APPLICATION OF A NEAR INFRADED REFLECTANCE SPECTROSCOPY (NIR) TO MEASURE PROTEIN, FAT AND MOISTURE IN FISH SAMPLES

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Quality control programs need methods for simple and rapid analysis of feeds and ingredients. Near Infrared Reflectance (NIR) and Transmission (NIT) Spectroscopy have been previously used for the analyses of fish carcasses (Gjerde and Mathias. 1987; Valdes et al.. 1989 and Downey. 1995). NIR was applied to measure percent fat, protein and moisture in fish samples used for food in zoological institutions. Fish samples were collected from 1992 to 1996 at the Wildlife Conservation Society (WCS), and samples were obtained froze (-30° C) from various distributors and included a total of 68 samples (16 herring, 4 whitebait, 12 capelin, 6 mackerel, 6 squid, 13 trout, 2 pollock and 7 smelt, 2 sardines). The 68 samples were analyzed within one month from arrival for percent protein, fat and moisture at the Zoo Nutrition Center of the WCS. Fish samples were thawed in a refrigerator overnight and ground in a food processor (Kenmore Short Order ll, Sears, Roebuck Co, Chicago, IL, 60684). Sub-samples were weighed, freeze-dried (Virtis Model 10-MR- TR Gardiner. N. Y, 12525) and percent moisture was calculated. The dried samples were then ground using a mill (Braun, commercial coffee grinder). Crude fat and crude protein were determined using AOAC methodology for meat (Ellis. 1984). Fat was determined by extraction of samples with petroleum ether, and protein was analyzed using a macro-Kjeldhal method with a copper catalyst and calculated as total nitrogen x 6.25. After the chemical analyses were completed, the dried ground samples were shipped to the Metro Toronto Zoo where NIR calibrations were developed.

Analytical moisture (residual moisture in freeze-dried samples) was determined (24 h, 100^0 C). Ground fish samples were scanned and near infrared Spectra stored using a scanner NIR spectrophotometer model 6500 (Perstorp Analytical, Maryland, USA) and NIR calibrations were developed for percent fat, protein and analytical moisture (AM). Prior to obtaining the NIR spectra, samples were taken from the freezer and left in a room where temperature was kept at 20° C for 3 to 4 h. Samples were stirred in the holding bags/bottles with a spatula and approximately 5g were loaded into a sample cup holder. Operation of the spectrophotometer and the collection and manipulation of spectra were performed using a software package supplied by the instrument manufacturer. Duplicate readings were recorded and stored for each sample and averaged before developing the calibrations. Prior to calibration development all spectra were examined to detect the presence of outliers or those samples whose spectra were different from the population. Spectra were converted to their second derivatives in order to minimize scatter effects due to differences in particle size. The calibrations for the different chemical parameters were developed using the full NIR spectrum by applying "partial least squares" (PLS.. Martens and Naes. 1987). In the PLS method the compression of the spectra data points and the regression steps are performed simultaneously. Thus, the PLS factors primarily described the NIR spectra that were relevant for modeling the variations in the chemical data of the fish samples. Using the PLS calibration method new sets of basis vectors are calculated which attempt to correlate the maximum variance in the spectral variance with the variance in the chemical parameters. Thus, more refined NIR calibration equations are obtained that are more representative of the variance

due to the analyte of interest {Valdes, 1993). Due to the limited number and great variability in the species of fish samples used in this study, the predictive capabilities of the calibration models generated using PLS were tested using a cross-validation method (Sharaf et al.. 1986). The accuracy (closeness to the laboratory primary method) was assessed by calculating the residual standard deviation (RSD) and the bias (non-random error), or the average difference between the NIR predicted and laboratory va1ues. Coefficients of determination (R2) and other statistical parameters were also calculated, for the relationship between NIR predicted and laboratory values (Table 1). The RSD and bias for %fat, %protein and %AM were 2.31, 0.05; 2.47,-0.18: and 1.14, 0.05, respectively. The result indicated that NIR predicted well the percent fat, protein and analytical moisture. However, the presence of sample outliers indicates that more research should be performed on these samples. Furthermore, more samples should be included in the calibration sets to add more universality and to account for more variability (e.g. fish species, locations, laboratory procedures). NIR is characterized by being fast and should be investigated further for analysis of chemical parameters in zoo feeds.

(key words: fish, composition, infrared spectroscopy)

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Table 1. Prediction of Chemical Parameters In Fish Samples By NIR

PARAMETERS	PROTEIN	FAT	MOISTURE[1]
n	49	50	55
R^2	0.93	0.97	0.48
RSD (%)	2.47	2.31	1.15
Mean lab (%)	62.97	23.53	3.18
Mean NIR (%)	63.15	23.59	3.23
Bias (lab-NIR) (%)	-0.18	-0.05	-0.05
Slope	1.00**	0.97**	1.02**
Range lab (%)	45.0-83.4	5.4-49.3	0.5-5.6
Range NIR (%)	44.5-79.7	2.2-49.3	1.3-5.2
Outliers[2]	13	12	7

1 Analytical Moisture (residual moisture in freeze-dried samples)

2 Outliers: samples that have unique character so as to make them recognizably (statistically) different from a designated sample population