

# Comparison of Sample Handling and Data Treatment Methods for Determining Moisture and Fat in Cheddar Cheese by Near-Infrared Spectroscopy<sup>†</sup>

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Various sample handling and data treatment methods were compared for determining moisture and fat in Cheddar cheese by near infrared (near-IR) spectroscopy. Reflectance and transmittance spectra were collected from grated samples, and reflectance spectra were also collected from intact samples with a fiber optic probe. Calibrations were developed using partial least-squares (PLS) and multiple linear regression (MLR) techniques. For grated samples, PLS regression of first-derivative reflectance data yielded a calibration that gave the lowest standard error of prediction (SEP) for moisture (0.335%), while a PLS regression of log 1/R spectral data performed best for determining fat (SEP = 0.330%). Acceptable results for moisture were also obtained from a calibration based on MLR of first-derivative transmittance spectra (SEP = 0.356%), but determining fat by transmittance yielded high SEP values. Use of a fiber optic probe with intact cheese blocks gave less reliable results than were obtained from grated samples.

**Keywords:** *Near-infrared spectroscopy; Cheddar cheese; moisture; fat*

## INTRODUCTION

Most analytical procedures for measuring moisture and fat in cheese are time consuming and destructive to the sample. Near-infrared (near-IR) spectroscopy has been shown to be useful for direct, rapid, and non-destructive quantitation of major components in solid and semisolid foods. However, development of near infrared methods for use with cheese has been limited.

Although mid-infrared spectroscopy has been used for several years to determine protein, fat, and lactose in milk, and the near-IR spectral properties of dairy foods were investigated more than 35 years ago (Goulden, 1957), only in the past few years has much research been conducted with respect to near-IR analysis of dairy products. Near-IR spectroscopy has been used to measure moisture, protein, fat, lactose, and other constituents in dried dairy products including whey powder (Baer et al., 1983a), milk powders (Baer et al., 1983b), and dried milk and cheese powders (Ereifej and Markakis, 1983). Near-IR spectroscopy has also been coupled with multivariate statistical techniques to classify milk powders according to the level of heat treatment to which they were exposed during processing (Kelly et al., 1989).

Near-IR reflectance spectroscopy using a fiber optic probe, coupled with principal component analysis, has been used to monitor the enzymatic phase of milk coagulation during cheesemaking (Saputra et al., 1992). This was achieved by measuring changes in the macropeptide concentration in the milk.

With respect to compositional analysis of cheese, Frank and Birth (1982) measured moisture in cheese

and protein and fat in a dried cheese product by near-IR reflectance. Due to the extremely strong water absorption band around 1940 nm, only wavelengths below 1700 nm were used to quantitate moisture. Their results indicated the potential for successfully measuring cheese composition by near-IR spectroscopy. Frankhuizen and van der Veen (1985) used a commercially available filter monochromator near-IR instrument to determine moisture, fat, protein and other constituents in several dairy products, including European-style soft cheeses. Using this system, moisture contents of Edam and Gouda cheese were successfully predicted with a standard error of prediction (SEP) of less than 0.3%. Weaver (1984) used a similar instrument to measure the moisture content of cottage cheese. A standard error of 0.4% with respect to the reference method was obtained.

More recently, Wehling and Pierce (1988, 1989) used a commercial, filter monochromator near-IR reflectance instrument to determine moisture and fat in Cheddar cheese. Spectral data in log 1/R format were correlated with gravimetric analyses by multiple linear regression (MLR), and the resulting equations used to analyze an independent set of validation samples. A comparison of near-IR and vacuum oven moisture determinations gave an SEP of 0.38%. Comparison of near-IR and Roesse-Gottlieb fat determinations resulted in an SEP of 0.44%.

On the basis of these results, there is continued interest in the analysis of American-style hard cheeses by near-IR spectroscopy. The purpose of the present work was to further evaluate the use of near-IR spectroscopy for Cheddar cheese analysis by determining if the use of a scanning near-IR spectrometer, coupled with derivative mathematics and full-spectrum regression methods, could give improved results over discrete wavelength measurements using a log 1/R data treatment. Also, a comparison of transmittance and reflectance measurements, including the use of a fiber optic probe, was made.

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## MATERIALS AND METHODS

**Selection of Samples.** Cheddar cheese samples (ca. 454 g blocks) were obtained from area retailers. Variation was maximized by including samples of all commercial products available in the Lincoln, NE, area, and samples from the University of Nebraska Dairy Processing Plant. To maximize variability in chemical composition, cheeses of different ages were used including mild, medium, and sharp Cheddars. Product codes were monitored to avoid duplication of samples. All samples contained annatto extract as a coloring agent.

**Determination of Moisture and Fat.** Cheese samples were grated such that individual shreds were ca. 1 mm diameter by 20 mm long and thoroughly mixed. Moisture was determined by vacuum oven drying. Two to three grams of grated cheese was accurately weighed into a tared aluminum dish and the cheese spread into a uniform layer. The sample was placed in a vacuum oven at room temperature and the pressure in the oven reduced to 75 Torr. The temperature was then slowly increased to 95 °C, and the samples were maintained at this temperature for 14 h (Method 926.08; AOAC, 1990). The dried samples were then removed from the oven, cooled to room temperature in a desiccator, and weighed.

Fat in the grated cheese samples was determined by solvent extraction using the Roese-Gottlieb method (Method 933.05; AOAC, 1990) with one modification. Extraction flasks were not centrifuged but were allowed to rest until the aqueous and organic phases separated.

**Near-Infrared Spectroscopy.** Spectroscopic data were collected with an NIRSystems Model 6500 scanning spectrometer (NIRSystems Division of Perstorp Analytical, Silver Spring, MD). Spectral collection accessories included a fiber optic remote probe capable of diffuse reflectance measurements, and a sample transport module fitted with a high fat/high moisture cell that permitted both diffuse reflectance and transmittance spectra to be obtained. For instrument control, data collection and calibration development, the spectrometer was interfaced to an IBM Model 50Z personal computer (IBM Corp., Armonk, NY) running the Near Infrared Spectral Analysis Software (NSAS) package (version 3.16; NIRSystems, Inc.) under MS-DOS.

Because of the effect of temperature on spectral response (Wehling and Pierce, 1988), all samples were allowed to reach room temperature ( $25 \pm 2$  °C) prior to collection of spectra. The sample transport temperature control unit was set at 26 °C. Diffuse reflectance spectra were collected over a range of 400–2500 nm in a log  $1/R$  format, while transmission spectra were collected from 400–1100 nm using log  $1/T$ . All spectra were collected at 2 nm intervals.

Diffuse reflectance spectra of each cheese sample were first collected with the fiber optic probe at two different locations on the intact block of cheese. The individual spectra were then averaged by the software. The remote probe sampled a 5 cm  $\times$  5 cm area; therefore, a 4 cm  $\times$  4 cm  $\times$  1.5 cm piece was cut from each of the two locations on the cheese block where the probe was used to collect data. These pieces were then grated, mixed and analyzed for moisture and fat by standard methods as previously described. The remainder of each block of cheese was grated and filled into a polyethylene bag (5.7 cm  $\times$  20.3 cm, part NR7060, NIRSystems). The bag was then compressed between the two quartz windows of the high fat/high moisture cell, and the cell placed into the sample transport mechanism. A spectrum was obtained by collecting and averaging 32 individual spectral scans over the length of the filled sample cell as it was moved through the infrared beam by the transport mechanism. The bag was then removed, turned over, reinserted into the cell, and scanned a second time. The two spectra were averaged. Each sample was scanned in both reflectance and transmittance modes. After both sets of spectral data were collected, samples for gravimetric analysis were obtained by slicing the bag open and removing a 3 cm  $\times$  15 cm strip from the center of the cheese.

**Calibration Development and Validation.** Calibration sample sets were individually selected for the fiber optic probe reflectance data, the reflectance data from the high fat/high moisture cell, and the transmittance data from the high fat/

high moisture cell. The calibration sets were chosen by the use of a subroutine of the NSAS software package. The algorithm based its selection of the calibration samples on a standardized  $H$  statistic (Mahalanobis distance; Mahalanobis, 1936), which indicates how different a sample's spectrum is from the average sample in the set. The spectra used for both calibration and validation were those obtained by averaging the duplicate spectral readings from each cheese sample. The calibration samples selected were inspected to ensure that the extremes of moisture and fat contents were included in the set. The validation sets were composed of those samples not used for calibration.

Calibration equations were created from each set of spectra by use of multiple linear regression to select wavelengths and relate log  $1/R$  or log  $1/T$  values to moisture and fat contents. Both forward stepwise and all possible regressions algorithms were applied. These regression procedures have been described in detail by Draper and Smith (1981). The all possible regressions algorithm allowed a maximum of 70 wavelengths, evenly spaced across the near-IR spectrum, to be considered for regression. Multiterm equations using first and second derivatives of the spectral data were also developed. The optimum number of wavelengths for inclusion in the calibration equations was determined by comparing regression results for multiple correlation coefficient ( $R$ ), standard error of calibration (SEC), partial  $F$  values for each term in the equation, and the overall  $F$  of regression (Workman and Mark, 1992). Additionally, these statistical parameters were used to select the optimum segment and gap values for calculating the derivatives (Williams, 1987).

A partial least-squares (PLS) regression algorithm was also used to develop calibration equations using log  $1/R$ , log  $1/T$ , and derivatized data. Underivatized spectra were scaled to a mean of zero and variance of one before regression. The optimum number of terms for inclusion in a PLS calibration equation was selected based on the standard errors of cross validation, which should be minimized (Workman and Mark, 1992), along with the  $R$  and SEC values obtained from the regressions.

The standard error of prediction (SEP; Workman and Mark, 1992) of each calibration equation was determined by predicting moisture or fat values for the appropriate validation sample set.

## RESULTS AND DISCUSSION

**Reflectance with the High Fat/High Moisture Cell.** A set of 30 samples was selected for calibration development when using the high fat/high moisture cell in the reflectance mode. The calibration samples had a mean moisture value of 36.94% with a standard deviation of 1.42%, while the mean fat content was 32.47% with a standard deviation of 0.92%. Twenty-two samples were used for validation of the moisture calibrations and had a mean value of 37.25% and a standard deviation of 0.93%. Nineteen samples, whose fat contents fell within the range of the calibration samples, were used to validate the fat calibrations. This sample set had a mean of 32.31% and a standard deviation of 0.87%.

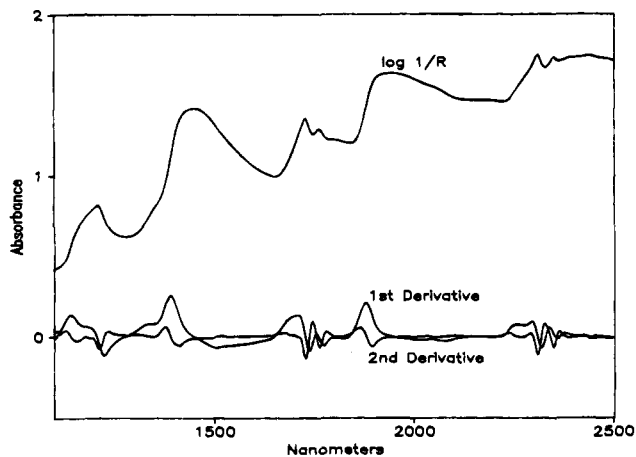
The reflectance spectrum of a representative Cheddar cheese sample is shown in Figure 1 in log  $1/R$  and derivative formats. Two strong absorption bands due to water are evident in the spectrum. A band arising from a combined  $-OH$  stretch and deformation is centered at 1942 nm, and a band arising from the first overtone of an  $-OH$  stretch occurs at 1450 nm (Osborne and Fearn, 1986). Sharp absorption bands due to lipids occur from a combined  $-CH$  stretch and deformation at 2310 nm, a first overtone of a  $-CH$  stretch at 1726 nm, and a  $-CH$  stretch second overtone at 1208 nm. While absorption bands arising from the coloring agents were observed in the visible region, they had no significant influence on the near-IR spectrum.

**Table 1. Calibration and Validation Results from Various Near-IR Procedures for Determining Moisture in Cheddar Cheese Using Reflectance Measurements from the High Fat/High Moisture Cell**

regression type	spectral treatment	wavelength (nm) or no. of PLS terms used	calibration results		validation results	
			R	SEC (%)	r	SEP (%)
MLR <sup>a</sup>	log 1/R	1180	0.972	0.335	0.916	0.374
		1400				
		1660				
		1680				
MLR <sup>a</sup>	1st deriv <sup>c</sup> of log 1/R	1100	0.984	0.276	0.925	0.353
		1170				
		1380				
		1660				
MLR <sup>a</sup>	2nd deriv <sup>d</sup> of log 1/R	1338	0.982	0.276	0.914	0.385
		1522				
PLS <sup>b</sup>	log 1/R	6 factors <sup>e</sup>	0.968	0.398	0.895	0.416
PLS <sup>b</sup>	1st deriv <sup>c</sup> of log 1/R	8 factors <sup>e</sup>	0.995	0.175	0.933	0.335
PLS <sup>b</sup>	2nd deriv <sup>d</sup> of log 1/R	4 factors <sup>e</sup>	0.974	0.344	0.901	0.404

<sup>a</sup> Multiple linear regression. <sup>b</sup> Partial least squares regression. <sup>c</sup> Segment = 20 nm, gap = 20 nm. <sup>d</sup> Segment = 10 nm, gap = 10 nm.

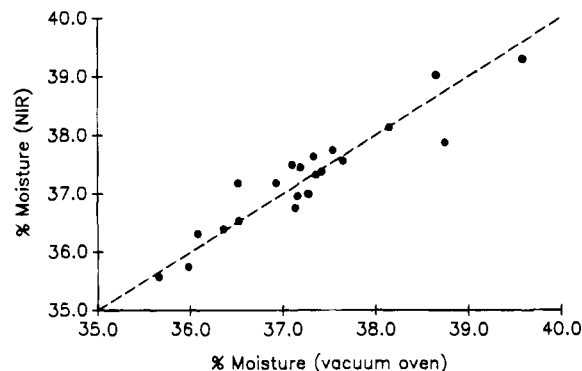
<sup>e</sup> Spectral range = 1100–2500 nm.



**Figure 1.** Diffuse reflectance spectrum of a representative Cheddar cheese sample, obtained with the high fat/high moisture cell, over a spectral range of 1100–2500 nm. The spectrum is shown in log 1/R, first derivative of log 1/R, and second derivative of log 1/R formats.

Calibration and validation results for moisture, obtained from reflectance measurements using the high fat/high moisture cell, are shown in Table 1. The results represent the calibrations that provided the lowest SEP's for each regression/data treatment combination. A maximum of four wavelengths was used in the multiple linear regression equations in order to prevent overfitting the data. It can be observed that application of a first-derivative treatment to the spectral data slightly lowered the SEP values as compared to the log 1/R or second-derivative treatments. The first derivative treatment may eliminate the baseline shifts observed in the log 1/R spectra of different samples. Using the full first-derivative spectrum in a calibration developed by PLS regression further decreased the SEP and gave the best overall results for near-IR moisture measurement. The calibration performed equally well with both mild and sharp cheese samples. Figure 2 shows a scatter diagram comparing the near-IR values obtained from the validation samples by this calibration and the oven moisture results. Use of PLS regression did not improve the SEP values when using log 1/R or second derivative treatments.

Calibration and validation results for fat, as measured by reflectance with the high fat/high moisture cell, are shown in Table 2. One sample in the calibration set was observed to be a consistent outlier with respect to its residual, and was deleted during calibration development. Data in the log 1/R format were found to give



**Figure 2.** Comparison of moisture values for the validation sample set as predicted by the optimized near-IR calibration, with those determined by vacuum oven drying.

the lowest SEP values; derivatization provided no improvement in the results. Use of PLS regression methods improved the SEP values for all spectral data formats, with the combination of PLS regression and log 1/R spectral data providing the best results overall. For the validation set, Figure 3 shows a scatter plot comparing the fat values predicted by near-IR using this equation, and the gravimetric fat values. Again, differences in aging did not affect performance. It should also be noted that truncating the spectra at 1850 nm improved the PLS regression results. Due to the high fat content of cheese, the lipid bands in the 2310 nm region are so strong that their intensities are nonlinear with respect to fat concentration (Figure 1). Eliminating this nonlinear region of the spectrum improves the calibration performance.

**Transmittance with the High Fat/High Moisture Cell.** A set of 24 samples was selected for calibration development when using the high fat/high moisture cell in the transmittance mode. The calibration samples had a mean moisture value of 37.06% and a standard deviation of 1.44%, while the mean fat content was 32.41% with a standard deviation of 0.88%. Twenty-one samples were used for validation of the moisture calibrations and had a mean value of 37.19% and a standard deviation of 0.90%. Eighteen samples whose fat contents fell within the range of the calibration samples were used to validate the fat calibrations. This sample set had a mean of 32.31% and a standard deviation of 0.87%.

A transmission spectrum of a representative cheese sample is shown in Figure 4 in both log 1/T and derivative formats. The prominent feature in this

**Table 2. Calibration and Validation Results from Various Near-IR Procedures for Determining Fat in Cheddar Cheese Using Reflectance Measurements from the High Fat/High Moisture Cell**

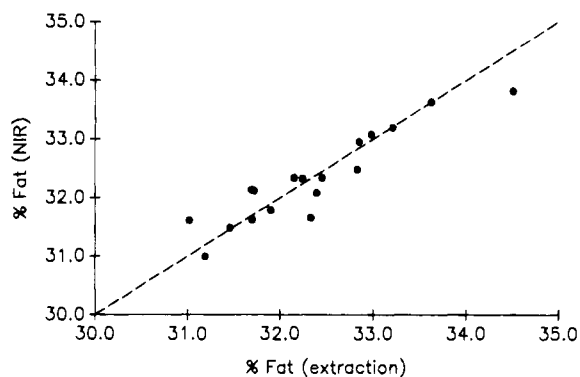
regression type	spectral treatment	wavelength (nm) or no. of PLS terms used	calibration results		validation results	
			R	SEC (%)	r	SEP (%)
MLR <sup>a</sup>	log 1/R	1398 1712 1772 1866	0.911	0.409	0.929	0.337
MLR <sup>a</sup>	1st deriv <sup>c</sup> of log 1/R	1204 1452 1656 1728	0.935	0.352	0.871	0.439
MLR <sup>a</sup>	2nd deriv <sup>d</sup> of log 1/R	1344 2260	0.910	0.396	0.786	0.551
PLS <sup>b</sup>	log 1/R	8 factors <sup>e</sup>	0.955	0.321	0.928	0.330
PLS <sup>b</sup>	1st deriv <sup>c</sup> of log 1/R	5 factors <sup>e</sup>	0.950	0.318	0.882	0.418
PLS <sup>b</sup>	2nd deriv <sup>d</sup> of log 1/R	5 factors <sup>e</sup>	0.929	0.377	0.850	0.514

<sup>a</sup> Multiple linear regression. <sup>b</sup> Partial least squares regression. <sup>c</sup> Segment = 20 nm, gap = 20 nm. <sup>d</sup> Segment = 10 nm, gap = 10 nm. <sup>e</sup> Spectral range = 1100–1850 nm.

**Table 3. Calibration and Validation Results from Various Near-IR Procedures for Determining Moisture in Cheddar Cheese Using Transmittance Measurements from the High Fat/High Moisture Cell**

regression type	spectral treatment	wavelength (nm) or no. of PLS terms used	calibration results		validation results	
			R	SEC (%)	r	SEP (%)
MLR <sup>a</sup>	log 1/T	920 940 970	0.963	0.419	0.912	0.370
MLR <sup>a</sup>	1st deriv <sup>c</sup> of log 1/T	928 960	0.967	0.386	0.919	0.356
MLR <sup>a</sup>	2nd deriv <sup>d</sup> of log 1/T	920 946	0.964	0.404	0.894	0.405
PLS <sup>b</sup>	log 1/T	7 factors <sup>e</sup>	0.976	0.379	0.901	0.392
PLS <sup>b</sup>	1st deriv <sup>c</sup> of log 1/T	3 factors <sup>e</sup>	0.970	0.375	0.912	0.371
PLS <sup>b</sup>	2nd deriv <sup>d</sup> of log 1/T	3 factors <sup>e</sup>	0.970	0.380	0.907	0.379

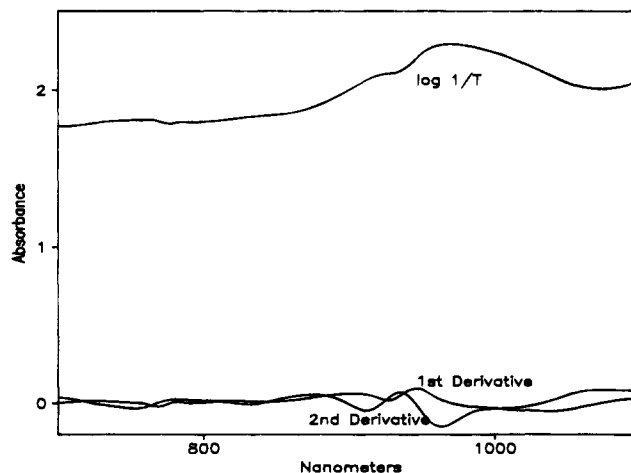
<sup>a</sup> Multiple linear regression. <sup>b</sup> Partial least squares regression. <sup>c</sup> Segment = 10 nm, gap = 10 nm. <sup>d</sup> Segment = 30 nm, gap = 10 nm. <sup>e</sup> Spectral range = 800–1070 nm.



**Figure 3.** Comparison of fat values for the validation sample set as predicted by the optimized near-IR calibration, with those determined by the Roese–Gottlieb extraction method.

shorter wavelength region is the absorption band centered at 970 nm arising from the second overtone of an –OH stretch in the water molecule (Osborne and Fearn, 1986). The shoulder at 928 nm on the side of the water band is due to lipid in the sample and arises from the third overtone of a –CH stretch.

Calibration and validation results for moisture, obtained from transmittance measurements using the high fat/high moisture cell, are shown in Table 3. One sample in the calibration set was observed to be a consistent outlier with respect to its residual and was deleted during calibration development. As with the reflectance measurements, a first-derivative data treatment slightly lowered the SEP values, as compared to the log 1/R or second-derivative treatments. Again, the first-derivative mathematics may be most successful in eliminating sample-to-sample baseline shifts, which can



**Figure 4.** Transmittance spectrum of a representative Cheddar cheese sample, obtained with the high fat/high moisture cell, over a spectral range of 700–1100 nm. The spectrum is shown in log 1/T, first derivative of log 1/T, and second derivative of log 1/T formats.

be large in the transmission mode. Use of PLS regression with the full spectra did not improve the SEP values, except for the second-derivative data treatment. However, the results were still not as good as a simple MLR calibration with either log 1/R or first derivative data. The best transmission calibrations for moisture gave SEP values only slightly higher than the best results obtained in the reflectance mode.

Calibration and validation results for fat, as measured by transmittance with the high fat/high moisture cell, are shown in Table 4. For all data treatments, the SEP values were substantially higher than those obtained

**Table 4. Calibration and Validation Results from Various Near-IR Procedures for Determining Fat in Cheddar Cheese Using Transmittance Measurements from the High Fat/High Moisture Cell**

regression type	spectral treatment	wavelength (nm) or no. of PLS terms used	calibration results		validation results	
			<i>R</i>	SEC (%)	<i>r</i>	SEP (%)
MLR <sup>a</sup>	log 1/ <i>T</i>	890 914 966	0.851	0.497	0.744	0.576
MLR <sup>a</sup>	1st deriv <sup>c</sup> of log 1/ <i>T</i>	922 928 1072	0.873	0.461	0.817	0.555
MLR <sup>a</sup>	2nd deriv <sup>d</sup> of log 1/ <i>T</i>	764 920	0.893	0.416	0.763	0.577
PLS <sup>b</sup>	log 1/ <i>T</i>	5 factors <sup>e</sup>	0.864	0.502	0.738	0.585
PLS <sup>b</sup>	1st deriv <sup>c</sup> of log 1/ <i>T</i>	3 factors <sup>e</sup>	0.867	0.472	0.763	0.595
PLS <sup>b</sup>	2nd deriv <sup>d</sup> of log 1/ <i>T</i>	3 factors <sup>f</sup>	0.869	0.469	0.760	0.599

<sup>a</sup> Multiple linear regression. <sup>b</sup> Partial least squares regression. <sup>c</sup> Segment = 10 nm, gap = 10 nm. <sup>d</sup> Segment = 30 nm, gap = 10 nm. <sup>e</sup> Spectral range = 800–1070 nm. <sup>f</sup> Spectral range = 750–1070 nm.

when working in the reflectance mode. The use of PLS regression did not improve the predictions. Similar results were obtained by Isaksson et al. (1992) when working with homogenized meat products. They found that transmission measurements were equal to or better than reflectance for measuring moisture but that reflectance performed better than transmittance for determining the fat content. They attributed this to the fact that the lipid absorption bands in the 700–1100 nm spectral region are much less distinct than in the longer wavelength region.

**Reflectance with the Fiber Optic Probe.** Calibrations using reflectance measurements obtained from the fiber optic probe were also developed and validated. Spectra obtained in this manner were quite noisy at wavelengths above 1800 nm (data not shown), as the transmission efficiency of the optical fibers is lower at longer wavelengths. Therefore, only wavelengths shorter than 1800 nm were used in calibration development. While moisture calibrations with low SEC values could be developed, when they were applied to the validation set, the SEP values obtained ranged from 0.562 to 0.632%. These values are substantially higher than those obtained with the high fat/high moisture cell in either reflectance or transmittance modes. Results of fat determinations were somewhat better, with a two-wavelength MLR calibration using log 1/*R* data yielding an SEP of 0.424%. However, the results were still not as good as those achieved with the high fat/high moisture cell. While use of the fiber optic probe seems to hold some potential for rapid at-line analysis without sample preparation, more work is necessary before the technique becomes practical, as current results are not acceptable.

**Conclusions.** If moisture and fat both need to be measured in Cheddar cheese, reflectance measurements from grated samples allow reliable determination of both constituents. A first-derivative spectral treatment gave the lowest SEP values for moisture, while un-derivatized log 1/*R* measurements gave the best results for fat. For both constituents, PLS regression equations gave slightly lower SEP values than did calibrations developed by multiple linear regression. On the basis of our results, it should also be possible to develop successful calibrations for other varieties of hard cheeses and reduced fat cheeses. If only moisture is to be determined, transmittance measurements can provide results nearly equal to those of the reflectance method. Again, a first-derivative data treatment produced the lowest SEP values, but PLS regression offered no improvement over MLR when working with transmission measurements. Transmittance is not as successful

for measuring fat content as is reflectance. While reflectance measurements obtained from intact samples with a fiber optic probe showed some promise, the measurements were not as reliable as those obtained from grated samples with the high fat/high moisture cell. Further research is needed to make the fiber optic probe useful for cheese analysis.

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Received for review June 3, 1994. Accepted September 27, 1994.\*

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\* Abstract published in *Advance ACS Abstracts*, November 1, 1994.