

Predicting sodium chloride content in commercial king (*Oncorhynchus tshawytscha*) and chum (*O. keta*) hot smoked salmon fillet portions by short-wavelength near-infrared (SW-NIR) spectroscopy

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Abstract

Partial least square (PLS) based short-wavelength near-infrared (SW-NIR) prediction models for salt content in commercial hot smoked fillet portions of king or Chinook (*Oncorhynchus tshawytscha*) ($N=140$; 212–468 g) and chum (*O. keta*) salmon ($N=120$; 137–356 g) were developed. Spectra were collected in the diffuse reflectance mode (600–1100 nm). The total salt content ranged from 1.66 to 5.95% w/w for king and 2.15 to 5.69% w/w for chum salmon. The moisture ranged from 50.7 to 71.6% w/w for king and 55.5 to 69.7% w/w for chum salmon. An optimal PLS model for salt required eight latent variables for king salmon ($R^2=0.83$, SEP=0.32% w/w) and eight latent variables for chum salmon ($R^2=0.82$, SEP=0.25% w/w).

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1. Introduction

Ready-to-eat smoked aquatic food products such as hot smoked salmon in controlled atmosphere packaging are important commercial products with an increasing domestic and international market. On a per pound basis, the wholesale value of salted salmon roe is higher than cured salmon, but on a volume basis, the value of the cured and/or smoked muscle is greater. The international harvest of salmon (including aquaculture) exceeds 4000 million pounds with an estimated wholesale market value of over 10,000 million dollars (Johnson, 2001). Approximately 15% of the harvested salmon is cured and/or smoked with a value at first sale of between 2000 and 3000 million dollars (Huang, Cavinato, Mayes, Bledsoe, & Rasco, 2002; Johnson, 2001).

Two procedures are commonly used to produce smoked fish: cold smoking and hot smoking. Hot smoking

applies both heat and smoke to cook the product. Current FDA guidelines pasteurize hot smoked products by subjecting them to a thermal process equivalent to 145 °F (62.8 °C) for at least 30 min (FDA, 2001).

Most smoked aquatic food products are distributed at refrigeration temperatures, but may not be held at temperatures low enough to inhibit the growth of microbial pathogens. These products pose food safety risks if not properly processed and stored and are currently under heightened scrutiny by the FDA (Huang et al., 2002; Busta, Bledsoe, Flick, Gram, Herman, Jancke, & Ward, 2001; Herman, 1999).

A key hazard analysis critical control point (HACCP) preventive measure for smoked fish is adequate salt content. The FDA requires a level of water phase salt (WPS) sufficient to inhibit the germination of *Clostridium botulinum* spores with the current regulatory criteria of: $\geq 3.5\%$ WPS for vacuum packaged product; $\geq 3.0\%$ WPS for product with ≥ 100 ppm nitrite held in vacuum or modified atmosphere packaging; or $\geq 2.5\%$ WPS for products packaged in an air atmosphere (FDA, 2001). The level of salt added during brining, the

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brining regime, curing process, and dehydration steps during the curing and smoking process all affect the final WPS content of a smoked fish product. In addition to a sufficiently high salt content, storage temperature (≤ 38 °F) is one of the most important hurdles contributing to the stability and safety of smoked fish products (FDA, 2001).

Accurate salt measurement is critical for HACCP control for smoked fish products considering the heightened regulatory scrutiny these products are currently being subjected to. Common methods for salt analysis include hydrolysis-titration (AOAC 973.09), a chemical test kit (“Quantab” method) (Hilderbrand, 1992), and an electrochemical method (AOAC 976.18). The recently proposed draft Codex method involves aqueous extraction of salt from a sample and titration with standardized silver nitrate (Karl, Akesson, Etienne, Huidobro, Lutén, Mendes, Tejada, & Oehlenschläger, 2002). These analytical methods for salt are time consuming and difficult to run in a production setting, and they yield analysis results with questionable accuracy (Huang, Cavinato, Mayes, Bledsoe, & Rasco, 2001). Furthermore, these analytical methods require the sample to be destroyed. Non-destructive methods for salt detection show promise and will greatly help processors manufacture safe and high quality products (Huang et al., 2001; Rogers, Cavinato, Mayes, Bledsoe, Huang, & Rasco, 1999; Ellekjaer, Hildrum, Naek, & Isaksson, 1993). Short-wavelength near-infrared (SW-NIR: 600–1100 nm) spectroscopy can remotely analyze fish tissue through the skin and scales permitting analysis of intact whole fish, fish fillets or steaks (Huang et al., 2002; Wold & Isaksson, 1997; Lee, Cavinato, Mayes, & Rasco, 1992).

Moisture and salt concentration can be measured in the SW-NIR. The OH stretch of water is clearly visible at approximately 960 nm. These spectral features are chosen for calibration models for moisture and lipid (Rasco, Miller, & King, 1991). Salt is measurable indirectly (Begley, Lanza, Norris, & Hruschka, 1984) as a consequence of the 960 nm water band shifting to a longer wavelength (Lin & Brown, 1992). Application of a rapid such SW-NIR method could be used to control salt content during processing, thereby improving product consistency and quality. The method could also be used to quickly determine whether product in commerce contains a level of WPS meeting regulatory requirements, or to predict whether a product could pose a microbial food safety risk during storage. Neural network (back propagation neural network (BPNN) models yielded slightly better predictive results for salt and moisture (Salt: $R^2=0.82$, $RMS=0.55$; moisture: $R^2=0.95$, $RMS=2.44$) than PLS models (Salt: $R^2=0.78$, $RMS=0.63$; moisture: $R^2=0.94$, $RMS=2.65$) for cold smoked Atlantic salmon (*Salmo salar*) (Huang et al., 2002).

The objective of this study was to evaluate the ability of short-wavelength near-infrared (SW-NIR, 600–1100 nm) spectroscopy to predict salt content in hot smoked king or Chinook (*Oncorhynchus tshawytscha*) and chum (*O. keta*) salmon fillets prepared under commercial processing conditions using existing and newly developed chemometric calibration models.

2. Materials and methods

2.1. Sample preparation

Hot smoked salmon fillet portions from fourteen production lots (king or Chinook) (*Oncorhynchus tshawytscha*) ($N=140$, weighing 212–468 g) and twelve production lots of chum (*O. keta*) ($N=120$, weighing 137–356 g) were kindly donated by a commercial producer in Seattle, Washington and shipped frozen to the lab by an overnight delivery service. The frozen salmon samples thawed at room temperature. Spectra were collected when samples reached room temperature (ca. 20 °C). At least two spectra were acquired on each fillet portion with one sample site being at the thickest area of the piece.

2.2. Spectra collection

SW-NIR spectra were recorded in diffuse reflectance mode with a ProSpectra spectrophotometer (Textron Systems, Wilmington, MA). The probe was placed in direct contact with the thickest area on the interior surface of each fish portion. This is the location where the salt content is presumed to be the lowest. Spectra were collected at 0.5 nm intervals from 600 to 1100 nm. Each spectrum was the average of 100 scans with 30 ms exposure time for each sample. In addition, one or more spectra were collected in a similar fashion at other locations on the fillet portion.

2.3. Reference analysis

After spectral acquisition, a ca. 5.000–10.000 g sample was collected for reference analysis from the same area. Sodium chloride concentration was determined in duplicate by AOAC method 973.09. Ca. 0.6000–0.8000 g was used for each determination.

2.4. Statistical analysis

Data analysis was performed using Delight version 3.2.1 (Textron Systems, Wilmington, MA). Because spectral features in SW-NIR often appear to be quite overlapped, data pre-processing algorithms such as binning, smoothing and second derivative transformation, and multivariate statistical analysis techniques were

employed to analyze the data (Martens & Naes, 1989). For this study, partial least square (PLS) calibration methods were developed. PLS methods utilize information from the entire spectra in a calibration model. Leave-one-out-cross-validation was used to evaluate the quality of the model (Martens & Naes, 1989). In this technique, all but one sample was used to build a calibration model. This procedure was repeated for each sample in the data set. Correlations between spectral features and chemical reference values are used to predict analyte concentrations in test samples. The predicted values were then compared with the reference values. The standard error of prediction (SEP) was used to indicate the predictive performance of the calibration models: X = NIR method value, Y = reference method value, and n = the number of samples.

$$SEP = \sqrt{\frac{\sum_{i=1}^n (X - Y)^2}{n - 1}}$$

3. Results and discussion

The range of salt content between fillet portions produced under commercial conditions from twenty-six production lots is shown in Table 1. For king salmon, total salt ranged from 1.66 to 5.95% (w/w). For chum salmon, total salt ranged from 2.15 to 5.69% (w/w). The mean total salt for king salmon was $3.51 \pm 0.86\%$ (w/w), similar to the mean total salt of chum salmon [$3.53\% + 0.72\%$ (w/w)]. The mean total salt content for the thickest part of the fillet portion was 3.36% for king salmon, and 3.18% for chum salmon. The mean water phase salt (WPS) for king salmon was $5.19 \pm 1.29\%$ (w/w), lower than for chum salmon [$5.26 \pm 1.10\%$ (w/w)].

The distribution of salt among fillet portions for each species is shown in Fig. 1 for king salmon and Fig. 2 for chum salmon. For king salmon, > 70% of samples were between 2.44–4.39% total salt. For chum salmon, > 70% of samples were between 2.47–4.08% total salt. For WPS of king salmon, 95% of samples were above

3.5% (w/w). For WPS of chum salmon, 97% of samples were above 3.5% (w/w).

The distribution of salt between various locations on a single king or chum salmon fillet portion was studied. Two samples were analyzed from each of king or chum salmon fillet portion with one from the thickest area and the other from thinnest area. Sodium chloride concentration of each sample was determined by AOAC method 973.09. For king salmon, the difference of salt content between the sample from the thickest area and the sample from the thinnest area is shown in Fig. 3, with a mean difference value of approximately 0.4%. Results for chum salmon showed similar trends. Salt can vary by more than 1% between different locations on a single fish portion depending upon the condition of the fish, fish thickness, brining time, and the brining method used. Salt gradients exist in smoked fish

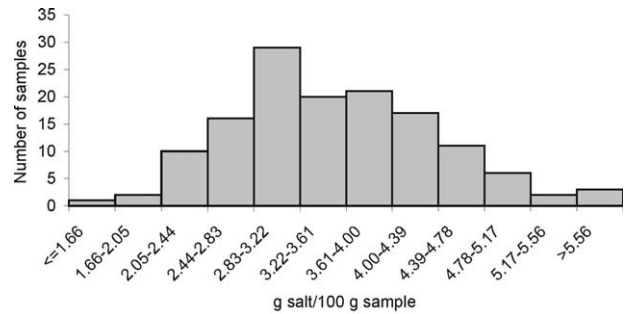


Fig. 1. Salt content in hot smoked king salmon fillet portions.

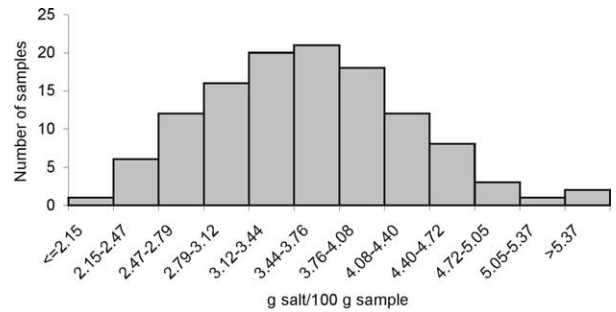


Fig. 2. Salt content in hot smoked chum salmon fillet portions.

Table 1
Salt and moisture content for hot smoked king and chum salmon samples

	Number of samples	Component	Min	Max	Range	Mean	S.D.
King	140	Salt (%)	1.66	5.95	4.29	3.51	0.86
		Water (%)	50.68	71.57	20.89	64.16	3.59
		WPS (%)	2.39	8.66	6.27	5.19	1.29
Chum	120	Salt (%)	2.15	5.69	3.54	3.53	0.72
		Water (%)	55.50	69.69	14.19	63.83	3.28
		WPS (%)	3.14	7.97	4.83	5.26	1.10

S.D.: standard deviation; WPS: water phase salt (salt/ salt + water).

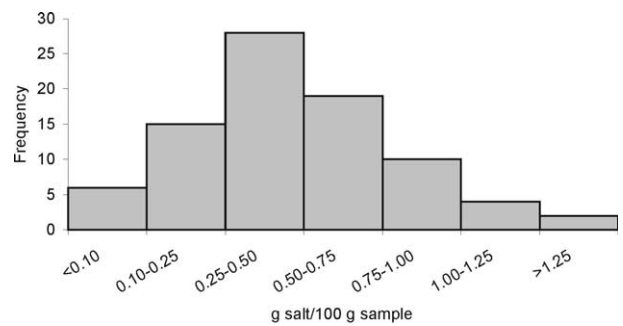


Fig. 3. Frequency distribution for the largest salt content difference within a single king salmon fillet portion.

samples, even when brined fish are permitted to equilibrate for a significant period of time. At least twenty-four hours are required for the center of a thin slice (4–5 mm) of Atlantic salmon (*Salmo salar*) to reach at least 90% of its equilibrium salt concentration (Wang, Correia, & Tang, 1998a). Additional factors affecting salt uptake rate and the final equilibrium salt concentration in a brined fish portion include the post-mortem condition of the fish (Wang, Tang, & Correia, 1998b), species, and whether a sample had been previously frozen.

Representative SW-NIR spectra collected for hot smoked king salmon portions are shown in Fig. 4. Spectra recorded on chum salmon showed similar features. The prominent water band at about 985 nm arises from the $2\nu_1 + \nu_3$ 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 (Phelan, Barlow, Kelly, Jinguji, & Callis, 1989).

Representative second derivative spectra for hot smoked king salmon portions are shown in Fig. 5. Second derivative transformation spectra for chum salmon were similar. Second derivative technique is often used to process NIR data. It helps to separate overlapping absorption bands, remove baseline shifts, and increase apparent spectral resolution. Besides the peak at about 985 nm, weaker absorption bands arising from $2\nu_1 + \nu_2 + \nu_3$ and $3\nu_1 + \nu_3$ were observed near 840 nm and 750 nm (Weyer, 1985) in the second derivative spectra. Salt has no specific absorption band in the NIR region, but detection for sodium chloride is possible due to its effect on the shape and position of the water band (Huang et al., 2002;

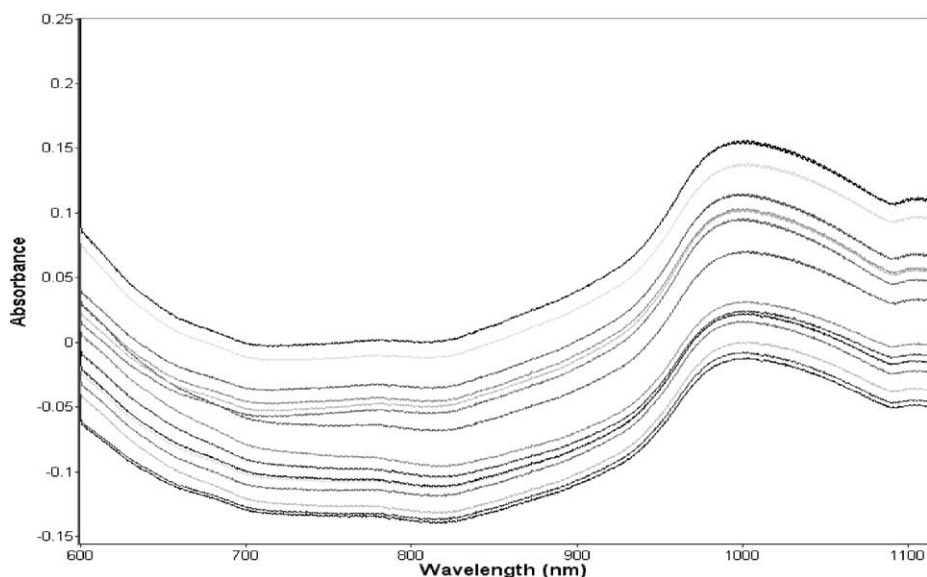


Fig. 4. Representative reflectance spectra for hot smoked king salmon.

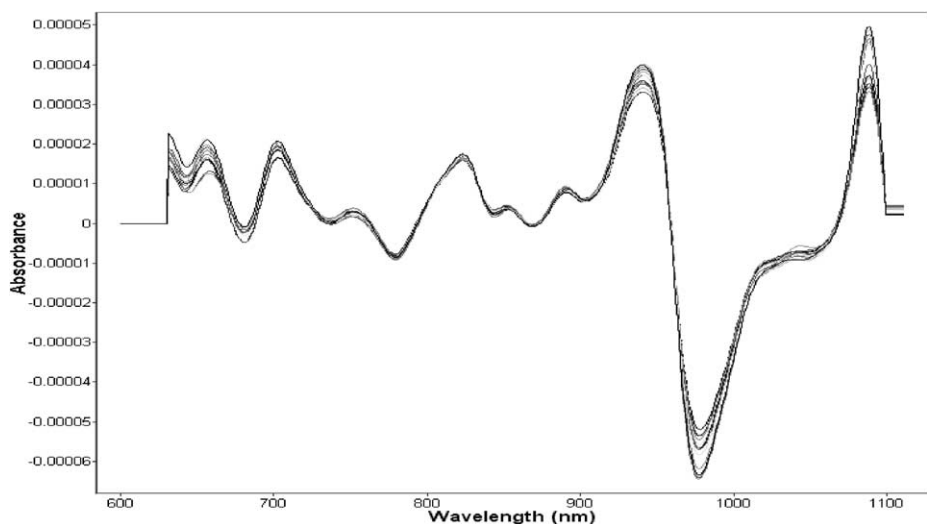


Fig. 5. Second derivative transformation of hot smoked king salmon spectra.

Huang et al., 2001; Lin & Brown, 1992; Begley et al., 1984).

Short-wavelength near-infrared spectroscopy can provide measurements of salt content in the muscle of Pacific king and chum salmon non-invasively. This analysis was based upon the use of multivariate statistics to find wavelength or spectral regions that would correlate with salt content as determined by AOAC methods.

A summary of the statistical results of chemometric models for the king and chum salmon is provided in Table 2. PLS models were developed for salt determination of king and chum salmon, respectively. Validation results for predicting salt content in king salmon yielded a higher coefficient of determination ($R^2=0.83$) and a higher standard error of prediction ($SEP=0.32\%$) than for the chum salmon ($R^2=0.82$, $SEP=0.25\%$). This may due to the wider salt range of king salmon (1.66–5.95% w/w) than the salt range of chum salmon (2.15–5.69% w/w). Figs. 7 and 8 show the comparison between the actual and the predicted salt values for king and chum salmon with both models yielding similar results.

An optimal PLS model required eight latent variables for king salmon and eight latent variables for chum salmon. PLS is a multivariate regression method using full spectrum, which was mainly designed for predictive analysis of complicated problems that are “data rich” but with scarce theoretical information (Peiris, Dull, Leffler, & Kays, 1998). Choosing the optimal number of

latent variables is very important in PLS methods. If the model is constructed with too few latent variables, it will be inaccurate because not all the information is used. In contrast, too many latent variables will decrease the prediction accuracy and can lead to over fitting (Peiris et al., 1998). Fig. 6 shows the standard error of prediction (SEP) of hot smoked king and chum models for different latent variables. The SEP decrease sharply with the first eight latent variables for king and chum models. After eight latent variables, the SEP decrease slightly. This figure illustrates that the optimal number of latent variables for both king and chum PLS model was eight. Requiring as many as eight latent variables for king and chum salmon could reflect the chemical and physical complexity inherent with these samples. For the PLS model of king salmon, these eight latent variables accounted for 84% of the sample variance. For the PLS model of chum salmon, these eight latent variables accounted for 87% of the sample variance.

4. Conclusions

The salt content of the hot smoked Pacific king and chum salmon can vary by more than 1% between the dorsal and ventral sections of a single sample. Non-invasive SW-NIR reflectance methods were developed for predicting salt content in hot smoked Pacific salmon

Table 2
Statistical summary of PLS calibration for salt content in hot smoked king and chum salmon samples

Sample	N ^a	L.V. ^b	Salt	
			R ²	SEP ^c (%)
King	140	8	0.83	0.32
Chum	120	8	0.82	0.25

^a Number of treatments.
^b L.V., latent variable.
^c SEP, standard error of prediction.

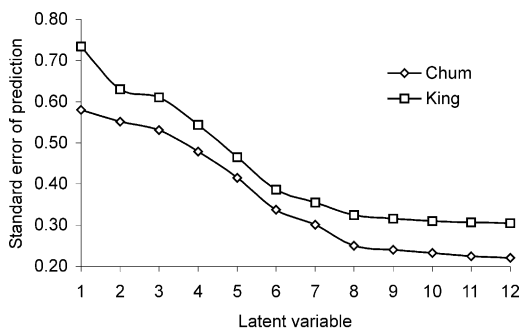


Fig. 6. Standard error of prediction for hot smoked king and chum salmon.

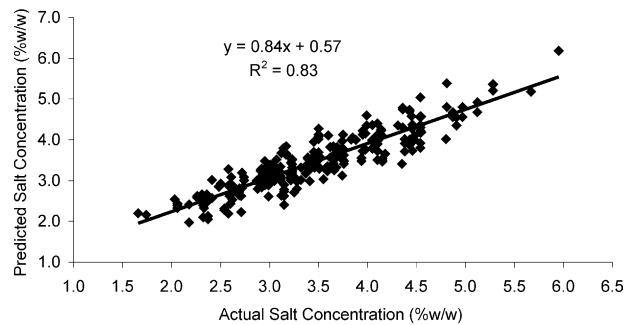


Fig. 7. Comparison between the actual and predicted salt concentration for hot smoked king salmon.

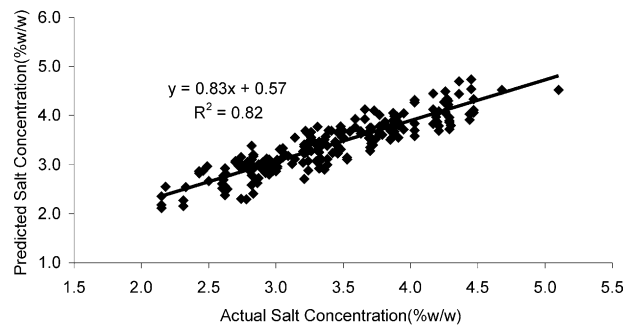


Fig. 8. Comparison between the actual and predicted salt concentration for hot smoked chum salmon.

yielded results similar to those reported for cold smoked Atlantic salmon (*Salmo salar*) (Huang et al., 2002). Partial least square (PLS) calibrations produced acceptable prediction results for king salmon ($R^2=0.83$, SEP=0.32%) and for chum salmon ($R^2=0.82$, SEP=0.25%).

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