Beebe and Kowalski, 1987; Naes and Martens, 1987), several discrete NIR wavelengths were chosen to construct a calibration. These wavelengths are chosen such that absorbances of the analyte and overlapping absorbances of interferents are accounted for. In the PLS method (Geladi and Kowalski, 1986), all wavelengths in the spectrum are used for calibration, and each wavelength is weighted according to its ability to predict the analyte concentration in the presence of interfering components.

Evaluation of the ability of NIR spectroscopy to determine analyte concentrations can be made by a calibration/prediction procedure in which a representative set of samples is used to construct a calibration model. The calibration model is then used to predict the concentrations of analytes in samples of a separate prediction set. The errors in the analyte concentration for samples composing the prediction set are an indication of the predictive ability of the model(s) constructed as part of the NIR method.

Further evaluation of the NIR method(s) can be done by observation of the chosen wavelengths (from MLR) or the highly weighted wavelengths in the regression coefficient spectrum (from PLS). It can then be determined whether these wavelengths properly account for analyte

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and interferent absorptions through examination of analyte and interferent model spectra. Unfortunately, these analyses can be rather difficult, because several constituents might have highly overlapped absorbances. Furthermore, the use of second-derivative spectra can complicate these interpretations, because side lobe bands are created for each single band in the NIR spectrum.

The purpose of the present study was to evaluate the usefulness of the NIR spectral region from 900 to 1800 nm for the analysis of fish muscle that has received minimal sample pretreatment. The intent of this research is to provide a basis for an NIR method that can be adopted in fish processing, fish grading, or aquaculture operations for rapid analysis and for quality control purposes.

## MATERIALS AND METHODS

Postjuvenile rainbow trout (*Oncorhynchus mykiss*) spawned in the winter of 1985 of two different strains were housed in the headwater of an outdoor raceway at the University of Washington hatchery (November 1987–May 1988). The water source for the hatchery, taken directly from Lake Washington, circulated through the raceway at approximately 0.5 $ft^3/s$. The lake water temperature was monitored daily and varied from 6 to 23 °C during the course of this study. The rainbow trout were anesthetized with MS 222 (tricaine methanesulfonate, Argent Chemical Laboratory, Redmond, WA) and then individually tagged for identification with two Floy tags (Floy Tag and Manufacturing Inc., Seattle, WA). The fish were fed an Abernathy production diet supplemented with 50 ppm 5% asthaxanthin (dwb) (Carophyll pink, Hoffman La Roche, Inc., Nutley, NJ) ad libitum on a daily basis.

Animals were sacrificied over a period of 6 months at times that corresponded to prespawning, spawning, and postspawning conditions. Selecting animals at these time periods would yield the widest range of constituent values. All animals were of market size when harvested. Fish were weighed, eviscerated, glazed, and stored at −30 °C. Flesh samples (1–2-cm plug) were taken for NIR analyses from the section of the fish midway between the gill and the insertion of the dorsal fin and midway between the lateral line and the base of the dorsal fin on the left side. These samples were stored, sealed in Whirl-Pak bags (2 oz, VWR Scientific, Seattle, WA), at −40 °C prior to NIR analysis. For proximate analysis, samples that were analyzed corresponded to the same section of the fish (given above) but from the right side of the animal.

Moisture content was determined by freeze-drying the tissue. Crude lipid content was measured by extracting freeze-dried samples with petroleum ether for 12 h (AOAC, 1984, No. 24.005), and protein nitrogen (% protein = % N × 6.25) was determined by a Kjeldahl method using ca. 500 mg of freeze-dried sample and a copper selenium catalyst ($CuSeO_3$, EMKX0014K1, Fisher Scientific, Pittsburgh, PA). These methods of analysis were chosen because they are widely used in the field of aquaculture. The estimated errors of these reference methods, determined as the pooled standard deviation of duplicate or triplicate measurements for each sample, were 0.93% for moisture, 0.27% for crude lipid, and 4.0% for protein nitrogen. Average concentrations determined by the reference methods were used for each sample in establishing the calibration models.

NIR reflectance spectral data were collected by using a Technicon InfraAnalyzer 500E grating instrument (Technicon, Tarrytown, NY) with a nominal resolution of 10 nm. Spectral reflectance readings were obtained at 4-nm intervals over the region 900–1800 nm. Thin cross sections of frozen tissue (less than 3 mm thick) were taken from the center of the plug of tissue (see above), applied to a small (approximately 5 cm) circular ceramic plate (which acted as a diffusely reflecting background), and allowed to thaw to room temperature. Samples were flattened with a metal spatula to a thickness of less than 1 mm; however, samples were not of uniform thickness. Although the fish tissue samples themselves were not highly scattering, the ceramic background provided a basis for diffuse reflectance measurements; the instrument used in this work operates only in the reflectance mode.

**Table I. Statistics of Constituent Values in Calibration and Prediction Sets**

| | Distribution of Analyte Concentrations in Calibration and Prediction Sets | | | | | |
|---|---|---|---|---|---|---|
| | calibration set | | | prediction set | | |
| | moisture | lipid | protein | moisture | lipid | protein |
| mean | 73.8 | 12.8 | 79.4 | 74.2 | 13.1 | 80.1 |
| SD | 1.9 | 2.6 | 5.4 | 1.6 | 2.3 | 5.3 |
| range | 70.4–77.6 | 7.84–17.5 | 68.9–87.6 | 71.5–76.7 | 8.8–17.0 | 69.3–87.9 |

| | Correlation Coefficients between Different Analytes in Calibration and Prediction Sets | | | |
|---|---|---|---|---|
| | calibration set | | prediction set | |
| | lipid | protein | lipid | protein |
| moisture | −0.4513 | 0.3113 | −0.2978 | 0.3620 |
| lipid | NA[a] | −0.4005 | NA | −0.4997 |

[a] NA, not applicable.

Four reflectance spectra were obtained for each sample. After the first spectrum was taken, the samples were rotated 90° for the second spectral reading. The third and fourth spectra were obtained by flipping the sample over and repeating this procedure. Spectra from 42 different samples were taken. The spectral region from 1352 to 1448 nm was not included for quantitative analysis because of an instrumental anomaly that occurs in that region. Reflectance spectra were converted to log (1/reflectance) spectra (or pseudoabsorbance spectra) prior to multivariate analysis.

Second-derivative (log 1/reflectance) spectra were used for analysis to reduce the effects of baseline offset in the spectra. The second-derivative operation caused a truncation of the NIR spectral range from 924 to 1776 nm. In addition, a multiplicative scatter correction (MSC) (Geladi et al., 1985) was applied to the second-derivative spectra to reduce the effect of nonreproducible sample thickness.

A multiple linear regression (MLR) and a partial least-squares (PLS) calibration were conducted for each analyte (Beebe and Kowalski, 1987; Geladi and Kowalski, 1986). MLR calibrations involved the stepwise selection of four analytical wavelengths, which constituted a linear additive calibration model for each analyte:

$$\text{concn} = k_1\lambda_1 + k_2\lambda_2 + k_3\lambda_3 + k_4\lambda_4 + k_0 \quad (1)$$

$k_1$, $k_2$, $k_3$, $k_4$, and $k_0$ are regression coefficients and $\lambda_1$, $\lambda_2$, $\lambda_3$, and $\lambda_4$ represent the absorbances of the analytical wavelengths chosen by the stepwise method.

For the PLS calibrations (CPAC, Seattle, WA) (Beebe and Kowalski, 1987; Geladi and Kowalski, 1986), the optional number of spectral factors was determined by the method of cross-validation (Sharaf et al., 1986). Analysis of cross-validation results indicated that four spectral factors were necessary for each of the three PLS calibrations. Mean-centered NIR spectra were used for all PLS calibrations.

For all analyses, approximately half of the individuals were selected at random for inclusion in the calibration set for each sampling period and were used to construct the MLR or PLS calibrations. The remaining samples constituted the prediction set, which was used to test the validity of the calibrations.

The samples that had the maximum and minimum values for each analyte were placed in the calibration set, and the remaining samples were chosen such that samples in both sets were distributed over the range of concentrations for each analyte. Table I, which shows the mean, standard deviation, range, and cross-correlations for all three analytes in the calibration and prediction sets, indicates that the sample population is well-represented in both the calibration and prediction sets.

As a result, two statistics were obtained from each analysis: the root mean square of estimate (RMSEE), which is the root mean squared error of predicted analyte concentrations of the calibration samples, and the root mean square error of prediction (RMSEP), which is the root mean squared error of predicted analyte concentrations of the prediction samples. The RMSEE

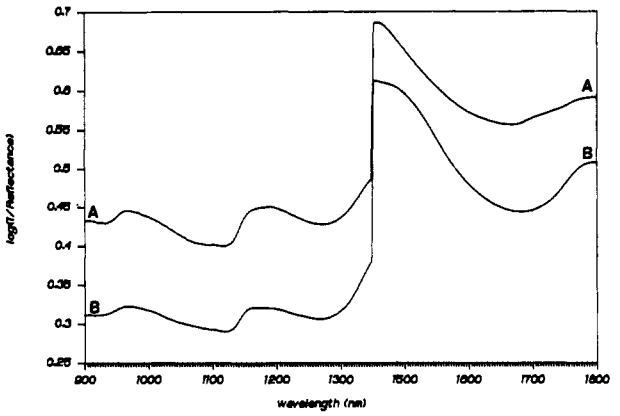


**Figure 1.** NIR diffuse reflectance spectrum of fish meat (A) and NIR spectrum of water (B). Spectra are scaled and offset for clarity.

value is indicative of calibration fit, and the RMSEP value is indicative of the predictive ability of the calibrations.

NIR spectra of water, lyophilized fish muscle, lyophilized lipid-free fish muscle, and a solvent-extracted crude lipid fraction of visceral fat, used as controls, were obtained by the same procedure as the spectra of fish muscle samples. These spectra were useful for interpreting the wavelengths chosen in the MLR calibrations and the most prominent wavelengths in the PLS regression coefficient spectra.

## RESULTS

The use of four MLR wavelengths and four PLS factors in each calibration implies the presence of four major independent variations in the NIR spectra of the samples. The correlations between known analytes in the samples (i.e., moisture and lipid) were low (Table I). Therefore, each of the known analytes contributes one source of variation in the spectra. The fourth variation could have been caused by temperature variations in the samples during NIR analysis or sampling variations which were not adequately removed by the spectral pretreatment methods.

The NIR diffuse reflectance spectrum of a fish meat sample is shown in Figure 1A. This spectrum is very similar to the spectrum of water (Figure 1B) because water is the dominant component of the fish samples. Subtle but significant differences between these two spectra in the regions 950–1050, 1150–1300, and 1650–1800 nm are a result of the protein, lipid, and other nonaqueous components in the fish samples (Figure 3). If the water absorbances are not strong enough to cause nonlinearity in the spectra, the small differences that can emerge from the second derivative of the spectral data can be used to determine lipid and protein concentrations in the fish tissue samples (Figures 4–6).

Results of MLR and PLS calibration studies are given in Table II. Calibration and prediction errors (RMSEE and RMSEP) were consistently lower for the PLS method. The correlation coefficients for the PLS calibrations ranged from 0.908 for moisture to 0.691 for crude lipid content. The correlation coefficients for the MLR calibrations were slightly lower. The RMSEP values for the PLS predictions were 1.1% for moisture, 3.1% for crude lipid, and 5.4% for protein nitrogen. For both calibration methods, the moisture predictions were the best. Prediction errors for moisture were similar to the estimated error for the wet chemical measurements. However, the prediction errors for protein nitrogen and crude lipid determinants were significantly higher than the estimated errors of the laboratory measurements. These results were expected because protein and lipid absorbances are masked by strong water absorbances.

Figure 2, which shows the PLS calibration curve for moisture in the fish meat samples, indicates that good agreement with the calibration model was obtained ($r$ = 0.91). The second-derivative spectrum for pure water and the PLS regression coefficient spectrum for the moisture content in the fish samples are given in Figure 3. Because the spectrum of pure water is expected to be different from the spectrum of water in fish due to interactions of water with other components in the sample matrix, the water spectrum in Figure 3 is only an approximate model spectrum.

The model spectrum for water and the PLS coefficient spectra were fairly similar in the region from 1250 to 1800 nm. The strong water band at approximately 1150 nm, which was not in the PLS regression coefficient spectra, probably experiences interference from other analytes. The PLS coefficient spectrum shows sharp bands at longer wavelengths (1650–1750 nm), presumably because of strong lipid and protein interference bands in this region of the spectrum. A weaker water absorbance at 1750–1800 nm was observed in both the model and PLS coefficient spectra.

The four wavelength terms used in the MLR equation for moisture can be tentatively assigned to moisture absorbances and to protein and lipid interference absorbances. The 1732-nm term is correlated with strong water absorbance (Figure 3) and possibly to a protein interferent absorbance. The 1200-nm term is located where strong absorbances from both lipid and protein are known to occur and is probably used to compensate for these interferences. The 924- and 932-nm terms correspond to significant water absorbances (see water model spectrum, Figure 3). The strong OH overtone band region (1490 and 1580 nm) was not used for the MLR moisture calibration, possibly because the high concentration of water in the samples caused these absorbances to be nonlinear.

The PLS calibration curve for crude lipid content is shown in Figure 4. The calibration fit is rather poor. The spectrum of lipid extracted from fish viscera (Figure 5) indicates that significant lipid absorbances are in the regions 1100–1250 and 1650–1800 nm. In contrast, the PLS regression coefficient spectrum does not show any specific wavelength regions that are weighted much higher than others. It is possible that interference from water bands in the regions 1150–1170 and 1700–1800 nm limits the usefulness of these regions for lipid analysis. In the 1700–1800-nm region, this is indicated by the weakness of the PLS coefficient spectrum, where strong bands are observed in the spectrum for extracted lipid.

The poor performance of the lipid calibration may have been due in part to an inaccurate measurement of crude lipid content using extraction by a nonpolar solvent. This would have led to a low recovery of polar lipid and might have been responsible for the lack of highly integrated characteristic lipid bands in the PLS coefficient spectrum. However, gravimetric estimate of total lipid content in fish tissue by extraction in polar solvents [i.e., chloroform–methanol 2:1 (v/v)] can lead to erroneously high results due to the solubilization of low molecular weight nonlipid substances including nitrogeneous compounds, lipoproteins, and salt (Barnes and Blackstock, 1973) unless the lipid extracts are extensively purified.

Two of the chosen wavelengths for the lipid calibration, 1220 and 1692 nm, correspond to strong lipid bands (Figure 5). More specifically, $CH_2$ and $CH_3$ bands have been

**Table II. Calibration and Prediction Results for MLR and PLS Methods[a]**

| constituent | wavelengths selected, nm | r | calibration RMSEE[c] | calibration range, % | prediction RMSEP[d] | prediction range, % |
|---|---|---|---|---|---|---|
| | | MLR, Four Terms | | | | |
| moisture | 1732, 1200, 924, 932 | 0.893 | 0.8 | 70.4–77.6 | 1.5 | 71.5–76.7 |
| lipid[b] | 1144, 960, 1220, 1692 | 0.657 | 2.0 | 7.84–17.5 | 3.5 | 8.8–17.0 |
| protein[b] | 1064, 996, 1196, 1652 | 0.921 | 2.7 | 68.9–87.6 | 9.3 | 69.3–87.9 |
| | | PLS, Four Factors | | | | |
| moisture | | 0.908 | 0.75 | 70.4–77.6 | 1.1 | 71.5–76.7 |
| lipid[b] | | 0.691 | 1.9 | 7.84–17.5 | 3.1 | 8.8–17.0 |
| protein[b] | | 0.861 | 2.6 | 68.9–87.6 | 5.4 | 69.3–87.9 |

[a] Pooled standard deviation values for results from laboratory analyses were 0.93% for moisture, 0.27% for crude lipid (dwb), and 4.0% for protein nitrogen (by Kjeldahl, % P = % N × 6.25). [b] Values for crude lipid and protein nitrogen are on a dry weight basis. [c] RMSEE, root mean square error of estimate. [d] RMSEP, root mean square error of prediction.

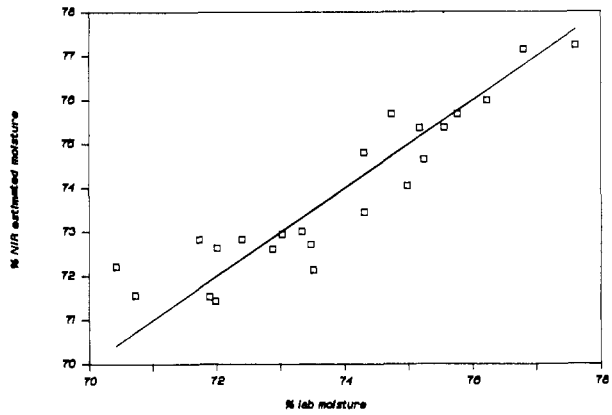


**Figure 2.** Calibration curve for PLS calibration of moisture content in fish meat.

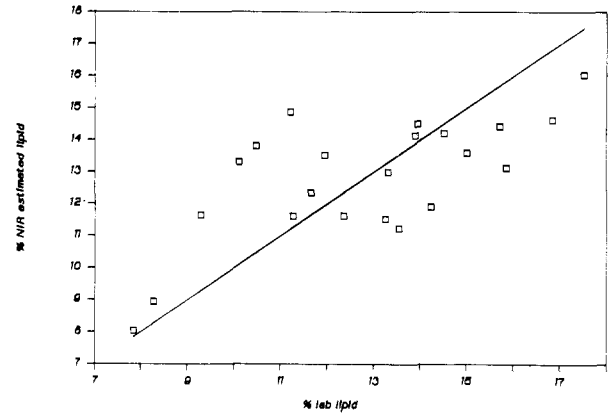


**Figure 4.** Calibration curve for PLS calibration of lipid content in fish meat.

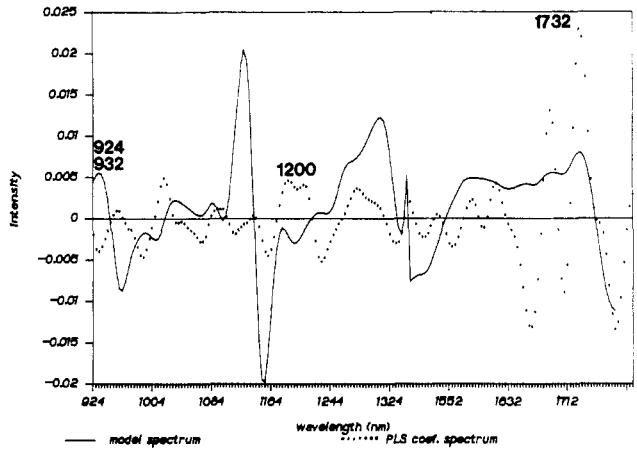


**Figure 3.** Second-derivative NIR spectrum of water (solid line) and PLS regression coefficient spectrum for water in fish meat (dotted line). Wavelengths used in the MLR calibration for moisture are labeled.

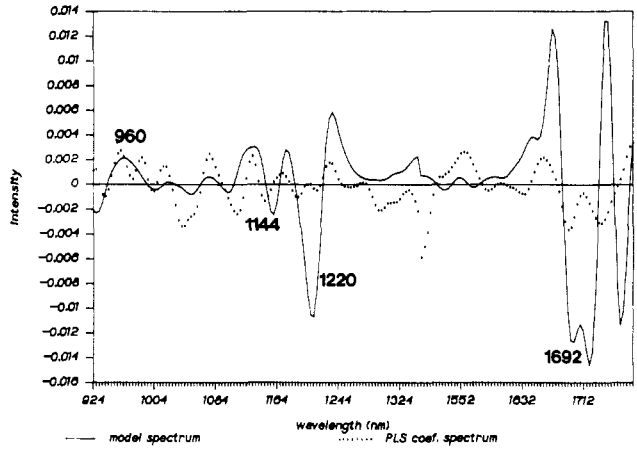


**Figure 5.** Second-derivative NIR spectrum of lipid extracted from a fish meat sample (solid line) and PLS regression coefficient spectrum for lipid in fish meat (dotted line). Wavelengths used in the MLR calibration for lipid content are labeled.

observed from 1100 to 1220 nm (Miller, 1989) and from 1690 to 1750 nm (Osborne and Fearn, 1986). One of the chosen wavelengths (960 nm) probably compensates for water interference; the other chosen wavelength (1144 nm) might compensate for protein or other interferents.

The PLS calibration curve for protein nitrogen content is given in Figure 6. For both the MLR and PLS studies, the prediction error (RMSEP) is significantly greater than the calibration error (RMSEE). This result suggests that overfitting of the calibrations may have occurred. In Figure 7, the model spectrum and PLS coefficient spectrum for protein (% N × 6.25, Kjeldahl analysis) in the rainbow trout tissue samples are presented. The model spectrum in this case was a spectrum of lyophilized solvent-extracted rainbow trout muscle. It should be noted that a number of nitrogen-containing compounds other than protein are present in fish tissue at relatively high levels. These include trimethylamine oxide, urea, taurine, small peptides and amino acids, nucleotides, and other purines at levels of 0.5–1.0% of the total weight of the muscle (Spinelli and Dassow, 1982). Nevertheless, the model spectrum should provide a good approximation for protein. The PLS coefficient and model spectra were very similar in the region from 1100 to 1250 nm. The large difference between the two spectra in the region 1250–1800 nm is probably caused by the presence of interfering water and lipid bands.

Protein absorbances are expected in the ranges 1000–1100 (second overtone NH stretching modes) and 1450–1600 nm (from first overtone NH stretching modes)

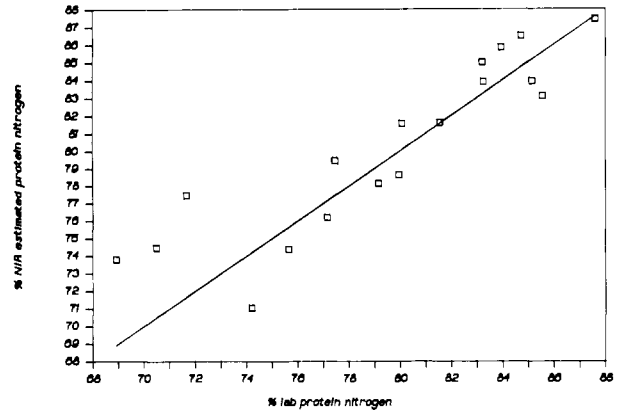


**Figure 6.** Calibration curve for PLS calibration of protein nitrogen content in fish meat.

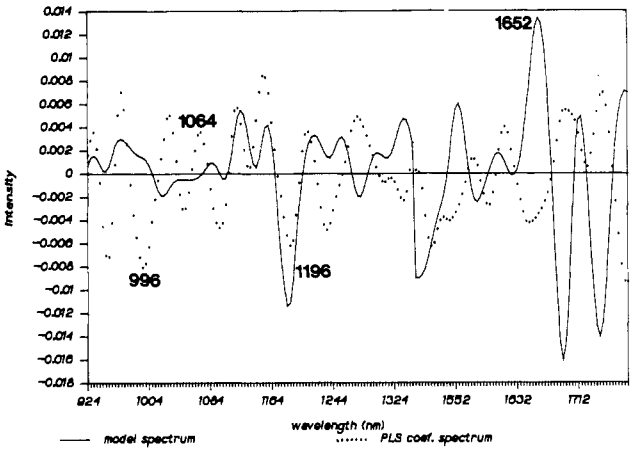


**Figure 7.** Second-derivative NIR spectrum of a freeze-dried, fat-extracted fish meat sample (solid line) and PLS regression spectrum for protein nitrogen in fish meat (dotted line). Wavelengths used in the MLR calibration for protein are labeled.

(Osborne and Fearn, 1986). The PLS regression coefficient spectrum has significant intensity in the CH region of the NIR, presumably to account for protein absorbances, and in other regions to account for interferences. Two of the wavelengths chosen for the MLR protein calibration (1196 and 1652 nm) correspond to strong peaks in the protein model spectrum (Figure 7). Both of these terms may correspond to CH absorbances in protein or compensate for lipid interferences. The 1064-nm term probably corresponds to the second overtone NH band for protein, and the 996-nm term probably corresponds to interferent absorbances.

## DISCUSSION

On the basis of the results of this study, this NIR method could be used to provide a reliable measurement of moisture content in thin slabs of fresh or thawed rainbow trout meat (RMSEP = 1.1%). The RMSEP for crude lipid content and protein nitrogen were relatively high (3.1% and 5.4%, respectively). Even so, because of the ease of this method, lack of sample preparation, and the ability to determine the concentration of multiple analytes with one measurement, it could be employed in grading programs and quality monitoring in cases where only approximate values are needed.

The calibration and prediction errors in these analyses originate from several sources. Reflectance spectroscopy in the NIR region requires the collection of sufficient light energy from the sample to determine sample absorbance. As a result, for poorly scattering samples (like fish muscle tissue), a low signal to noise ratio is obtained and weaker overtones of fundamental IR bands must be used. Reflectance measurements have been noted to be less sensitive than transmittance in the NIR region by some investigators (Bjarno, 1981).

Errors in the MLR or PLS calibrations could also be due to the empirical nature of the standard methods used to quantitate lipid and protein, the difficulties in obtaining a representative sample, and inherent problems associated with conducting the standard analytical methods (Darwish et al., 1989; Bjarno, 1982). Obtaining a representative sample for confirmatory tests for NIR calibrations is a recurrent problem (Gjerde and Martens, 1987). Measured moisture, lipid, and protein concentrations were affected by sampling procedure, although the variance due to sampling itself was found to be small when compared to the variance caused by nonhomogeneous distribution of analytes within the fish. In general, however, the results of these analyses compare well with those of others (Gjerde and Martens, 1987).

Another source of error in these experiments that could have contributed to the prediction and calibration errors for protein and lipid analyses is interference from water absorbances. Not only is water a strong absorber in the NIR region, but it is also the dominant component in the samples analyzed in this study. As a result, useful spectral data from other analytes were masked.

It is also possible that the concentrations of several constituents were perturbed by the NIR sampling procedure. The presence of extraneous surface moisture, the occurrence of water evaporation, segregation, and migration, and the loss of tissue lipid may have occurred during the sampling procedure. Therefore, an accurate NIR estimation of water or lipid content in the fish tissue samples may have been difficult to accomplish. In addition, the effect of variable sample thickness may not have been completely corrected for by the multiplicative scatter correction method employed here.

Gjerde and Martens (1987) obtained a predicted correlation of 98% for moisture and fat and 69% for protein nitrogen on a raw meat (as is basis) in their study. Their results were similar to ours for both moisture and fat. However, correlation for protein in lyophilized samples was better than ours (91%), but the predicted protein content on a wet weight basis was less accurate than what we obtained. Using mid-IR transmittance spectroscopy on emulsified fish tissue samples, Darwish et al. (1989) found that spectral and chemical methods for individual analyses agreed within ±1%. They performed standard chemical methods on tissue emulsions rather than whole tissue samples, which reduced the problems other researchers have had with inherent sample variability. Darwish et al. (1989) were also working in a different IR region (5500–6700 nm).

Improvements in this analytical method could be made through better control of the NIR sampling procedure. If sample thickness can be controlled, and the migration or loss of analyte during sample preparation is limited, better results will be obtained. However, the sampling procedure required would be more complicated and labor intensive and could limit the ease with which this method could be employed in the field. If the short-wave NIR region (600–1000 nm) can be used with a silicon detector, diffuse transmittance measurements of materials with high water content (or strong absorbances) would be possible. In this case, no sample preparation would be necessary. Preliminary results from our laboratory indicate that it will

be possible to analyze intact, whole fish by using SW-NIR reducing errors introduced by sampling and increasing the attractiveness of NIR methods to the targeted users. Despite the relatively high RMSEP for protein (% N × 6.25, Kjeldahl method), the method presented here would provide reasonable estimates for moisture, protein, and, to a limited degree, crude lipid for quality control purposes, fish grading programs, and for monitoring husbandry practices at aquaculture and hatchery facilities.

In summary, a near-infrared reflectance (NIR) method was developed to measure the proximate composition of small, thin slabs of fresh or frozen rainbow trout muscle by using partial least-squares (PLS) and multiple linear regression (MLR) calibrations. Results indicate that a good prediction of moisture and approximate predictions of protein and lipid are possible by the NIR method presented here. As a result, this method could be used for applications in which sampling time must be reduced and where only approximate constitute values are required.

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