PHAZIR™

Determination of moisture in salmon and salt cod.

By S. K. Schreyer

Abstract

Polychromix offers self-contained spectrometers that can be rapidly deployed for analysis of properties, such as moisture, fat or protein in agricultural materials. The goal of this paper is to demonstrate the PHAZIRs capability of quantitatively measuring moisture in raw fish and in processed fish such as salt cod. Issues related to sample preparation and calibration model generation unique to fish samples are explored. Ultimately a PLS model for determination of moisture, based on loss on drying (LOD) protocols was developed as a demonstration PHAZIR application.

Introduction

The determination of components such as fat, moisture and protein is highly desirable for many agricultural and food materials. Rapid identification of these parameters, with minimal sample preparation would be an optimal scenario for many food manufacturers and processors in order to assess quality of the material. In particular, current methods of moisture content of fish follow AOAC guidelines. The guidelines recommend solvent extraction and forced-air oven methods on ground samples, requiring both time and specialized equipment. NIR methods do not require sample preparation methods, can be done in real time, and on-site.

Moisture determination in food can be difficult due to the inherent inhomogenity of these materials. In particular, dried salt cod, has an uneven distribution of moisture due to the uneven thickness of the sample. Salt tends to be unevenly distributed; with lower salt content in the thicker loin part of the muscle and a higher salt content on the surface of the material.

Sampling of the moisture content can be difficult, as the sample material is uneven, making good physical contact between the instrument and the surface difficult. Near-infrared (NIR) spectroscopy is very sensitive to moisture, and has been used to assess moisture in ground agricultural materials for over 25 years. Water has strong, specific optical absorptions near 925, 1425, and 1925 nm. These arise from NIR photons exciting the vibrational modes of water. Other absorptions in the NIR correspond to other "functional groups" in the molecules, like CH_3, CH_2, CH, OH, NH_2. There are a few challenges to building first principles calibration models for water in the NIR. The presence of water has competing effects on the transparency of the host material. It fills voids increasing transparency, and absorbs light at other wavelengths decreasing transparency. By conducting a series of experiments, it is possible to discover mathematical transformations that minimize these effects and result in accurate water calibrations.

Other difficulties inherent in testing fish (and other foodstuffs) are interferences from fat distribution, uneven muscle surfaces, skin, deformities, bloodspots, maturity of the fish, location, and time from death, all of which have been shown to change moisture content and NIR spectra.

All these issues affect the ultimate accuracy of any PLS model to determine moisture. However a rapid method of determining moisture is highly desirable as moisture determination of a salt cod sample, for example, has a direct result on the price obtained on the market. Moisture in food samples affects the quality of the product. In food, moisture can result in off-flavors as the water enables enzymatic activity, and bacterial or mold growth. Therefore, rapid assessment of moisture is needed to control quality, as well as determine market price.

Partial Least Squares (PLS) is a method commonly used in the NIR community to correlate values, like water content, with spectral changes in the spectrum. Because it is a correlation method, the resulting accuracy depends upon low-noise spectra, representative samples and on the accuracy of the reference method.

Experimental

Prior to building the calibration model, sample preparation was required to provide compatibility with the reference method. In order to ascertain the most reproducible and reliable sampling method, various methods were utilized and compared. This included taking cross sectional cuts from the salmon and the salt cod fillets, and collecting data from various positions on these cross sections. The LOD was done for a sample from each of these sample sets, using the Mettler Toledo Moisture balance (HG63). Spectra collected at each of these samples were also utilized to generate PLS models for moisture, based on the LOD.

The process of building the calibration was thus multi-fold, and based on the conditions determined in the preliminary study for the cross sectional samples: initial weight recorded to be used as basis for weight loss due to drying; collection of replicate spectra across various positions on sample; drying for set period of time at a given temperature (see below), and; re-weighing of sample after drying. This process was then repeated for a given number of times, until drying is complete.

The drying method was adapted from the recommended conditions for the Mettler Toledo Moisture Balance for salmon. Weighing of the cooled samples was done using an analytical balance. Spectra were collected through a glass slide using PHAZIR 1018.

Sample Preparation

Salmon:

A fillet of sample was sliced into 4 cross sectional pieces (top layer, 2 middle layers and a skin layer). A 3-gram piece was cut from the loin area nearest the backbone from each layer and used for subsequent analyses.

Salt cod:

A large piece of salt cod was split lengthwise into a tail section and a head section. The tail section was layered into 3 cross sectional pieces (top, middle and skin layer). A 3-4 gram sample was cut from each cross section from the area nearest the backbone.

Similarly the head section was divided into the loin (thickset area nearest the backbone), and an off-loin section (thinner section). The loin was divided into 3 cross sectional areas (top, middle and skin layer); the other section was split into 2 cross sectional layers (top and skin layer). As previous, a 3-4 gram sample was cut from each layer and used for subsequent drying and spectra collection.

Moisture Analysis

Based on the Mettler Toledo recommendations, a 3-4 gram sample of either the salmon or the salt cod was cut from each of the cross sectional pieces. The sample was weighed (initial weight recorded), and dried from 2, 5 or 10 minutes at 120°C. Samples were cooled and re-weighed, prior to spectra collection. The time sequence for drying was: initial drying time of 2 minutes as moisture loss is more rapid, repeat drying periods of 5 minutes, and a final sequence of 2-10 minute periods to complete moisture loss in the sample.

To estimate the moisture in the samples, the initial weight of the sample and the weight after drying was recorded. The difference was used to determine the percent moisture loss of the sample, based on percent of the original weight.

NIR Measurement

Spectra were taken using a stock PHAZIR 1018 using the "Collect M1018" application. The spectrometer was stabilized for 30 minutes and wavelength calibrated before spectra were collected. The reference spectrum is a 10-scan average of a sintered Teflon puck. Each sample spectrum is the result of 5 scans through a glass side. The sample spectra are taken of 3 independent locations over the surface.

Materials

0.47 lb Fresh salmon fillet (Farm raised, Canada)
1.05 lb Salted cod, skin on (Canada)
Glass microscope slides

Equipment

LOD balance (Mettler Toledo HG63)
PHAZIR™ 1018, firmware v. 2.27
PHAZIR Method Generator™ v. 1.5.8.8

Results and Discussion

Sample preparation was found to be critical for the resulting NIR spectra collection. Initial studies performed on the cross sectional samples indicated sufficient differences in spectra, resulting in a loss in predictability of PLS models. Spectra obtained from the cross sections are shown in Figure 1, and clearly show the variability of the scans obtained for salmon (A) and salt cod (B).

This lack of homogeneity of fish samples has been well documented in the literature, and arises from a variety of reasons. For salmon, differences in spectra collected from different locations are due to the differences in fat content going from head to tail, from the skin to the backbone, and from the lateral line to the belly.[1] For salt cod, other issues arise also from the salt content of the samples. The salting process results in an uneven distribution of the moisture content, with higher moisture in the thicker loin portions and lower moisture in the thinner sections. The surface of the fillet is drier and contains a layer of partially hydrated salt.

Added to the inherent inhomogenities of the fish samples is the small sample size actually used for testing purposes. Given the recommended sample size of 3-4 g (Mettler Toledo), the sample is not wholly representative of the moisture content of the remainder of the fish.

Preparation of uniform samples is important for NIR. The effective sampling volume for PHAZIR 1018 is between 1 and 24 mm^3. This results in a greater sensitivity of the NIR measurement to inhomogeneities on the scale of 0.5 mm^3 to

50 mm^3.   Therefore to compensate somewhat for these inherent inhomogenities, models developed from the salmon and the salt cod samples were based on a sample cut from the thickest part of the fillet (loin), and from the top layer.

Typically, samples from fish or meat (materials with substantial inhomogenities in the sample), are ground to compensate for the differences, then testing is performed. Due the inherent nature of testing using a handheld NIR instrument, where testing is ideally performed on samples as-is, and in a rapid and time-effective manner, our alternative method was utilized to minimize sample preparation. To compensate for at-use inhomogenities, it is recommended that future models utilize averaging to improve precision.

To obtain good contact between the sample surface and the PHAZIR, a glass slide was used to maintain uniform contact over the sample. Glass is commonly used for NIR spectroscopy as it is transparent and has a flat surface that allows for uniform presentation of the sample to the spectrometer. The primary source of presentation variation here is surface flatness.

A subset of the raw spectra generated from PHAZIR is shown in Figure 2. The absorption band, centered on 1440 nm, is assigned to water. The strength of the band increases with moisture content. The spectra are offset from each other, this is likely to be due to changes in the material transparency with moisture and packing. Discrepancies also arise due to the heating progresses, as some protein denaturation occurs, causing shifts in the bands associated with C-H (centered at 1180 nm) Preprocessing was applied to the spectra to remove variations that are not related to the property of interest. Preprocessing conditions were optimized to separate the different moisture levels, and to minimize PLS prediction errors. The best preprocessing was found to be Standard normal variates (SNV) to compensate for particle size differences, followed by Savitsky-Golay 1st derivative (5 point window, 2nd order polynomial). The preprocessed data is shown in Figure 3.   PLS is chosen as the calibration method because the response to water in powders is inherently non-linear in diffuse reflectance measurements due to changes in particle refractive index, absorption and particle size. For the PLS model the wavelength range was truncated, and only the region between 1070-1703 nm was used. A 5-factor PLS model was generated from the top layer sample collected from salt cod. Leave-one-out cross-validation using Phazir MG resulted in root-mean square error (RMSE) for the training data set of 1.7% moisture LOD, and a correlation (R^2) of 0.997, as shown in Figure 4. The results shown are for a PLS model made from spectra collected from the top layer of salt cod. Within sample precision was found to be 0.5% LOD.
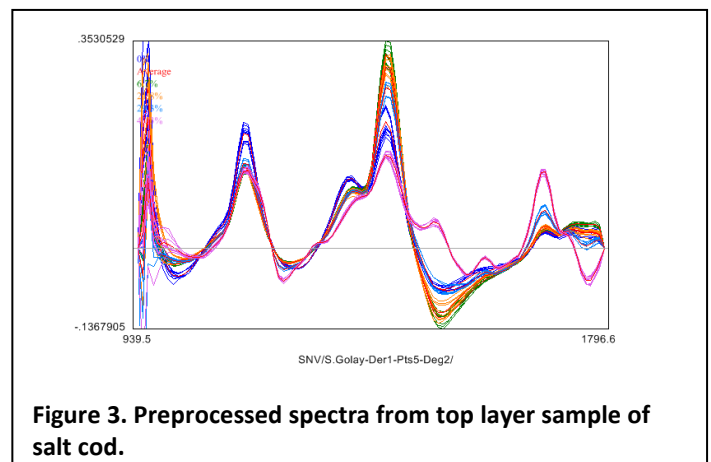
Results obtained from PLS models generated from data collected from all layers, and from averaged spectra obtained
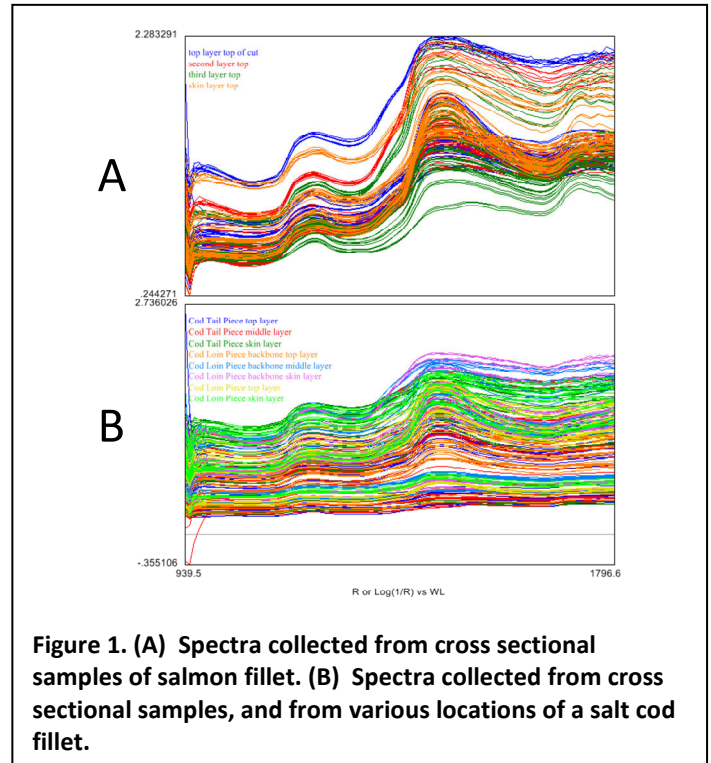
Figure 1. (A) Spectra collected from cross sectional samples of salmon fillet. (B) Spectra collected from cross sectional samples, and from various locations of a salt cod fillet.
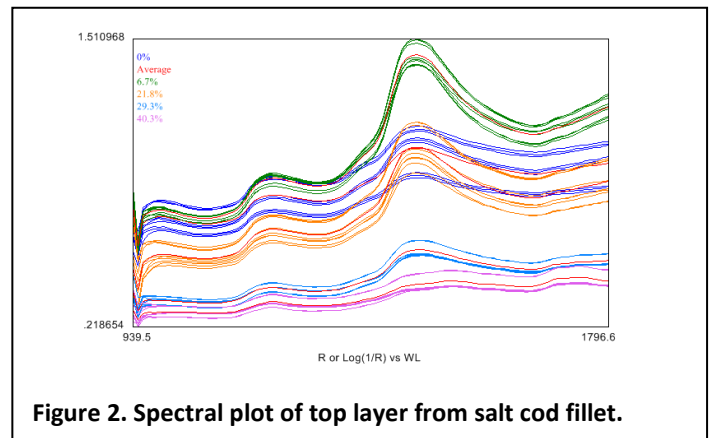
Figure 2. Spectral plot of top layer from salt cod fillet.

Figure 3. Preprocessed spectra from top layer sample of salt cod.

PHAZIR™

Moisture Determination in Fish v1.0
© 2008 Polychromix Inc.

from the top layer only, for salmon and for salt cod are summarized in Table 1.

Results obtained in the Table can be compared to literature. Literature results include models made using either the whole fish or minced, homogenized samples. One study using whole farmed Atlantic salmon and NIR spectroscopy with an optical fibre probe resulted in a RMSECV of 0.98% for moisture determination.[2] The study involved 49 salmon, using a larger sampling area.

In another example, Wold *et. al.*[1] obtained RMSE(CV)'s of 1.42% moisture based on a point section (similar to the sample size in this study), using transflectance NIR imaging of salt cod. Best results in this study were obtained by imaging the whole fish, to give a RMSE(CV) of 0.71. Wold also addressed the issue of inhomogenity of the samples, when they compared the RMSE(CV) results of a homogenized sample (RMSECV=0.6%), to that of an intact sample (RMSECV=1.9%). The results obtained for our study (RMSECV=1.7%) are similar to the literature, but need only the simpler sampling procedure of a handheld device.

Figure 4. Plot of % moisture LOD for top layer of salt cod.

Table 1 RMSE(CV) results for salmon and salt cod, based on PLS models.

Sample	Data from	Factors in model	RMSECV, % LOD
Salmon	All layers	6	5.5
	Top layer	4	2.4
	Average of top layer	4	0.5
Salt cod	All layers	5	5.0
	Top layer	5	1.7
	Average of top layer	3	1.8

Conclusions

Accurate and precise calibrations for moisture in fish can be created on the PHAZIR. Accuracy is comparable to the reference method, and has the advantage of rapid assessment in prediction. The handheld NIR application has the advantage of precision and short analysis time.

Given that these results are based on a single sample, accuracy can be improved by models utilizing multiple samples. As well, accuracy can be improved by averaging several scans and using this in the prediction models.

---

[1] JP Wold, I-R Johansen, KH haugholt, J Tschudi, J Thielemann, VH Segtnan, B Narum and E Wold, *J. Near Infrared Spectrosc.*, **14**, 59-55 (2006).

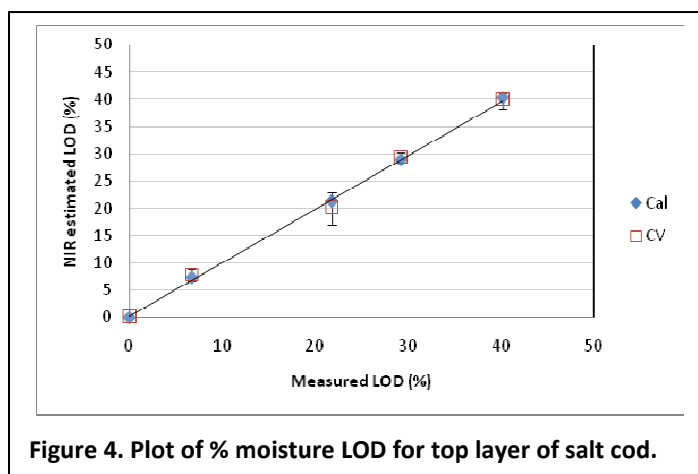[2] JP Wold, T Isaksson, *J. Food Sci.*, **62**(4), 734-736 (2006).

Polychromix
www.polychromix.com

30 Upton Drive
Wilmington, MA 01887
(978) 284-6000