JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Noncontact Salt and Fat Distributional Analysis in Salted and Smoked Salmon Fillets Using X-ray Computed Tomography and NIR Interactance Imaging

VEGARD H. SEGTNAN, MARTIN HØY,* ODDVIN SØRHEIM, ACHIM KOHLER, FRANK LUNDBY, JENS PETTER WOLD, AND RAGNI OFSTAD

Centre of Biospectroscopy and Data Modelling, Nofima Food, Matforsk AS, Osloveien 1, N-1430 Ås, Norway

To be able to monitor the salting process of cold smoked salmon, a nondestructive imaging technique for salt analysis is required. This experiment showed that X-ray computed tomography (CT) can be used for nondestructive distributional analysis of NaCl in salmon fillets during salting, salt equilibration, and smoking. The combination of three X-ray voltages (80, 110, and 130 kV) gave the best CT calibrations for NaCl, with a prediction error (root mean square error of cross-validation, RMSECV) of 0.40% NaCl and a correlation (R) of 0.92 between predicted values and reference values. Adding fat predictions based on NIR interactance imaging further improved the NaCl prediction performance, giving RMSECV = 0.34% NaCl and $R = 0.95$. It was also found that NIR interactance imaging alone was able to predict NaCl contents locally in salted salmon fillets with RMSECV = 0.56% and $R = 0.86$.

KEYWORDS: Computed X-ray tomography (CT); NIR interactance; imaging; NaCl; smoked salmon

INTRODUCTION

Salted and smoked salmon is a traditional Norwegian food product that is consumed worldwide. The product is normally cold smoked, that is, at temperatures around 25 °C, without subjection to further heat treatment. The product normally has a shelf life of approximately 6 weeks at 4−5 °C. NaCl is the main microbial inhibitor in smoked salmon, partly because of lowered water activity and partly because of the antimicrobial effects of sodium (*1, 2*).

Sodium has received a lot of negative attention during the past decade due to its apparent effect on human health. The average sodium content of Norwegian smoked salmon lies around 2−4 g/100 g of product. The recommended maximum daily intake of NaCl is 5 g, whereas the average daily intake for Norwegian adults today is 10 g (from The Norwegian Directorate of Health).

Reduction of NaCl contents in a product such as cold smoked salmon may have a negative impact on several quality attributes. First, it may affect the taste of the product. Different salt replacers mimicking the taste of NaCl are on the market, but alteration of the taste is likely when the amount of NaCl is reduced. Second, NaCl reduction may also reduce the microbial shelf life unless other process changes are introduced. Third, NaCl reduction is likely to alter the textural properties of the product, due to the effect of NaCl on the structuring of proteins and water molecules. Thus, reducing the NaCl content of smoked salmon is not trivial.

Traditionally, Norwegian smoked salmon is dry-salted; that is, the raw fillets are covered with dry salt and left for salt diffusion for a certain number of hours. The only parameter that can be adjusted to change the salt content is the duration of dry salt contact, but this process does not lead to specified and controllable salt contents for all parts of all salmon fillets. Birkeland et al. studied the effect of raw material characteristics and processing parameters on the end quality of smoked salmon (*3, 4*). Generally, the salt content of the finished product depends on the thickness and the fat content of the fillet (*5*), and these characteristics vary significantly both within and between fillets. The salt diffusivity is (among other things) dependent on water content. Because water content and fat content are negatively correlated in fish fillets, a lower salt content is generally expected with increasing fat content (*6*). Thus, parts of the fillet will be significantly oversalted to meet the minimum requirements in all parts of the product. To reach the lower limit for NaCl in all parts of all fillets, it is necessary to know more about the NaCl diffusion dynamics in all parts of the fillet. To be able to monitor the salting process, a nondestructive imaging technique for salt analysis is required.

Adding NaCl to meat increases the density of the sample because the density of NaCl is approximately twice the density of salmon fillet. Thus, NaCl can potentially be detected nondestructively using computed tomography (CT) (*7*). Håseth

* Author to whom correspondence should be addressed (e-mail martin.hoy@nofima.no).

10.1021/jf802080s CCC: $40.75 © 2009 American Chemical Society
Published on Web 02/13/2009

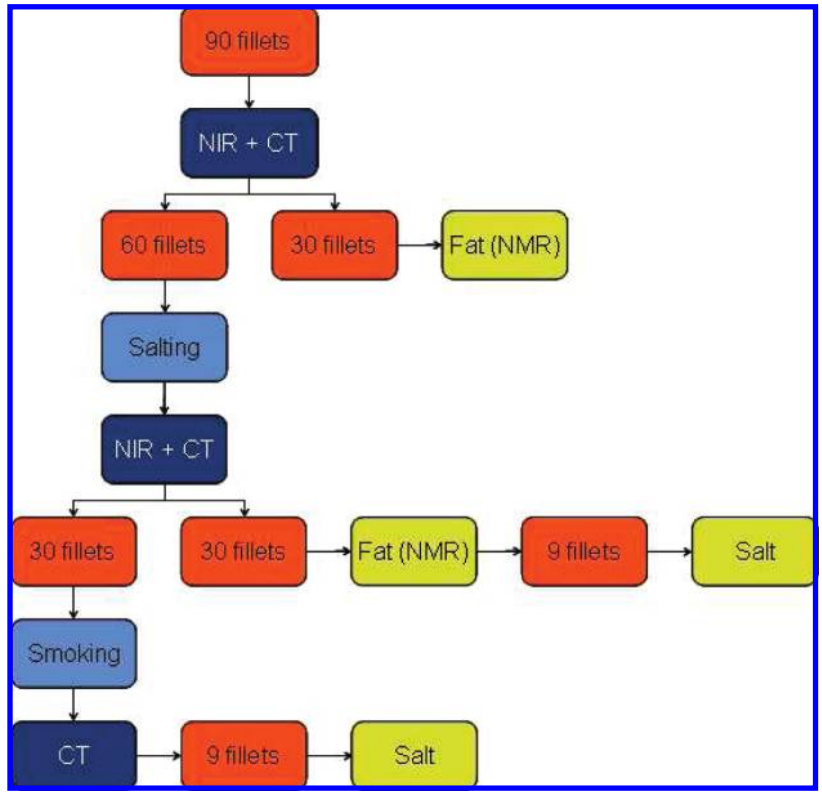

Figure 1. Experimental flowchart.

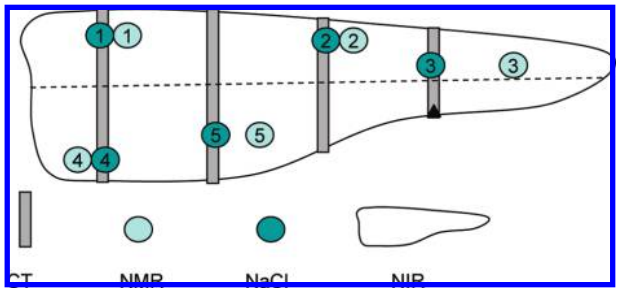

Figure 2. Sampling regimen of the experiment, showing four CT lines, five plug positions for NMR fat analysis, and five plug positions for NaCl analysis.

Table 1. Summary of Reference Data for Fat and NaCl in Plugs from Raw, Salted, and Salted/Smoked Salmon Fillets

	min	max	mean	SD	plugs
% fat in raw fillets	3.84	26.22	13.92	6.16	150
% fat in salted fillets	3.81	29.64	16.07	7.63	150
% fat in all fillets	3.81	29.64	14.99	7.00	300
% NaCl in salted fillets	1.61	6.74	3.39	1.20	45
% NaCl in salted/smoked fillets	1.55	5.79	3.42	1.11	45
% NaCl in all fillets	1.55	6.74	3.41	1.15	90

et al. (*8*) studied the feasibility of using CT for NaCl determination in ground pork and dry-cured ham and achieved a prediction error of 2.8% NaCl for ground pork with widely varying contents of fat, protein, and water. It was seen that protein and fat variation strongly affected the accuracy of the NaCl modeling. Adding chemical information on fat or protein contents in the modeling reduced the prediction error to 1.6%, whereas modeling samples with similar fat and protein contents gave a prediction error of 0.2% NaCl. One voltage level was used for this experiment (110 kV). The same group also tested the effect of using three voltages (80, 110, 130 kV) for the same

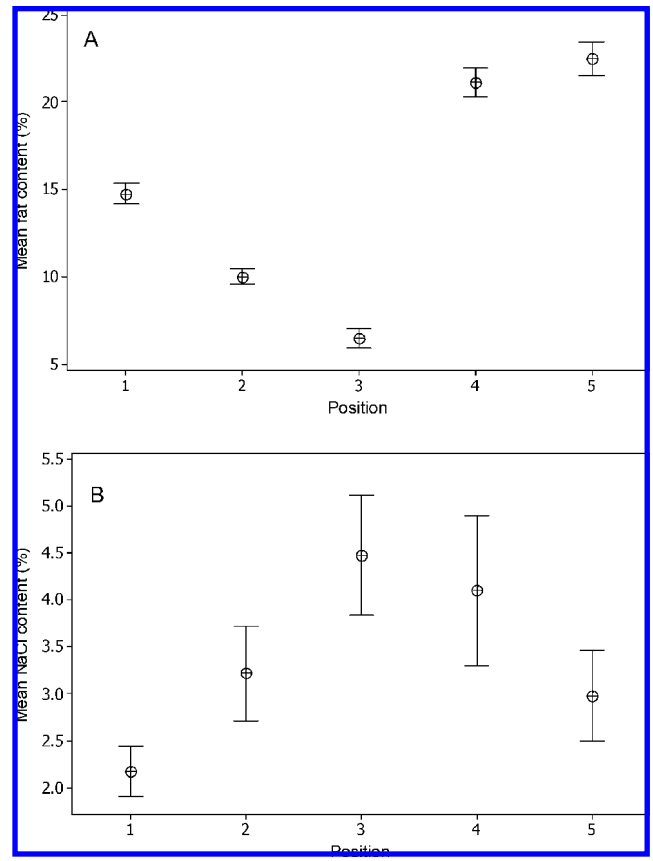

Figure 3. Mean values for fat (**A**) and NaCl (**B**) as a function of plug position (referring to the numbering in **Figure 2**). Mean values are given with 90% confidence limits.

samples (*9*) and found that this reduced the prediction error to approximately 1% NaCl. Adding fat contents information reduced the error even further to 0.2−0.3% NaCl.

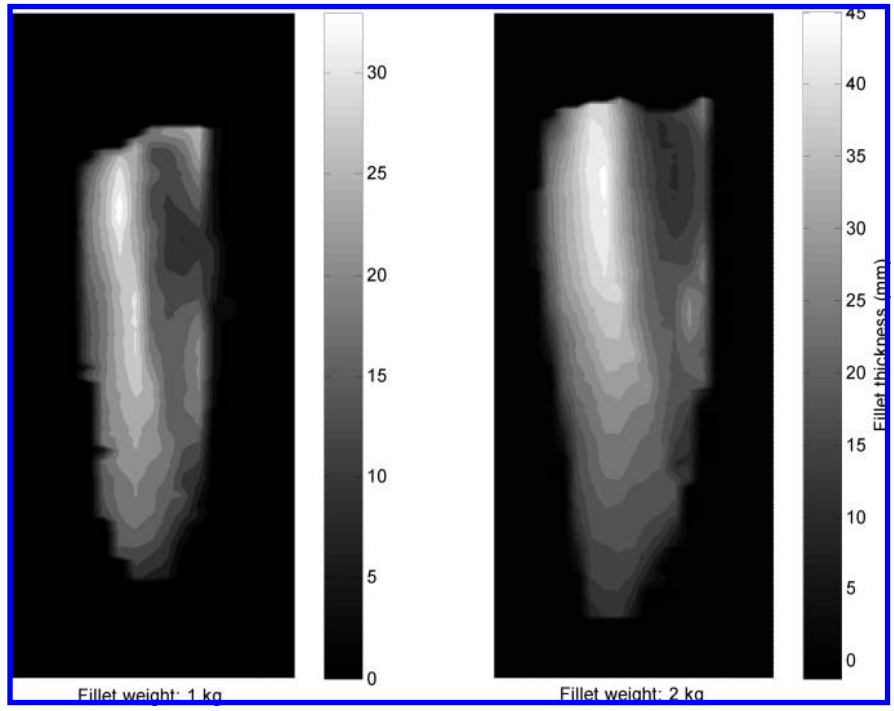

Figure 4. Thickness data for a small salmon fillet (left) and a large fillet (right).

Despite the fact that NaCl molecules are not infrared active, several studies have been published on the use of near-infrared spectroscopy (NIR) for prediction of NaCl contents in foods (*10*). NaCl affects other structures in the food matrix, for example, water and protein molecules, and these effects can be measured with NIR spectroscopy and related to the NaCl contents. Huang et al. (*11*) studied the feasibility of NIR reflection spectroscopy (600−1100 nm) for NaCl prediction in cold smoked salmon and achieved a prediction error of 0.55% NaCl and a correlation between predicted values and reference values of 0.91 using neural networks. The same group later published a study on hot smoked salmon fillets, reporting prediction errors between 0.25 and 0.32% NaCl and correlations around 0.91 using PLS regression.

The main goal of this experiment was to utilize nondestructive analytical techniques for the study of salt distribution in salted and smoked salmon. The following three approaches were tested and compared: (1) salt distributional analysis using CT, (2) salt distributional analysis using NIR interactance, and (3) salt distributional analysis using CT and fat values from NIR interactance measurements.

MATERIALS AND METHODS

Samples. Forty-five salmon (*Salmo salar*) representing three different weight classes were slaughtered and filleted by a Norwegian company (Bremnes Seashore AS, Bremnes, Norway). The weight classes were 3−4, 4−5, and 5−6 kg, and 15 salmon were selected from each class. The 90 fillets were subjected to measurements, salting, and smoking, according to the experimental flowchart shown in **Figure 1**. The figure also shows where and how many nondestructive instrumental (CT and NIR) measurements and (destructive) reference measurements were made. First, 30 raw fillets were used for fat reference analysis. The remaining 60 fillets were dry-salted for 8, 16, and 24 h and were left for equilibration for 5 days. The salting was performed according to a full factorial experimental design, whereby all three weight classes were subjected to all three salting times. Also, the two fillets from the same fish were always subjected to different salting durations. After salt equilibration, 30 salted fillets were used for reference analysis. The last remaining 30 fillets were smoked for 4 h at 25 °C.

Sampling and Reference Analysis. Figure 2 shows the sampling strategy used in the experiment. From each fillet, 5 or 10 cylindrical plugs were cut out using a core sampler and used for fat and/or NaCl reference analysis. The locations of the plugs were chosen to span the internal fat and salt variation for each fillet. Each plug had a diameter of 15 mm and a length according to the thickness of the salmon fillet at the point where it was taken. The plug was removed from the skin, and the fat content of the plug was measured using a low-field ^{1}H NMR instrument (MARAN Ultra, 23 MHz, Oxford Instruments). The instrument was calibrated with refined salmon oil prior to analysis. Except for temperature standardization, no further sample preparation was performed. When the plug was too long for the NMR equipment, it was split in two prior to analysis, and the weighted-average fat content of the two parts was used further on. The plugs were contained in Teflon containers during analysis.

The NaCl content was determined as water-soluble Cl using titration with a Corning 926 chloride analyzer (Corning Medical and Scientific, Halstead, Engdahl, A.; Kolar, K. Metodeforskrift 22.6.93 Koksaltbestämning med Corning 926 Chloride Analyzer, Köttforskningsinstitut i Kävlinge, 1993).

NIR Interactance Imaging. All raw and salted fillets were scanned using the noncontact interactance imaging instrument Qmonitor (Qvision AS, Oslo, Norway). Light from 12 halogen lamps is focused onto a line on the conveyor belt. Between the light sources and the imaging detector is a blackened plate that prevents detection of pure surface reflection from the sample. The detected light is collected from a line approximately 2 cm away from the illuminated line. Thus, only photons that have traveled this distance horizontally are detected. The NIR scanner is able to collect approximately 10000 spectra per second. Fifteen equally spaced wavelengths ranging from 760 to 1040 nm were collected from 60 points across the conveyor belt. In this experiment, 3000−4000 spectra were collected from each fillet (conveyor belt spectra omitted). The individual spectra were subjected to log-transformation and standard normal variate (SNV) transformation (*12*) prior to averaging and subsequent calibration.

A modified version of the interactance scanner was used for topographical measurements of a selection of salmon fillets. A laser line was focused over the breadth of the conveyor belt, and the responses were measured using the same detector system as the one used in the interactance scanner. The topographical instrument was calibrated using a series of plastic cylinders of known height.

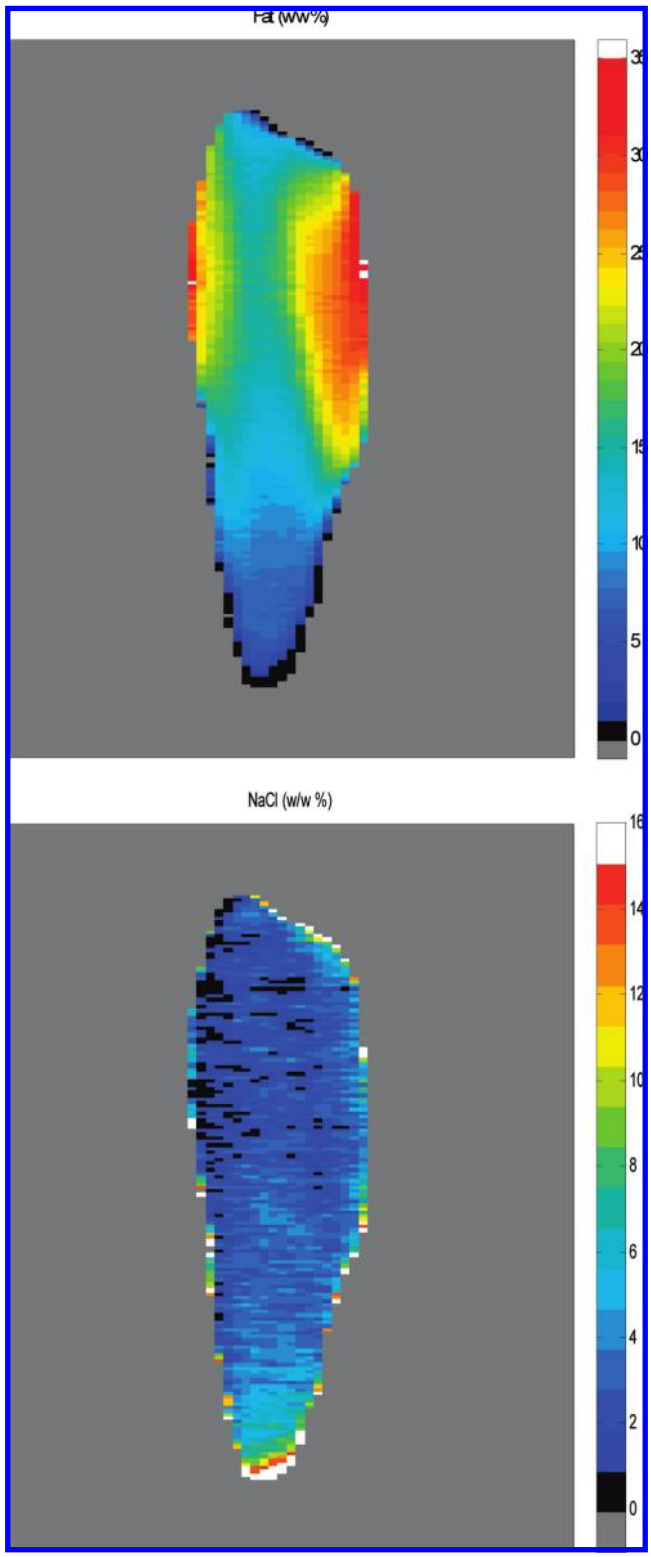

Figure 5. Prediction images for fat (top) and NaCl (bottom) using NIR interactance imaging.

Computed Tomography. Four 10-mm slices were scanned with CT and are shown in gray in **Figure 2**. The resolution of the scans was 2.56 pixels/mm, and the reconstruction filter was B50s. The laser line of the CT instrument was aligned with a cut that was made on all fillets prior to analysis. This was done in order to measure at the same positions prior to salting, after salt equilibration, and after smoking. The first (or rightmost) CT slice was scanned at this cut, and the consecutive slices were scanned 75 mm from the previous slice. The instrument used was a Siemens Somatom Emotion CT scanner (Siemens AG, Erlangen, Germany) at 106 mA and 1 s rotation time. Three

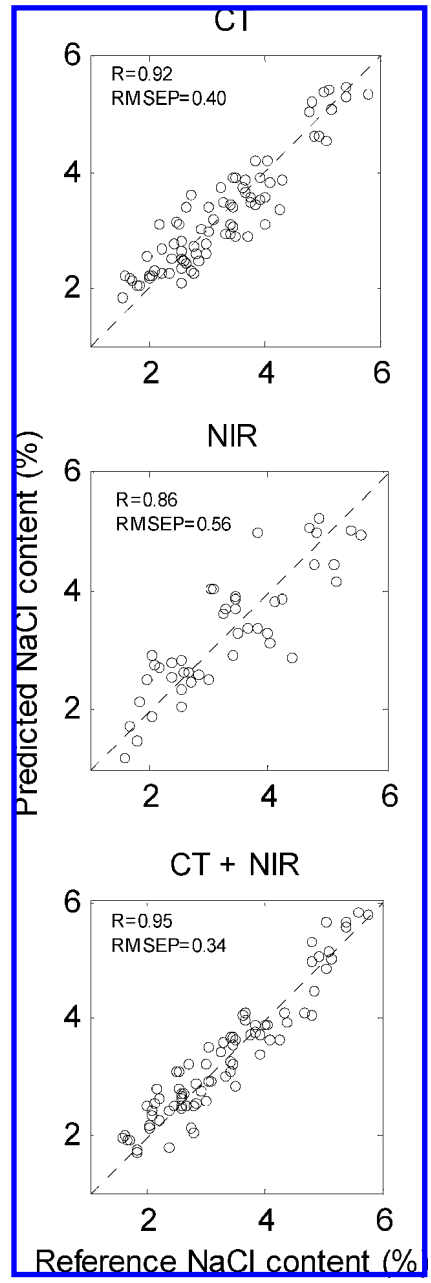

Figure 6. Predicted NaCl contents plotted against reference contents. Models are based on computed tomography (top), NIR interactance (middle), and the combination of the two techniques (bottom).

voltages were used: 80, 110, and 130 kV. These settings were chosen on the basis of experience from earlier experiments (*9*). The salt calibration plugs were taken at the same locations as the CT slices to match the instrumental signals and the reference values.

Data Analysis. NIR spectra were averaged in the reference plug regions after SNV correction. Models were built between the average NIR spectra and the plug fat and NaCl values. For the NaCl CT calibration, we had CT images obtained at three different voltages (80, 110, and 130 kV). The regions of the reference plugs were selected in each image, and the average CT value was computed for this region. For each plug, this gives three CT values (one from each voltage) that can be modeled against the salt content. Calibration models for fat and salt were built using partial least-squares regression (PLSR) (*13*), and the models were validated using full cross-validation (*14*). The data analysis was performed using Matlab 7.5 (The Mathworks Inc., Natick, MA).

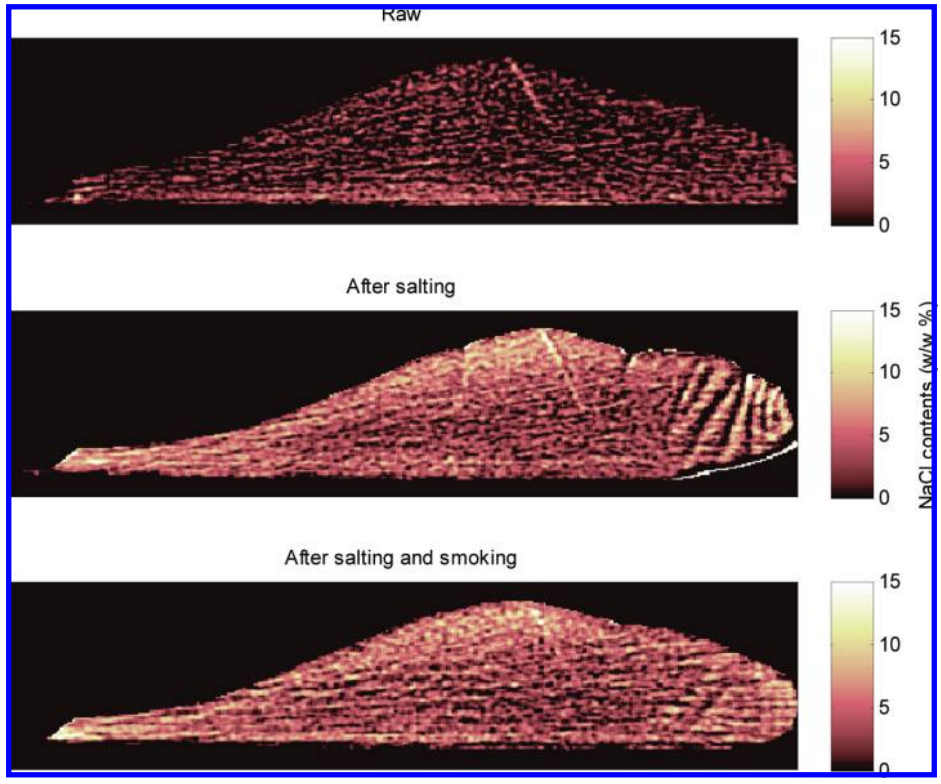

Figure 7. Prediction images for NaCl in a 1-cm slice of raw (top), salted (middle), and salted and smoked salmon (bottom) using computed tomography.

RESULTS AND DISCUSSION

The results from the fat and salt reference analyses of all fillet plugs are summarized in **Table 1**. The table shows that there is a large variation in the fat and salt contents of the plugs, ranging from 3.8 to 29.6% for fat and from 1.5 to 6.7% for salt.

The fat and salt contents are summarized per position in panels **A** and **B**, respectively, of **Figure 3** (position numbers refer to **Figure 2**). **Figure 3A** shows that the fat contents are generally higher on the belly side (positions 4 and 5) compared to the back side (positions 1−3). It also shows that the fat content generally increases from tail to head on the back side (positions 3, 2, and 1, respectively). Note that the variation in fat within a fillet is much larger than the variation between the fillets (the deviations per position is small). **Figure 3B** shows that the salt variation within each position is large compared to the variation between positions, at least when compared to the small variations shown in **Figure 3A**. One should keep in mind, however, that each plug position represents three different salmon weight classes (and consequently fillet thicknesses) and three different salting durations. Thus, a larger variation in salt content was expected. Comparing **Figure 3B** clearly indicates an opposite relationship between fat and salt contents, but this relationship needs to be investigated in more detail. Looking at plugs 1−3 only, the correlation between fat and salt is relatively high ($R = -0.78$). If we include the belly plugs as well, the correlation is reduced to −0.17. For the belly plugs only, the correlation between fat and salt contents is close to zero. The reasons for these differences are most likely due to the physiological differences within the fillet. In addition to having higher fat contents than the back, the belly part is always much thinner than the back part. Due to the difference in thickness, the uptake (diffusivity) of salt in the belly part of the fillet does not seem to be limited by low water content (high fat) in the same way as the rest of the fillet. Topographical maps of a small and a large fillet are shown in **Figure 4**.

Salt Distributional Analysis Using CT. CT has been shown to be feasible for salt distributional analysis in dry-cured ham during processing (*8*, *9*). In the present study, three voltages were used. Both salted and salted/smoked fillets were included in the modeling. The average CT responses in the salt plug regions for the three voltages were modeled against the salt reference values for the same plugs using PLS regression. It is known that the information obtained from CT images at different voltages is slightly different (*9*). We found that the use of only one or the combination of two of these voltages provided approximately the same prediction performances. The average prediction errors (root mean square error of cross-validation, RMSECV) were approximately 0.6%, and the correlation between the CT predictions and the reference values ranged from 0.78 to 0.80, using one or two voltages. Using all three voltages provided significantly better results. With all three voltages, the RMSECV was reduced to 0.40, and the correlation coefficient increased to 0.92. Jackknifing (*15*) was used to test the significance of the three energy levels, and the test showed that all three were significant on a 5% level.

Salt Distributional Analysis Using Online NIR Interactance. The fat prediction performances of the noncontact NIR interactance system used in the present experiment is published elsewhere (*16*) and will not be presented in detail here. In summary, prediction errors of 1.95 and 1.96% fat in raw and salted salmon fillets, respectively, with corresponding correlations between predicted fat values and reference values of 0.95 and 0.97, were obtained in this parallel study. We do have NIR interactance spectra from all regions of all the salted fillets. It is thus possible to investigate the feasibility of this NIR system for simultaneous fat and salt analysis.

The same strategy as used for fat calibration (*16*) was used for salt calibration; that is, spectra from the salt plug regions were averaged and modeled against the individual salt reference values for the same plugs. Because we did not obtain NIR

spectral images for salted and smoked fillets, only salted fillets were used in these calibrations. The NIR prediction model gave an RMSECV of 0.56 and an R of 0.86 for the NaCl content. This is a little bit better than one- or two-voltage CT models, but significantly poorer than the performance of the three-voltage CT model. It should be mentioned, however, that CT of the type used in our experiment is hardly applicable for practical online industrial analysis. In an industrial context, an NIR interactance system could be a good choice of technique, as it is able to measure fat and NaCl simultaneously. Examples of fat and NaCl prediction images obtained using NIR interactance models are shown in **Figure 5**.

Salt Distributional Analysis Using CT and Fat Predictions from Online NIR Interactance. What has been seen earlier is that the CT responses for salt are affected by the fat contents of the samples (*8*, *9*). To investigate this phenomenon, we calculated the predicted fat content of the salt plugs based on the NIR interactance measurements and added this fat information into the CT modeling process. Jackknifing showed that the fat variable contributed significantly to the prediction model. The model with three CT energies and predicted fat values gave an RMSECV of 0.34 and an R of 0.95.

Reference NaCl values are plotted against predicted values for these three systems in **Figure 6**. The figure shows that the combination of CT and NIR fat predictions (bottom panel) provides the smallest deviations from the target line. The top and bottom panels of **Figure 6** are based on salted and salted/smoked fillets, whereas the middle panel represents the salted samples only.

Figure 7 shows an example of salt prediction images based on the CT plus fat model. The images are taken at the same position of the same fillet in the raw state (top panel), after salt equilibration (middle panel), and after smoking (bottom panel). The images represent a cross section corresponding to the line that is located closest to the head in **Figure 2**. It can be seen that the unsalted fillet (upper panel) also contains significant amounts or NaCl, as predicted by our model. The true salt content of a raw salmon fillet should be close to zero (0.1−0.2% NaCl), indicating that our model gives erroneous predictions for unsalted fillets. Of course, salt prediction for raw fillets involves a severe extrapolation of the model, which was based on salted and salted/smoked samples. For the salted and salted/smoked fillets, **Figure 7** shows how the salt is distributed throughout the salmon fillets.

Conclusions. This experiment has shown that CT can be used for nondestructive distributional analysis of NaCl in salmon fillets during salting, salt equilibration, and smoking. The combination of three X-ray energy levels (80, 110, and 130 kV) gave the best CT calibrations for NaCl, with RMSECV = 0.40% NaCl and R = 0.92. Adding fat predictions based on NIR interactance imaging further improved the NaCl prediction performance, giving RMSECV = 0.34% NaCl and R = 0.95. It was also found that NIR interactance imaging alone was able to predict NaCl contents locally in salted salmon fillets with RMSECV = 0.56% and R = 0.86.

For further analysis and understanding of these measurement systems, NaCl analysis should also be performed on raw fillets to improve the CT model performance in the low-salt samples and regions in future experiments. Also, NIR interactance images should be collected for salted and salted/smoked fillets to further investigate the possibilities of performing online NIR analysis of salt in salted and smoked salmon.

ACKNOWLEDGMENT

Tom C. Johannessen, Karin Solgaard, Karen W. Sanden (Matforsk), and Knut Dalen (Department of Animal Science, University of Life Sciences, Ås, Norway) are acknowledged for skilled preparation and analysis of the samples.

LITERATURE CITED

(1) Sofos, J. N. Antimicrobial effects of sodium and other ions in foods: a review. *J. Food Saf.* **1984**, *6* (1), 45–78.
(2) Padan, E.; Krulwich, T. A. Sodium stress In *Bacterial Stress Responses*; Storz, G., Hengge-Aronis, R., Eds.; ASM Press: Washington, DC, 2000; pp 117−130.
(3) Birkeland, S.; Bencze Rørå, M. B.; Skåra, T.; Bjerkeng, B. Effects of cold smoking procedures and raw material characteristics on product yield and quality parameters of cold smoked Atlantic salmon (*Salmo salar* L.) fillets. *Food Res. Int.* **2004**, *37*, 273–286.
(4) Birkeland, S.; Bjerkeng, B. The quality of cold-smoked Atlantic salmon (*Salmo salar*) as affected by salting method, time and temperature. *Int. J. Food Sci. Technol.* **2005**, *40*, 963–976.
(5) Cardinal, M.; Knockaert, C.; Torrisen, O.; Sigurgisladottir, S.; Morkore, T.; Thomassen, M.; Vallet, J. L. Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Res. Int.* **2001**, *34* (6), 537–550.
(6) Schwartzberg, H. G.; Chao, R. Y. Solute diffusivities in leaching processes. *Food Technol.* **1982**, *36* (2), 73–86.
(7) Frøystein, T.; Sørheim, O.; Berg, S. A.; Dalen, K. Salt distribution in cured hams, studied by computer X-ray tomography. *Fleischwirtschaft* **1989**, *69* (2), 220–222.
(8) Håseth, T. T.; Egelandsdal, B.; Bjerke, F.; Sørheim, O. Computed tomography for quantitative determination of sodium chloride in ground pork and dry-cured hams. *J. Food Sci.* **2007**, *72* (8), E420–E427.
(9) Håseth, T. T.; Høy, M.; Kongsro, J.; Kohler, A.; Sørheim, O.; Egelandsdal, B. Determination of sodium chloride in pork meat by computed tomography at different voltages *J. Food Sci.* **2008**, *73* (7), E333−E9.
(10) Ellekjær, M. R.; Næs, T.; Hildrum, K. I.; Isaksson, T. Determination of NaCl of sausages by near infrared spectroscopy. *J. Near Infrared Spectrosc.* **1993**, *1*, 62–75.
(11) Huang, Y.; Cavinato, A. G.; Mayes, D. M.; Bledsoe, G. E.; Rasco, B. A. Nondestructive prediction of moisture and sodium chloride in cold smoked atlantic salmon (*Salmo salar*). *J. Food Sci.* **2002**, *67* (7), 2543–2547.
(12) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and detrending of near infrared diffuce reflectance. *Appl. Spectrosc.* **1989**, *43*, 772–777.
(13) Martens, H.; Næs, T. Partial least squares regression In *Multivariate Calibration*; Wiley: Chichester, U.K., 1989; pp 116−165.
(14) Stone, M. Cross-validatory choice and assessment of statistical predictions. *J. R. Stat. Soc.* **1974**, (2), 111–133.
(15) Westad, F.; Martens, H. *J. Near Infrared Spectrosc.* **2000**, *8*, 117–124.
(16) Segtnan, V. H.; Høy, M.; Lundby, F.; Narum, B.; Wold, J. P. Fat distributional analysis in salmon fillets using non-contact near infrared interactance imaging: a sampling and calibration strategy *J. Near Infrared Spectrosc.* Submitted.

Received for review July 9, 2008. Revised manuscript received November 3, 2008. Accepted January 5, 2009. The study was financially supported by the European Commission under the sixth Framework Programme for RTD (TRUEFOOD - "Traditional United Europe food" - Integrated project FOOD-CT-2006-016264), the Norwegian Research Council, and the Agricultural Food Research Foundation of Norway. The information in this document reflects only the author's views and the Community is not liable for any use that may be made of the information contained herein.

JF802080S