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Determination of Sodium Chloride in Meat by Near-Infrared Diffuse Reflectance Spectroscopy

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Near-infrared (NIR) spectroscopy was used to measure the amount of salt (NaCl) in canned cured hams. Calibration with 20 samples produced a high correlation ($r = 0.96$) between salt contents determined by chemical analysis and by NIR using the second derivative of log (1/reflectance) values at 1806 nm. Salt contents of nineteen unknown samples were predicted with a standard error of prediction of 0.17% NaCl. Salted beef, salted fresh ham (uncured), and salted water model systems demonstrated that the ability to measure salt by NIR is due to the shift in the water spectrum caused by salt-induced changes in the amount of hydrogen bonding.

Near-infrared (NIR) reflectance spectroscopy is a growing technique for the rapid compositional analysis of foods. The method was developed to replace slow, laborious, conventional methods such as the Kjeldahl method for protein analysis. Ben-Gera and Norris (1968) used transmission spectroscopy in the NIR range to determine the fat and moisture contents of meat products. More recently, Kruggel et al. (1981), Martens et al. (1981), and Lanza (1983) used NIR reflectance spectroscopy to determine the moisture, fat, and protein contents of fresh meats.

We now describe the use of NIR reflectance to measure the amount of salt (NaCl) added to meat products. We used canned cured ham to develop a calibration of NIR data to chemically determine salt values, and we prepared salted beef, salted fresh ham, and salted water samples to study the spectrochemical basis of the method.

The NIR reflectance analysis of salt in meat is based on the change in the water component of the meat spectrum. Bernal and Fowler (1933) showed that the addition of electrolytes change the spectrum of water in the infrared overtone region. Luck (1974) showed that temperature variations caused similar spectral changes and linked these changes to variations in the amount of hydrogen bonding. We show here that the best calibration of NIR data to salt content occurs at a point in the meat spectrum where the salt-induced changes in the water spectrum can be mathematically isolated from other spectral variations.

Because the NIR method can measure several constituents quickly, cheaply, accurately, and simulanteously, it can become a valuable tool for the quality control of processed meats.

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Table I. Salt and Fat Contents of Sample Sets

	no. of samples	% NaCl (w/w)	% fat (w/w)
fresh ham	20	0–4.8	7.4, 20.9, 24.9
beef	26	0–10.7	16.9, 17.4, 28.8
cured ham	20	2.3–3.5	1.4–6.5
cured ham	19	2.3–3.5	1.4–6.5
water	13	0–5	

EXPERIMENTAL SECTION

Samples. Duplicate finely ground canned cured ham samples were supplied and analyzed by the USDA Food Safety and Inspection Service (FSIS) using the AOAC procedure for salt determination in meats (AOAC, 1980, 24.011). We prepared model salted meat samples of fresh ham (uncured) and ground beef by first grinding samples to a paste in a Robot-Coupe food processor and then adding known percentages (w/w) of dry NaCl gravimetrically. The amount of fat in the beef samples was varied by choosing different types of ground beef. The amount of fat in the fresh ham samples was varied by carving portions of a fresh ham to include more or less fat prior to grinding. The fat levels were varied to ensure that the addition of salt could be measured independently of varying levels of protein, water, and fat. Aqueous salt solutions were prepared (w/w) with certified ACS grade NaCl and distilled deionized water. The salt and fat contents of the samples are summarized in Table I.

Instrumentation. Diffuse reflectance (R) was measured on all meat samples with a Neotec Model 6350 research composition analyzer. This instrument is a computer-based system with a single-beam scanning monochromator and a lead sulfide detector. The monochro-

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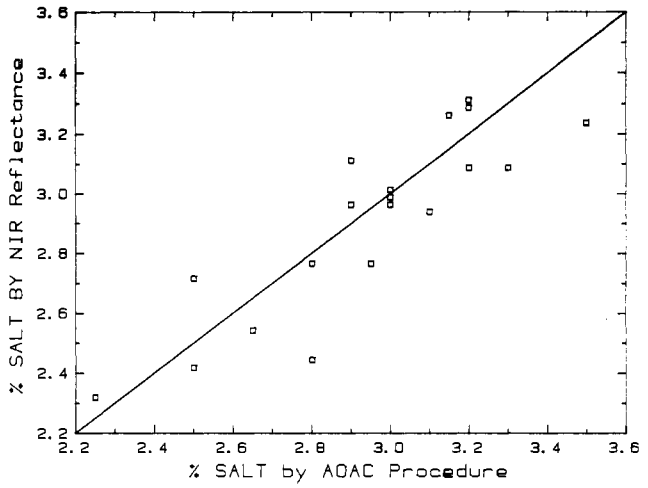

Figure 1. Scatter plot of NIR-predicted vs. chemically predicted salt in canned cured ham.

mator scans the range from 1100 to 2500 nm in 0.2 s, and data are recorded at 2-nm intervals. Each spectrum used here is the average of 50 scans, with a total measurement plus recording time of 45 s. We used a ceramic disk as a reference sample to correct the sample spectra for the system response. All corrected sample spectra were recorded as log $(1/R)$.

The aqueous NaCl solutions were run in the transmission mode on a computerized Cary Model 14 single-beam scanning spectrophotometer [described in Norris and Barnes (1976) and Norris et al. (1976)]. Spectra from 1500 to 1900 nm were recorded at 0.4-nm intervals, with a total measurement plus recording time of 40 s.

Samples were run at room temperature (22–23 °C), and the spectra of two reloads of each sample were averaged for subsequent data analysis.

Data Treatment. All NIR quantitative analyses were performed by a single-term linear regression analysis program where each log $(1/R)$ spectrum was smoothed and transformed to its second derivative with respect to wavelength. Norris (1983) describes this technique in detail. At each wavelength, a computer program regressed the transformed NIR values against the chemically determined salt values and calculated the correlation coefficient. The program then reported the wavelength that produced the highest correlation. The amount of smoothing and the gap parameter of the derivative were also varied to optimize the correlation. If S_w is the log $(1/R)$ value at wavelength w, then the second derivative is defined by $S''_w = S_{w-g} - 2S_w + S_{w+g}$, where g is called the gap and is measured in nanometers.

RESULTS AND DISCUSSION

We calibrated the second derivative NIR data to the chemically determined salt values in each of the sets of meat samples. The results for cured ham show the NIR method to be effective with processed meats. The results with the fresh ham and beef, together with an analysis of the derivative spectra of salted fresh ham and salted water, show the spectrochemical basis of the measurement.

In the experiment with cured hams, a calibration set of 20 samples and a prediction (or validation) set of 19 samples were used. The best wavelength for calibration was 1806 nm (r = 0.960, SEC = 0.12% NaCl). (The SEC is the standard error of estimate for the calibration regression.) The sampling precision for five reloads of the sample cell was 0.14% NaCl, indicating that the primary source of error in the measurement is sampling error. The regression equation obtained from the calibration set predicted the

Table II. Correlation of Salt Content to Second Derivative of Near-Infrared Spectra at 1806 nm

	r	SEC[a]	gap, nm
fresh ham	0.984	0.29	18
beef	0.997	0.22	14
cured ham	0.960	0.12	18
cured ham		0.17[b]	18

[a] Standard error of calibration. [b] Standard error of prediction.

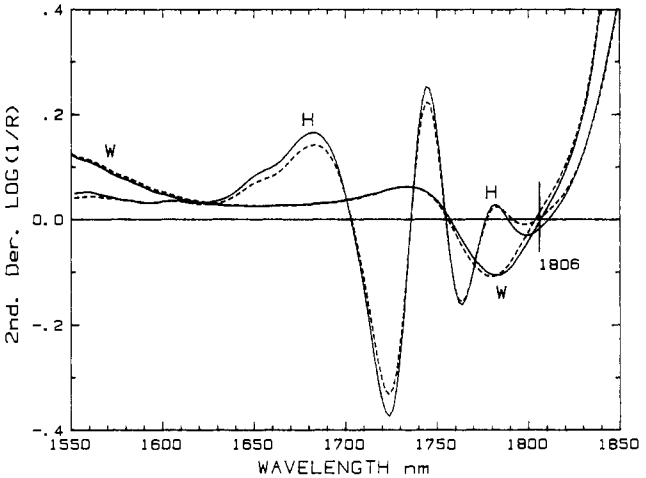

Figure 2. Second derivative spectra of fresh ham (H) with 0.0% and 4.8% added salt and water (W) with 0.0% and 5.0% added salt. The spectra of samples without added salt are dashed.

salt content of the 19 independent ham samples with a standard error of prediction (SEP) of 0.17% NaCl with a negligible bias of −0.04% NaCl (Figure 1). The SEP and the bias are the standard deviation and the mean of the differences between the NIR-determined and chemically determined salt values.

The NIR reflectance analyses of the salted fresh meat samples are summarized in Table II. Correlations were higher for beef than for fresh ham because the beef samples had a higher range of salt concentration and a lower range of fat concentration. As with the cured hams, the wavelength selection process found the best correlation at 1806 nm. The behavior of the derivatives in the region near 1806 nm explains the wavelength selection. Figure 2 shows the derivative spectra for two fresh ham samples with 0.0% and 4.8% added salt and for water with 0.0% and 5.0% NaCl. The fresh ham spectra show differences in several places in the 1550–1850-nm region. The water spectra, however, show significant differences only in one place. The wavelength selection process chose a wavelength where large differences in the fresh ham spectra coincide with salt-induced differences in the water spectra, indicating that the NIR reflectance measurement detected the effect of salt on water in the fresh ham.

Differences in the water spectra at 1806 nm are caused by a wavelength shift in the absorption band that peaks at 1795 nm rather than by a change in intensity. To further explore the spectral effect of salt on water, we obtained the spectra of 13 samples of water with salt concentrations of 0%, 1%, 2%, 3%, and 5% and measured the peak wavelength of the derivative band near 1795 nm. The high correlation of these peak wavelengths with salt concentration (Figure 3) shows that salt in this concentration range shifts the water band significantly and linearly.

Second-derivative spectra, over the 1100–1900-nm region, for the samples from Figure 2 with no added salt and with 4.8% added salt (Figure 4), show large differences in the three wavelength regions around 1380, 1460, and 1800

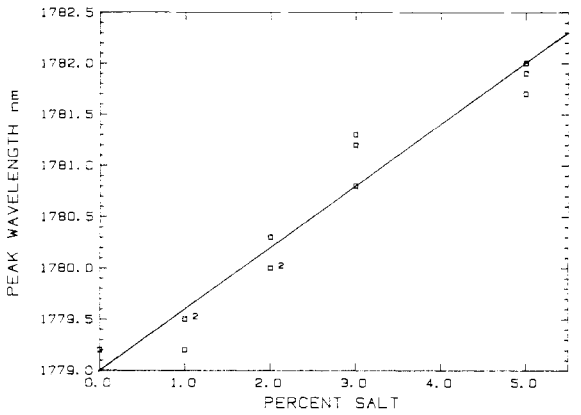

Figure 3. Regression of peak wavelength against salt concentration in water (r = 0.968). A number 2 indicates identical peak values for duplicate samples.

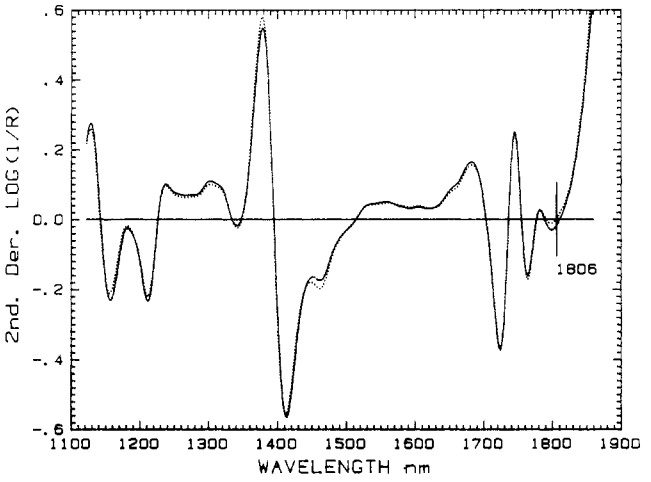

Figure 4. Second derivative spectra of fresh ham samples with 0.0% and 4.8% added salt and 20.9% fat. The spectrum of the sample without added salt is dotted.

nm, as well as small differences in other regions. These differences are very similar to those reported by Luck (1974) for the effect of temperature on the absorption spectrum of water. Temperature variations cause changes in the relative amounts of free and bonded OH groups, which can also be caused by changes in the salt level. The similarity of the effects of salt and temperature on water spectra makes it important to control one when measuring the other. How much improvement can be gained in the salt measurement by better control of temperature will be the subject of a subsequent paper. Figure 4 suggests that it might be possible to calibrate at a wavelength near 1380 or 1460 nm, but there the water band [see Norris (1983)] is strong enough to dilute the correlation with information about the water content. In the 1800-nm region, however, all absorbances are so low that only band shift information is relevant. (In plotting the two spectra of Figure 4, we multiplied the second-derivative values for the sample without salt by 1.110 so that the two spectra would have equal values at 1725 nm.)

With the meat samples, we tried correlating directly to single log $(1/R)$ values instead of using derivatives but obtained much lower correlation coefficients. The log $(1/R)$ spectra of seven fresh ham samples with 20.9% fat appear in Figure 5. The added salt correlated positively with an increase in log $(1/R)$ values at all wavelengths, indicating correlation to a change in the scatter coefficient. This change may have been caused by a change in refractive index of the water or by a change in the particle

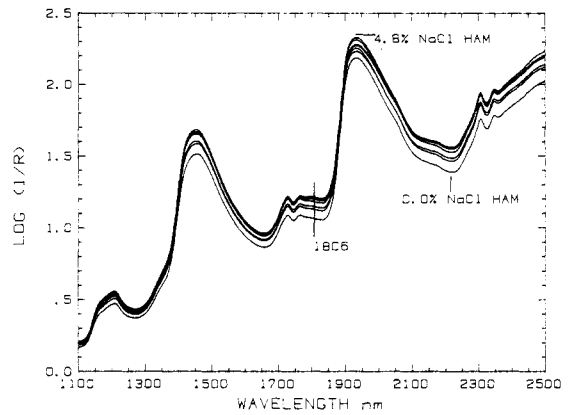

Figure 5. Spectra of seven samples of fresh ham containing 20.9% fat.

size caused by salt-induced swelling of the proteins. In a practical application, the scatter coefficient is affected by many factors, including fat, protein, and water contents, as well as by particle size and refractive index. Therefore, single log $(1/R)$ values alone would not be adequate for predicting salt content.

Two alternative data treatments used on the cured ham samples selected wavelengths that also measured the effect of salt on the water spectrum. Although the number of samples was small, we tried a data treatment involving a step-up multiple regression that searched all the log $(1/R)$ values rather than the derivatives. This procedure gave a three-term regression (wavelengths = 1806, 1790, and 1826 nm with coefficients −457, 884, and −547, respectively, constant term = 2.88) with essentially the same results as the derivative treatment (r = 0.970, SEC = 0.114, SEP = 0.158, bias = 0.02, reload error = 0.17). This combination of wavelengths and coefficients is very similar to combinations that produce a second derivative at 1806 nm. Results were similar with a data treatment that tested all possible three-term regressions and selected from every third recorded data point in each spectrum, except that overfitting was indicated by poorer predictions for some choices of the starting data point.

The NIR method has a direct relationship to the AOAC procedure used for chemical analysis of the cured ham samples. This procedure measures the chloride ion directly as the AgCl salt and assumes that all the chloride is associated with sodium. Because sodium and chloride are found in equal proportions in fresh ham tissue (Paul and Southgate, 1978) and added sodium is in the form of NaCl, that assumption is fairly accurate. The NIR procedure also measures the effect of the chloride ion because anions induce much larger changes in the NIR spectra of water than do cations (Luck, 1974). The two most prevalent anions in ham are chloride, 1670 mg/100 g, and phosphorus (phosphate), 280 mg/100 g (Paul and Southgate, 1978); chloride has the greater effect on the shift in the water spectrum because of its larger charge-to-size ratio (Luck, 1974).

With this experimental verification and theoretical justification of the ability of NIR to measure salt in cured ham, the NIR now has great potential for quality control in meat processing since it can give a simultaneous determination of water, protein, fat, and salt in 1 min.

Registry No. NaCl, 7647-14-5; water, 7732-18-5.

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Volatile Compounds from Heated Beef Fat and Beef Fat with Glycine

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One hundred forty-three compounds were isolated and identified from the dichloromethane extracts of heated beef fat and heated beef fat with glycine. Beef fat or beef fat with glycine was heated in a pressurized bottle at 200 °C for 4 h. The chemicals isolated from the heated samples using a simultaneous steam distillation/solvent (dichloromethane) extraction apparatus were subjected to gas chromatography and gas chromatography/mass spectrometry. The compounds identified included 15 *n*-alkanes, 12 *n*-alkenes, 13 *n*-aldehydes, 13 2-ketones, 12 *n*-alcohols, 11 *n*-alkylcyclohexanes, and 10 nitrogen-containing compounds. Formation mechanisms of some major products were proposed based on the radicals RCOO·, RCH_2O·, R·, and RO· formed from triglyceride.

Deep-fat frying is one of the most common cooking practices. Fat is used not only for transferring heat to cooking foods but also for giving flavor to foods, so the correct fat must be chosen in order to obtain satisfactory flavor. The fat found in meat seems to play an important role in the formation of cooked meat flavor (Yamamoto et al., 1970). Yamamoto et al. (1970) identified a series of fatty aldehydes in beef fat heated at 150 and 200 °C either under a nitrogen stream or in air. These carbonyl compounds may react with some nitrogen-containing compounds such as an amino acid to produce major cooked flavor chemicals such as heterocyclic compounds (Shibamoto, 1983). Buttery et al. (1977) identified many nitrogen-containing heterocyclic compounds (pyridines and pyrazines) in the volatile oils obtained from roasted lamb fat at 150 °C. These reports indicate that fat plays an important role in the formation of flavors in cooked foods.

Fats used for frying are usually heated over a prolonged period. Occupational cooking in a restaurant may involve the use of the same oil for more than a week. Fats that have been heated to high temperatures for long periods undergo chemical changes, including an increase in formation of carbonyl compounds.

In this study, compounds formed in heated beef fat and beef fat with glycine were isolated and identified in order to investigate a role of fat in flavor formation in cooked foods.

EXPERIMENTAL SECTION

Materials. The beef fat was obtained from the renal periphery of the beef carcass. The fat tissue was ground with small amounts of dry ice in a blender. The pulverized, solidified fat was placed in a glass container and heated in a water bath at 70–80 °C. The nonfat materials such as blood, muscle, and connective tissue were denatured by the heat and removed from the melted fat by filtration. The refined beef fat was weighed and stored in a freezer (–5 °C). Glycine was purchased from Eastman Kodak Co., Rochester, NY, and used without further purification. Authentic reference compounds were obtained from reliable commercial sources or were donated by Ogawa & Co., Ltd., Tokyo, Japan. 2-Butylpyridine and 2-pentylpyridine were synthesized according to Vogel (1962).

Sample Preparations. Beef fat (10.20 g) and glycine (9.95 g) were mixed in a pressurized bottle. The bottle was placed in an oven and heated at 200 °C for 4 h. The dark brown reaction mixture obtained was subjected to a simultaneous steam distillation (1 L of water) and solvent (dichloromethane, 200 mL) extraction. The dichloromethane extract was dried over anhydrous sodium sulfate. After the solvent was removed by using a Kuderna-Danish evaporative concentrator, the brown oily liquid obtained (381 mg) was analyzed with instrumental techniques. Additional experiments varying the quantities of beef fat and glycine (beef fat/glycine = 10/1 and 2/1 and beef fat alone) were conducted by using the same procedure as above, and the samples obtained from each experiment were also analyzed.

Isolation and Identification of Components in the Heated Samples. All samples were analyzed with Kovats gas chromatographic retention index [*I* (Kovats, 1965)] and gas chromatography/mass spectrometry (GC/MS) techniques as described previously (Yamaguchi and Shibamoto, 1980; Toda et al., 1982). The gas chromatographic retention index (Kovats index) and MS fragmentation pattern

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