Detection of Sodium Chloride in Cured Salmon Roe by SW−NIR Spectroscopy

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Salt and moisture content of cured salmon roe (ikura) was determined using short-wavelength−near-infrared (SW−NIR) reflectance spectroscopy (600−1100 nm). SW−NIR can be used to measure chloride species. Calibrations for salt in bulk samples of high-quality roe ($R^2 = 0.904$, SEP = 0.421%, RPD = 3.21) and average-quality roe ($R^2 = 0.711$, SEP = 1.13%, RPD = 1.81), crushed eggs ($R^2 = 0.857$, SEP = 0.509%, RPD = 2.62), and individual eggs ($R^2 = 0.731$, SEP = 0.698%, RPD = 1.98) were developed using partial least squares (PLS) regression models. The heterogeneous distribution of lipid and moisture in the individual eggs limit the sensitivity of this method; however, this method provides a rapid nondestructive method for high-value food products where destructive testing is expensive or impractical and for process control applications.

Keywords: *Ikura; salmon caviar; salt; moisture; short-wavelength−near-infrared spectroscopy; NIR; nondestructive analysis*

INTRODUCTION

Ready-to-eat cured and/or smoked aquatic food products are an important food product category, and the international market for them is huge and growing rapidly. The unique flavor and mouthfeel of caviars make them popular foods, and they are the most valuable aquatic food product on the world market on the basis of unit price. Salmon caviar (or ikura) from pink and chum salmon from Alaska alone has a value which exceeds $380 million annually at first sale (*1*).

Roe is harvested from mature female salmon when they return to spawn. The nutritional value of fish roe is extremely high. Salmon roe has about 20−38% protein and 10−20% fat, higher than the salmon flesh. (*2*). Fish roe are enveloped in an ovarian sack and are sterile when extracted from the fish, however individual eggs can become contaminated when the ovarian sack and connective tissue are removed and the eggs are separated from each other during the ikura manufacturing process.

Salmon roe is cured with salt to make salmon caviar or ikura. Salt content is closely tied to product sensory characteristics, but is also a critical factor for the product safety and quality. Salt is the primary, and in most cases, the only preservative used for ikura. The stability and safety of these products is achieved by controlling salt concentration to lower water activity and by altering the redox potential. The federal government requires manufacturers of roe products to have specific preventive measures in place as part of a HACCP plan (21 CFR Part 123) to control the growth and toxin production of *Clostridium botulinum* (*3*).

Ikura products with relatively low salt content (≤3.0% salt) are becoming increasingly popular on the world market because of changes in consumer preferences for these foods. However, lower salt products can support the growth of salt-tolerant psychrophilic foodborne pathogens including *C. botulinum* type E, *Escherichia coli, Listeria monocytogenes, Vibrio parahemolyticus*, and *Yersinia enterocolitica* (*3*). Few ikura products are pasteurized; however, current concern for food safety is increasing the interest in production and sale of these foods in pasteurized forms.

Some ikura is frozen during distribution. Frozen products are commonly thawed and held for several days before consumption or sale. Discernible quality degradation is apparent after previously frozen product is held refrigerated for 4 days or more. A greater problem is that ikura, particularly product packaged in glass jars, is presumed to be shelf stable and is often held unrefrigerated for extended periods by consumers and some retailers, creating a risk for microbial pathogen growth.

A key preventive measure for cured and/or smoked foods is adequate salt content, and the ability to accurately measure salt content is important. Total salt is commonly measured; however, this is a destructive test and takes anywhere from 5 min to several hours to complete depending upon the analytical method chosen. Unfortunately, there are no rapid, nondestructive analytical tests for salt. Current methods for salt include titration (*4*), a chemical test kit ("Quantab" method), an electrochemical method (*4*), and salt ana-

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lyzer or conductivity methods (*5*). The widely used salt analyzers are not very reliable in a production setting, and most methods fail to provide the desired precision of ± 0.5% in the salt measurement (*6*). Oftentimes, manufacturers err on the side of adding too little salt to avoid producing a product that is "too salty" tasting and less acceptable to consumers. Again, these analytical methods require the product to be destroyed.

Moisture content is a critical measurement for smoked and cured foods because it is the basis for water-phase salt calculations. Moisture determination is a destructive test. Standard methods that employ a drying oven take a minimum of 6 h to conduct (*4*). Microwave drying methods take as little as five minutes per sample but destroy the food in the process (*4*). Many food processors can conduct moisture analyses on site, however, these tests take time and may not provide immediate results which could be used to modify a production process to ensure safety or to improve product quality.

Short-wavelength near-infrared (SW−NIR: 700−1100 nm) spectroscopy has several advantages as a nondestructive method for measuring salt and moisture. First, SW−NIR allows the user to collect full spectra in less than a few seconds and to analyze food samples remotely using fiber-optic probes. Near-infrared light can travel over long path lengths and penetrate through fish skin and scales into the fish muscle tissue permitting analysis of intact whole fish, fish fillets, or steaks, and bulk samples of roe (*7*−*8*).

Both moisture and lipid concentrations can be measured by SW−NIR. The OH stretch of water and the methylene CH stretch of fat are clearly visible at approximately 960 and 930 nm, respectively. These spectral features are chosen for calibration models for moisture and lipid (*9*). Moisture and lipid are inversely correlated in fish muscle and in fish roe samples, and spectral features corresponding to each are selected in calibration models (*10*). Rasco and collaborators were the first to report the noninvasive analyses of whole fish by the SW−NIR method (rainbow trout (*Onchorynchus mykiss*) ranging in size from 66.5 to 883 g) (*10*). Recently, other researchers have confirmed SW−NIR to be an effective analytical method for moisture in fish and fish tissue. Isaksson et al. (*11*) used SW−NIR to measure lipid, moisture, and protein in Atlantic salmon (*Salmo salar*). Downey (*12*) used NIR interactance to predict the moisture content (prediction error (bias corrected) of 1.45%) of salmon by examining six sites on the dorsal and ventral surfaces of market-size Atlantic salmon (1 kg or greater). Wold et al. (*13*) confirmed a good correlation between SW−NIR and conventional reference methods on whole intact salmon (1.0−5.7 kg) finding root-mean-square error of cross validation values of 0.98 for moisture. The standard error for moisture (reference method) was 0.13 (*14*).

Salt is also measurable directly in the SW−NIR (*15*) as a shift in the water band to a longer wavelength (*16*). Research of Begley et al. (*15*) and Ellekjaer et al. (*17*) indicates that near-infrared spectroscopy has great potential for rapid and nondestructive analysis of salt in cured muscle food products. Although absorption in the NIR region is mainly due to overtone vibrations of C−H, O−H, and N−H functional groups, and sodium chloride has no specific absorption band in the NIR region, detection of the sodium chloride is possible because of its effects on the shape and position of the water band (*15, 18*).

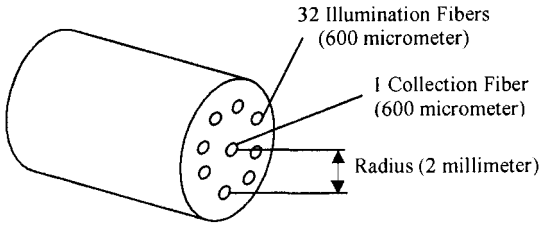

Figure 1. Size and spacing of the illumination and collection fibers on the end of the fiber-optic probe.

Correlating SW−NIR spectral measurements with chemical reference values can be a problem because of the nonuniform distribution of chemical constituents in intact tissue (e.g., fish fillets, fish steaks, and roe). However, when the distribution of analytes within the tissue is understood and taken into consideration, reliable spectral models can be developed (*12*). The prediction error of SW−NIR models for intact tissue and homogenized tissues can be similar if the nonhomogeneous nature of the tissue can be taken into account in the experimental design (*13, 14*). For example, Lee et al. (*10*) obtained a prediction error of 0.7% for lipid in intact fish muscle tissue, a result similar to that found by Sollid and Solberg (*19*) for homogenized salmon tissue.

The objective of this study was to develop a nondestructive short-wavelength near-infrared spectroscopic method for detecting salt and moisture in ikura.

MATERIALS AND METHODS

Roe Samples Preparation. Chum roe were recovered from hatchery return salmon (*Oncorhynchus keta*) at the Lummi Bay Hatchery, in Whatcom County, WA during the 1999 harvest season. Egg skeins were removed from mature fish, which had been killed with a blow to the head. The roe was recovered by making a cut with a Zack knife starting at the vent and continuing 25 cm up the side of the fish. The roe were immediately removed, placed in sterile polybags and then packed on gel ice and transported by air from Bellingham, WA to La Grande, OR on the same day. The skeins were sorted into a high quality lot and an average quality lot based upon the appearance of the skeins, degree of maturity, and the color and texture of the individual eggs within a skein. Individual eggs were separated from the egg skein and connective tissue by using commercial stainless steel ikura screens.

The high quality and average quality lots of roe were brined separately within 24 h of harvest. For each type of roe, 17 different brining treatments were conducted at 20 °C with samples brined in saturated salt from 0 to 20 min. The brined roe was then drained in commercial ikura curing baskets at 4 °C for 10 min. Samples were covered with plastic wrap and kept refrigerated until the spectra were recorded. Because of the fragile nature of this product, all spectra were taken within 48 h of preparation.

SW−NIR Spectral Analysis. *Salt.* Thirty-two solutions were prepared with salt concentrations from 0 to 15% (w/w) at increments of approximately 0.5%. Spectra were recorded in transmission mode using a 2-cm quartz cuvette. All samples were analyzed at room temperature (20 °C) with no additional temperature control.

Ikura. SW−NIR spectra were recorded in diffuse reflectance mode with a fiber-optic probe and a DPA-20 spectrophotometer (DSquared Development, Inc., La Grande, OR). Figure 1 shows a schematic representation of the end of the fiber-optic probe. The probe contained 32 illumination fibers (600 μm in diameter) arranged in a concentric circle, 2 mm away from a single collection pick-up fiber. The diameter of the probe was about 1 cm. Three separate sets of spectral measurements were taken on the roe: (1) roe as a bulk sample of intact eggs, (2)

crushed eggs, and (3) individual salmon eggs. Spectra were recorded between 600 and 1100 nm in 0.5-nm steps.

For roe bulk analysis, spectra of roe from both the high-quality and average-quality lots were measured. Approximately 25 g from each treatment was transferred to a 100-mL beaker. Spectra were collected in the diffuse reflectance mode by placing the fiber-optic probe in direct contact with the sample. The immersion distance of the probe into the sample was measured and kept constant between samples. The probe was positioned far enough from the bottom of the beaker containing the sample to avoid possible specular reflections. For each of the 17 treatments three spectra were collected. Before each individual spectrum was recorded, the sample was stirred and resampled to expose different eggs to the probe. Each individual spectrum consisted of 40 averaged spectra with a 400 ms acquisition time. Each sample was therefore analyzed in 16 s.

For crushed egg samples, spectral measurements were obtained on samples from the lots of high-quality cured roe only. Approximately 25 g of eggs was transferred to a 4-oz Whirlpak bag (Fisher Scientific, Pittsburgh, PA) and the eggs were crushed until all eggs were broken. Approximately 30 mL of this slurry was transferred to a 50 mL beaker. Spectra of crushed eggs were recorded in diffuse reflectance mode by placing the fiber-optic probe into the center of the sample. The immersion distance of the probe was again controlled to ensure reproducibility in sampling and to avoid specular reflections from the beaker walls. Three spectra were recorded for each sample. Prior to each spectrum collection, the sample was stirred in order to expose different volumes of the sample to the probe.

For measurement of spectra for individual eggs, 10 eggs from each treatment from a lot of high-quality roe were used. The fiber-optic probe was inverted, and eggs were analyzed one at a time by placing each egg on top of the inverted probe. Each individual spectrum consisted of 40 averaged spectra with 120 ms acquisition time. Each sample was analyzed in about 5 s. All samples were analyzed at room temperature (20 °C) with no additional temperature control.

Reference Analysis. Sodium chloride concentration was determined in triplicate by AOAC method 937.09. Ca. 1.000 g of roe was used for each determination. Moisture was determined in triplicate by AOAC method 952.08 with Ca 1.500 g used for each determination.

Data Analysis. Data analyses were performed using Delight 3.1a software package (DSquared Development, Inc., La Grande, OR). Principal component analysis (PCA) was used to explore the data and determine the number of latent variables that explain the variance in each data set. Partial least square (PLS) regression was used to correlate spectra with the reference values. Before the regression was conducted, all spectra were smoothed and transformed to the second derivative. Smoothing and the derivative parameters were varied to optimize the correlation. The number of PLS latent variables was also varied to obtain optimal correlation results.

Leave-one-out-cross-validation was used to evaluate the performance of the established calibration equations (*20*). For the bulk and crushed roe studies, all the three spectra corresponding to the same sample treatment were removed and a calibration model was built upon the remaining spectra. The model was then used to predict the salt and moisture concentration of the three removed spectra. This procedure was repeated for each sample in the data set. The predicted values were then compared with the reference values. The coefficient of variance (R^2), the standard error of prediction (SEP), and the RPD (ratio of the SEP to the standard deviation (SD) of the data set (*21*) were used to evaluate the quality of the model. The RPD provides a means for standardizing the SEP and evaluating the robustness of the model. For example, if the standard deviation of the original data set is 1.38 and the SEP is 0.284, the RPD is given by 1.38/0.284 = 4.86. A RPD greater than 10 indicates an excellent capability of the calibration model to predict a specific parameter. Values of 5−10 are adequate for quality control, whereas values of 2.5−5 are satisfactory for screening (for example, determining low, medium, and high concentrations). A RDP of 1 indicates that the standard deviation and the SEP are the same and that the calibration cannot be effectively used for predicting the parameter accurately.

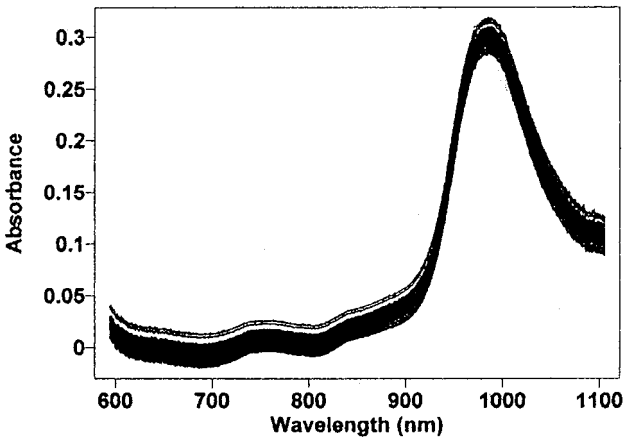

Figure 2. SW−NIR spectra of NaCl solutions.

For the individual egg analysis, 10 sets of spectra from each treatment were included to establish a model. Predictions were obtained by leave-one-out cross-validation and removing like spectra with each iteration.

RESULTS AND DISCUSSION

NaCl Study. The spectra of 32 NaCl solutions with concentrations varying between 0 and 15% (w/w) were determined to ascertain whether SW−NIR could detect and measure chloride species. Figure 2 shows representative spectra of these NaCl solutions. The prominent absorption at approximately 985 nm arises from the $2\nu_{1+}\nu_3$ overtone combination transition where ν_1 is the symmetric O−H stretch, ν_3 is the antisymmetric O−H stretch, and ν_2 is the O−H bending mode (*22*). The band is particularly broad because of the presence of two or more types of hydrogen-bonded molecular complexes (*23*) and the perturbation from the sodium and chloride ions (*16*). In addition, weaker absorptions arise from $2\nu_{1+}\nu_{2+}\nu_3$ and $3\nu_{1+}\nu_3$ and are observed near 840 and 750 nm, respectively (*24*).

A second derivative transformation of the spectra was calculated to eliminate baseline offsets due to cuvette placement and to improve spectral resolution. Spectral differences due to changes in NaCl concentration in the 980−990 nm region can be made more evident by subtracting the water spectrum from the data set (second derivative difference)(Figure 3). However the second derivative difference was used only for the purpose of enhancing spectral information and was not used to develop calibration models. The actual PLS calibration model was constructed on the second derivative absorbance transformation using 3 latent variables. The calibration model shown in Figure 4 is strongly weighted by the band at approximately 980 nm making information from this spectral region the most useful for predicting chloride concentration. An excellent correlation between actual and predicted NaCl concentrations (R^2 = 0.997, SEP = 0.242%, RPD = 18.91) was obtained. Even though sodium chloride has no specific absorption bands in the NIR wavelength region, salt analysis is possible because of the perturbation of the water band by sodium chloride (*16*). These results indicate that SW−NIR spectroscopy can be used for salt concentration measurements in aqueous solutions and

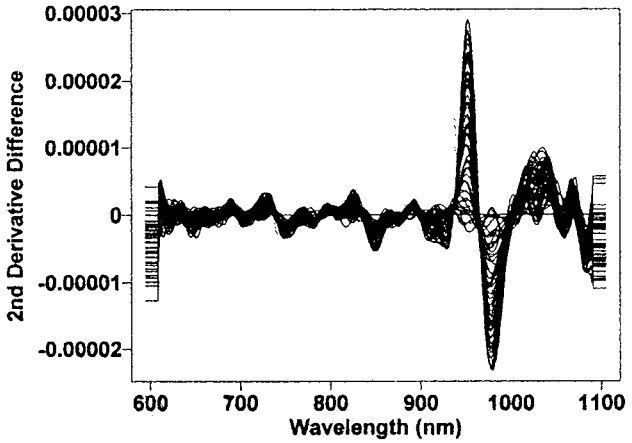

Figure 3. SW−NIR second derivative difference spectra of NaCl solutions.

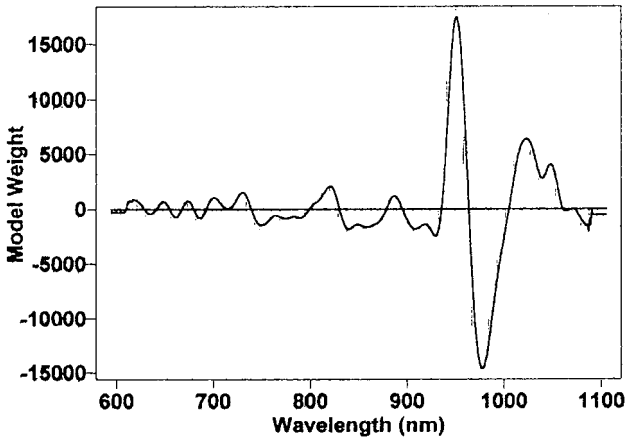

Figure 4. PLS calibration model for salt in NaCl solutions.

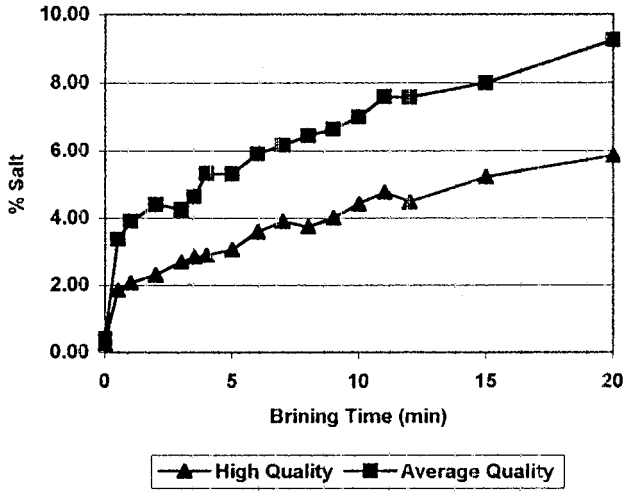

Figure 5. Difference in salt uptake rate for high- and average-quality chum salmon ikura.

provide a basis for the application of this technology to measuring salt concentrations in complex biological matrixes.

Roe Study. Salt uptake for high-quality and average-quality roe brined under the same conditions is shown in Figure 5. Salt uptake for average-quality chum salmon ikura was significantly faster than that of high-quality roe during the first minute (2% for high-quality roe and 3.8% for average-quality roe). After this point, high-quality and average-quality roe had similar salt

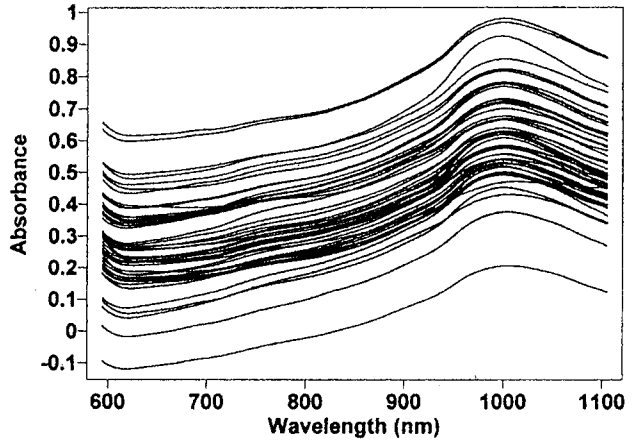

Figure 6. Diffuse reflectance spectra of high-quality chum salmon ikura, bulk properties.

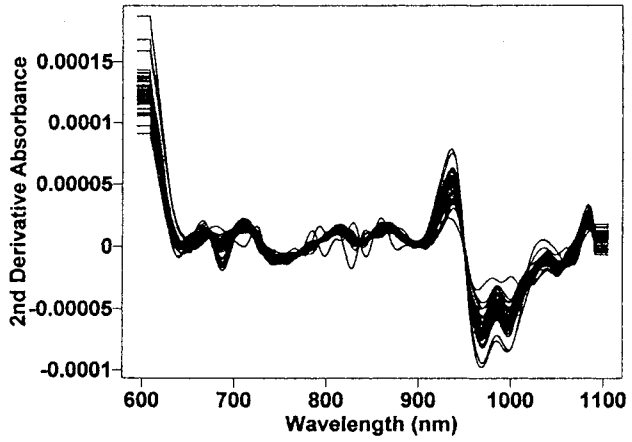

Figure 7. Second derivative transformation of spectra for high-quality chum salmon ikura, bulk properties.

uptake rates. The salt range was 0.27−5.85% for high-quality roe and 0.40−9.26% for the average-quality roe.

Diffuse reflectance spectra of high-quality chum salmon roe are shown in Figure 6. The broad absorption band at approximately 990 nm is related to the O−H second overtone as is the case for the NaCl solutions described above (Figures 2 and 3). In addition, the sloping nonzero baseline contributions are related to scattering effects (*25*) due to the translucent nature of the roe. Figure 7 shows how the second derivative transformation corrects for baseline offset and enhances changes in the spectral features of the water band in the 970−995 nm spectral region due to the addition of sodium chloride.

Principal component analysis (PCA) was conducted on the spectral data to determine the optimal number of latent variables to include in the chemometric model. For each data set the optimal number of latent variables was determined by plotting the % total explained variance vs the number of latent variables. For the high-quality roe data set six latent variables explain approximately 98% of the variance. Additional latent variables may model individual samples but do not improve the robustness of the overall model. This was demonstrated by the fact that when building the calibration model using a PLS regression, inclusion of more than six latent variables degrades the predictive ability of the model. The optimal number of latent variables was also confirmed by plotting the standard deviation of the error obtained through the cross-

Table 1. Statistical Summary of Calibration for Salt and Moisture Content in Chum Roe Ikura Using Partial Least Squares Regression

			salt			moisture		
experiment	N[a]	S[b]	R^2	SEP[c] (%)	RPD[d]	R^2	SEP[c] (%)	RPD[d]
high-quality bulk	17	51	0.904	0.421	3.21	0.876	1.13	2.82
	15	45	0.925	0.321	3.64	-	-	-
average-quality bulk	17	51	0.711	1.13	1.81	0.722	1.45	1.86
	16	48	0.903	0.592	3.21	-	-	-
high-quality and average-quality bulk combined	34	102	0.763	1.01	2.05	0.609	1.87	1.58
	31	93	0.800	0.875	2.19	0.724	1.57	1.89
crushed	17	51	0.857	0.509	2.62	0.842	1.27	2.51
	17	49	0.952	0.282	4.43	-	-	-
individual eggs	17	170	0.731	0.698	1.98	0.681	1.79	1.82

[a] Number of treatments. [b] Number of spectra. [c] SEP, standard error of prediction. [d] RPD, ratio of SEP to standard deviation of original data (see text).

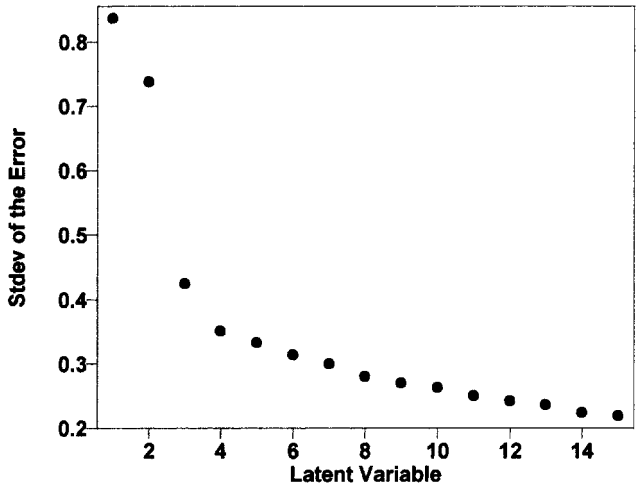

Figure 8. Standard deviation of the error vs. number of latent variables from PLS salt calibration of high-quality chum salmon ikura, bulk properties.

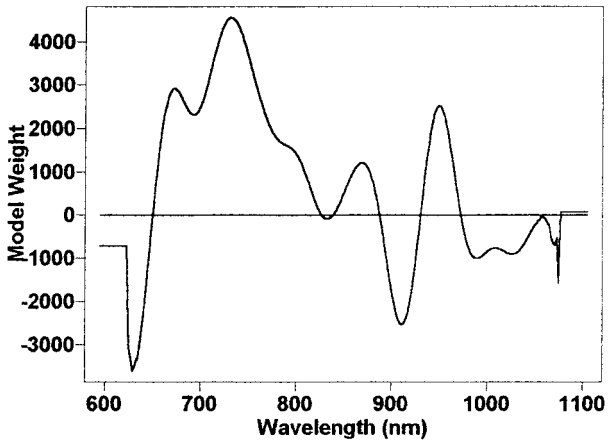

Figure 9. PLS calibration model for salt in high-quality chum salmon ikura, bulk properties.

validated PLS regression vs the number of latent variables. In Figure 8 a minimum in the standard deviation of the error is reached at six latent variables with no appreciable improvement when a larger number of latent variables is used. Figure 9 shows the PLS calibration model for salt for the high-quality roe. In addition to the water band, also observed for the sodium chloride solutions, contributions to the model come from the large lipid content of the eggs (at approximately 920 nm) and from carotenoid pigments (between 600 and 700 nm).

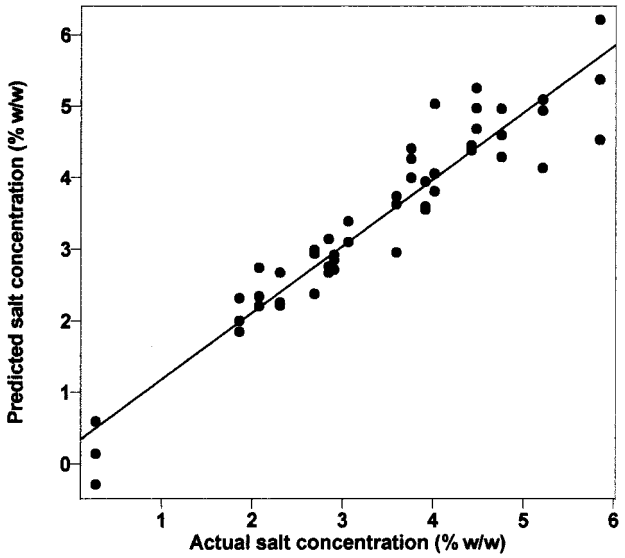

Figure 10. Comparison between the actual and predicted salt concentrations for high-quality chum salmon ikura, bulk properties.

Using a similar approach, a salt calibration model for the average-quality roe can be built with six latent variables. No substantial differences between calibration models constructed for high- and average-quality roe were observed.

Six latent variables were also used when constructing the moisture calibration models for both the high-quality and average-quality roes.

To determine whether a single calibration could be built to predict salt and moisture for both high- and average-quality roe samples, all spectra were combined and analyzed. Eight latent variables were needed for an optimal PLS regression. The additional two latent variables would most likely help explain differences between the quality of the eggs from the two grades.

A summary of the statistical results of all PLS regressions is provided in Table 1. For the bulk roe analysis, better results were consistently achieved for the high-quality product. For example, the cross-validation for predicting salt content yielded a higher correlation coefficient ($R^2 = 0.904$), a lower standard error of prediction (SEP = 0.421%), and a higher RPD (3.21) than for the average-quality roe ($R^2 = 0.711$, SEP = 1.13%, and RPD = 1.81). Figure 10 shows the comparison between the actual and the predicted salt values for the high-quality roe. Similar results were observed when constructing cross-validation models for moisture.

It is possible that the better results obtained for the high-quality roe could be explained by the fact these roe samples were more homogeneous that roe in the average-quality samples. Roe of higher quality would be of a more consistent maturity, appearance, and texture than product of lower quality. High-quality roe would also show no signs of autolysis or decomposition.

Removal of spectra associated with higher salt concentrations from both data sets yielded improved results. For example the elimination of two treatments corresponding to salt concentrations of 5−6% in the high-quality roe set improved the R^2 from 0.904 to 0.925 and lowered the SEP from 0.421% to 0.321%. Similarly, elimination of the highest salt concentration treatment from the average-quality roe data set (9.26%) improved the R^2 from 0.711 to 0.903 and lowered the SEP from 1.13% to 0.592%. These results suggest that the spectral properties of eggs at higher salt concentrations may be significantly different from spectral properties of eggs at a lower salt concentration. The sensory characteristics of high-salt ikura (> 5.0% total salt) are quite different from those of ikura at roughly 3.0% salt. At very high salt concentrations, chum salmon eggs become hard, desiccated, and translucent. Although none of the spectra recorded on the higher-salt eggs were identified as outliers, these eggs were clearly darker and desiccated.

Results obtained by merging both data sets yielded a poorer prediction ($R^2 = 0.763$, SEP = 1.01%, and RPD = 2.05). Removal of the three treatments associated with higher salt concentrations improved the calibration model slightly yielding a higher correlation coefficient ($R^2 = 0.800$) and a lower prediction error (SEP = 0.875%).

Prediction models for salt constructed on all spectra recorded on the crushed eggs yielded lower correlation coefficients, higher SEPs, and lower RPDs than those for models for bulk roe eggs from the same treatment. These results conflict with our hypothesis that spectra collected on more homogeneous samples would yield better correlations. Removal of spectra associated with samples 31 and 34 corresponding to salt concentrations of 0.27% and 3.92%, respectively, dramatically improved the calibration model (Table 1). However no statistical basis for the removal of these two samples could be determined.

Spectra of individual eggs were also collected to investigate whether the analysis of single eggs from a treatment would be representative of the bulk properties of a treatment. The prediction model for individual eggs had a significantly lower correlation coefficient, higher prediction error, and lower RPD than those for bulk roe (Table 1). This difference was most likely due to the variation in chemical composition of the individual eggs that could not be reflected in reference measurements. Reference measurements were, by necessity, taken from a much larger sample composed of numerous eggs. In addition, differences in the orientation of the lipid droplet among single individual eggs in each sample during spectral collection may contribute to increased noise for spectral measurements. There is an inherent nonhomogeneous distribution of the lipid and aqueous phases within the egg. Individual salmon eggs are approximately 5 mm in diameter and contain distinctive lipid droplets which are approximately 10−20% of the volume of the egg. These lipid droplets tend to congregate toward one pole of the egg. How the droplet is oriented during spectral acquisition would greatly affect whether salt or moisture would be "over sampled" or "under sampled" in any given spectral measurement. In a bulk sample of roe, the eggs tend to orient themselves with the lipid droplets up. In the experiment with individual eggs, we deliberately attempted to record spectra with the lipid droplet "up", "down", and laterally toward both sides as a means of reducing the effect of lipid droplet orientation. However, these efforts were not sufficient to compensate for the nonhomogeneous distribution of constituents within a single egg. Only when the near-infrared light could travel through several layers of eggs, could the inherent nonhomogeneity between individual eggs be lessened.

In conclusion, SW−NIR reflectance spectroscopy can be used for nondestructive determination of salt and moisture in cured salmon roe (ikura). Efficient salt calibrations were established for high- and average-quality roe for bulk samples of each product grade with measurement errors within the range currently experienced in commercial settings. Limitations of these models for eggs with a higher salt concentration may be due to major changes in the morphology of the eggs as salt concentration increases. However, these high salt concentrations greatly exceeded typical values for ikura and would not be expected in an industry setting.

The limited number of samples used in this study may raise some concerns as to the validity of the models developed. Access to large amounts of fresh salmon eggs is quite difficult and restricted to a very small window of time when the fish return to spawn. These eggs were recovered during a short commercial opening of the chum salmon fishery on Puget Sound and only a limited amount of product was available. Generally, during any given year, this fishery will be for a period of approximately 8 weeks. In addition, uncured salmon roe has a refrigerated shelf life of about 3 days, which further limits the time available to perform experiments. For the bulk roe study, seventeen different treatments were prepared. However, with the replicated spectral acquisition of subsamples for each treatment, we believe to have built sufficient variance within the data set to develop a sound calibration model. Further limitations for the analysis of salt and moisture in ikura are related to the heterogeneous distribution of constituents in the individual eggs, biological variation within the eggs which results in differences in the salt absorption between eggs within any given treatment, and variations in photon path length that results from such inhomogeneity. Most prominent is the variation in size and orientation of the lipid droplet inside individual eggs and how this affects spectral properties. This limitation was particularly evident when trying to predict salt and moisture content in a single egg. Currently there is no rigorous theory developed for the exact path of radiation propagation in samples containing a heterogeneous distribution of absorbers. Despite these difficulties, having a rapid nondestructive method which can predict salt and moisture content, even with limited accuracy, for high-value food products where destructive testing is expensive or impractical, and where the method could be applicable for process control, would be beneficial. This technology may become significant as a key element in the development of an automated continuous roe processing system which would reduce microbial contamination of the product and improve product consistency and quality.

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