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Effects of pH, Protein, Fat, and Calcium on Diffuse Reflectance of Milk

K. R. Lochte-Watson, F. A. Payne, R. S. Gates, C. L. Hicks

Abstract. *Cottage cheese and yogurt product quality and process control would improve if plant operators had a stable and sanitary device for pH measurement. The objectives of this research were to determine the effects of pH, protein, fat, and added calcium on diffuse reflectance of milk in the wavelength range of 350 to 990 nm and determine if milk pH correlates with diffuse reflectance over the range of treatments tested. Diffuse reflectance of milk was measured at pH levels in the range of 5.5 to 7.0 and at varying levels of fat, protein, and added calcium concentrations. Diffuse reflectance was found to decrease with pH, increase linearly with protein and fat concentration, be affected by milk pretreatment, and be affected by the interaction between calcium and protein. A pH prediction model which included reflectance data and product variables (fat, protein, pre-heat treatment) explained 74.4% of the variance and had a standard error of 0.29 pH units. Because milk constituents and pretreatment affect diffuse reflectance, this method would be limited in applicability to systems where factors such as fat, protein, calcium, and pretreatment would be relatively constant, such as in yogurt and cottage cheese production.*

Keywords. *Spectroscopy, Cheese, Reflectance, Milk, Sensor.*

Cottage cheese and yogurt product quality and process control would improve if plant operators had a stable and sanitary device for pH measurement. Although pH is the single most important factor in measuring culture progression during production, it is difficult to measure consistently in a production facility because of inherent variability within and between pH meters. During milk fermentation for yogurt production, a method for monitoring acid development would enable operators to adequately control the process mainly through temperature adjustments (Tamime and Robinson, 1985).

Acid-producing bacteria are used to manufacture cheese and yogurt. Lowering milk pH results in precipitation of calcium phosphate and subsequently causes denaturation and aggregation of casein micelles. For rennet cheese varieties, acid production by bacteria affects coagulation rate, protein denaturation, and retention of coagulant in the curd, which in turn affects the rate of proteolysis during ripening. Also affected by pH are cheese yield, cheese moisture content, rheological properties such as final texture, and growth of pathogenic bacteria (Fox, 1987). Optimal bacterial growth and phage detection in starter cultures could be obtained using an on-line pH monitoring method.

Current pH measurement in cheese and yogurt plants is performed using pH electrodes, titration methods, or pH indicator solutions. Electrodes used to measure pH in the plant are slow to stabilize and are moved from one vat to another resulting in potential sanitation problems. Titration and pH indicator solutions require drawing a sample and taking the measurement in the lab. Time delays between drawing and measuring the pH require that standardized curves be relied upon to predict current pH levels of fermenting milk containing dynamically growing bacteria.

Effects of pH on Milk Constituents

Milk proteins are classified as either casein (75-85%) or whey (15-25%). Electron microscopy indicates that casein micelles vary in diameter (80-300 nm) and are composed of discrete subunits ranging from 10 to 20 nm which are associated through calcium and/or complex calcium phosphate salt bridges (Brunner, 1976). Studies of casein micelle structure have yielded information about individual components and physical appearance, but the total structure of casein micelle is still unclear. Models have been proposed by Morr (1967), Slattery and Evard (1973), and Schmidt (1982).

Micellar casein dissociation and micelle voluminosity depend on pH (Tanford, 1968; Van Hooydonk et al., 1986; Wong et al., 1988). Visser et al. (1986) defined five stages of milk acidification between pH 6.5 and 4.5 at 43°C. Between pH levels of 6.6 and 5.5, casein micelles are found with a wide range of diameters. At a pH of 5.5, sufficient calcium and phosphate groups have been released such that the original and smaller casein aggregates become visible. At a pH of 5.2, an aggregation of corpuscle structure is observed. As the pH is lowered further, these aggregates seem to contract into smaller areas

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and finally individual casein particles are formed again, larger than the original casein micelle and different in character resulting from the loss of calcium phosphate.

Optical Properties of Milk

The effect of pH on optical properties of milk was not found in the literature; however, many studies on the optical properties of milk have been reported. Milk not only absorbs light at many wavelengths because of the large number of compounds present but also scatters light because of the colloidal dispersion of proteins, emulsion of fat, and distribution of particle sizes (Wong et al., 1988). The amount of light scattering in milk depends on the number and size of particles in suspension, incident radiation wavelength, and the difference in refractive index between the various particles and solvents (Goulden and Sherman, 1962; Haugaard, 1966; Walstra, 1967; Ashworth, 1969; Jeunet and Grappin, 1970; Flux et al., 1982; Payne et al., 1993). Recent interest in light absorption, fluorescence, and scattering in milk is largely quantitative rather than qualitative. Casein micelles are smaller than fat globules and thus tend to scatter less light. Walstra and Jenness (1984) suggested that casein micelles scatter more light at 400 nm (blue) than at 660 nm (red). Scattering measurements are hard to interpret, because of the non-homogeneity of casein micelle sizes. Light scattering by casein micelles greatly increases with colloidal calcium phosphate concentrations (Walstra and Jenness, 1984).

Protein and fat absorb light in the area of 220 to 380 nm and 400 to 520 nm, respectively (Wong et al., 1988). Protein and fat effects on absorbance at various wavelengths have been investigated with consideration given to various conditions (Goulden, 1957; Ben-Gera and Norris, 1968; Gruen and Tao, 1985; Casal et al., 1988; Payne et al., 1993; Kamishikiryo-Yamashita et al., 1994; Kuaye, 1994).

Fat globule size and concentration have been shown to have a large effect on absorption (Ben-Gera and Norris, 1968). Homogenization of milk results in more uniform fat globule size. Goulden (1957) reported transmission for homogenized milk to have distinct absorption peaks similar to water absorption peaks. Absorption peaks for fat were not detected because of the breakdown of fat globules as a result of homogenization. Payne et al. (1993) found diffuse reflectance at 820 nm varied linearly with fat concentration between the range of 1 to 5% fat.

Objectives

The research reported here is an investigation of the effects of pH on diffuse reflectance of cheese milk having a normal range of constituents. Since colloidal casein micelles and fat globule size and concentration affect diffuse reflectance, the effects of different protein and fat concentrations were also tested. As casein micelle size is affected by calcium content, the effect of calcium addition per protein content was also investigated. The objectives were to determine the effects of pH, protein, fat, and calcium on diffuse reflectance of milk in the wavelength range of 350 to 990 nm and to determine if diffuse reflectance parameters can be found which correlate with milk pH over the range of protein, fat, and calcium levels tested.

Materials and Methods

A laboratory system was assembled to measure diffuse reflectance at light wavelengths of 350 to 990 nm to determine if a relationship exists between pH and diffuse reflectance. Milk samples were prepared to mimic fat, protein, and added calcium levels normally encountered in cheese processing. Addition of calcium is common practice in the cheese making industry and was included because it affects protein structure. Milk temperature was not tested because reflectance spectra vary with temperature and most processes of concern are operated at constant temperatures.

Experimental Design

A 2 × 3 factorial split-spilt plot experiment with two replications was performed to test effects of fat, protein, calcium addition, and pH on diffuse reflectance spectrums of milk. Treatment for the whole plot experimental unit was two fat levels × three protein levels to provide six whole plots. Treatment for the sub-plot was three levels of calcium addition. Treatment for the sub-sub-plot was the adjustment to four target pH levels. Two fat concentrations, three protein concentrations, three levels of calcium additions, and four target pH levels were tested in two replications for a total of 144 samples (table 1).

Table 1. Experiment factors and levels tested

	Level			
Factor	1	2	3	4
Fat (%)	1	4		
Protein (%)	3.3	4.3	5.3	
Calcium (mmol/g protein)	0.00	0.03	0.06	
Target pH	5.5	6.0	6.5	7.0

Sample Preparation

Approximately 95 L of pasteurized, skim milk containing approximately 0.05% butterfat and 3.25% protein, and 4 L of cream containing about 40% butterfat were obtained from a local dairy processing plant (Winchester Farms Dairy, Winchester, Ky.). Half of the skim milk was condensed by low temperature evaporation (45-49°C) in a lab scale vacuum evaporator to a protein concentration of approximately 6%. An anti-foaming agent (Antifoam 1520-US, Dow Corning Corp., Midland, Mich.) was added at a concentration less than 13 ppm active silicon to reduce foaming. Condensed skim milk, skim milk, and cream were assayed for protein and fat content using a near-infrared analyzer (Dairylab 2, Foss Food Technology, Eden Prairie, Minn.). Condensed skim milk, skim milk, and cream were combined to obtain six samples of 9000 ± 10 g with specific protein and fat concentration. Each mixture was assayed for protein and fat content.

Protein assays were conducted using a near-infrared analyzer standardized at 3 to 4% protein using Kjeldahl Tests (DQC1 Services, Inc., St. Paul, Minn.). As condensed skim milk and target sample levels for protein exceeded the near-infrared analyzer's standardized range, a modification in analysis was performed. Protein levels above 4% were diluted with de-ionized water to obtain protein concentrations between 3 to 4%. After analysis by the near-infrared analyzer, results were calculated using the dilution ratio.

Fat assays were conducted using a near-infrared analyzer standardized at 3 to 5.5% fat using Babcock Tests (DQC1 Services, Inc., St. Paul, Minn.). For samples at target fat levels of 1%, the Babcock Test Method 18.8a (Richardson, 1985) was performed in the laboratory.

Each of the six combinations of fat and protein were divided into three 2700 g quantities. Calcium was added at the target levels of 0.00, 0.03, and 0.06 mmol/g protein. The milk was then divided into preweighed samples of 450 g and stored at 2°C until used.

pH Adjustment

Milk samples were placed in glass beakers, stirred and held in a 2°C water bath until thermal equilibrium was reached. Sample pH was adjusted by adding between 0 and 18 mL of either 1.0 M HCl or 1.0 M NaOH at 2°C. A constant dilution rate was assured by adding de-ionized water for a total added volume of 20 mL. After the pH was adjusted, the sample was held at 2°C with agitation for 10 min to allow for chemical equilibration. The sample was then heated in a 45°C water bath to 31°C and transferred to a 31°C water bath. After thermal equilibration at 31°C, the pH of the agitated sample was measured using a combination glass pH electrode (BN 8104, Orion Research Inc., Boston, Mass.) connected to an expandable ion analyzer (EA 940, Orion Research Inc., Boston, Mass.). The pH meter had a sensitivity of ± 0.01 pH units and was calibrated using a two-point calibration method. Buffers used included 7.0 and 4.0 standard solutions (Buffer salt 6.86, and Buffer salt 4.01; Fisher Chemical, Fisher Scientific, Fair Lawn, N.J.) at temperatures of 31°C.

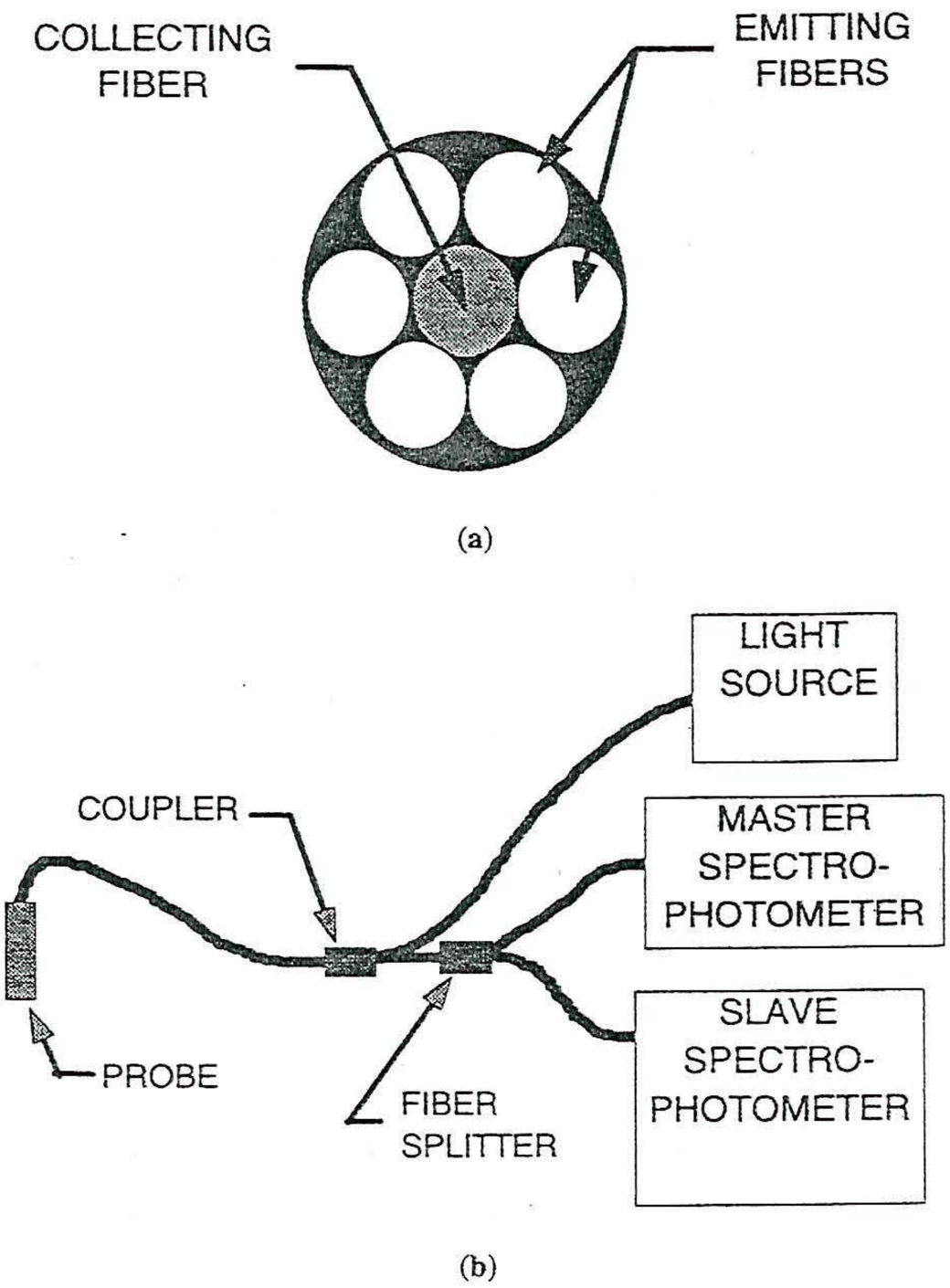

Figure 1–Schematic of probe design with (a) distal end in "bull's eye" configuration, and (b) fiber cables with fiber splitter.

Diffuse Reflectance Equipment

The equipment setup used to measure diffuse reflectance included a dual spectrophotometer, probe, and computer for data acquisition as detailed below.

Spectrophotometer. The diffuse reflectance of milk was measured with a dual miniature fiber optic spectrophotometer composed of a master and slave unit (model SD1000 with L1 lenses, Ocean Optics, Inc., Dunedin, Fla.). Both spectrophotometers had a holographic grating which dispersed light across a linear array of 1024 CCD (charge-coupled device) detector elements. The master unit had a bandwidth of 350 to 850 nm, a grating of 600 lines, and reported wavelengths from 323.47 to 882.29 nm. The slave unit had a bandwidth of 550 to 1000 nm with a GG475 Filter, a grating of 600 lines, and reported light wavelengths from 507.83 to 1020.82 nm. The spectrophotometer in combination with a 200 µm collecting fiber from the probe had a reported resolution of 2 to 3 nm.

Probe. The fibers at the distal end of the fiber optic probe were configured in a 6-1 bull's eye design as shown in figure 1a. Six 200 µm diameter fibers supplied light from a tungsten halogen light source (LS-1, Ocean Optics, Inc., Dunedin, Fla.) and one 200 µm fiber carried the reflected light back to a fiber splitter (fig. 1b). This probe configuration allowed diffuse reflection to be collected while excluding specular reflection at fiber-milk interface. The 200 µm fiber splitter divided the light for simultaneous measurement by the master and slave spectrophotometer detectors (fig. 1b). Optical fibers were of high water content, SR or HOH, for working in the ultraviolet-visual region. Spectrophotometer gratings and software settings compensated for measurements in the very near-infrared region (750-1000 nm).

Data Acquisition Equipment. The spectrophotometer was connected to an analog to digital conversion (ADC) board (CIO-AD16 Jr., Computer Boards, Inc., Mansfield, Mass.) installed in a 486-33 MHz computer (Gateway 2000, North Sioux City, S.Dak.). The data acquisition software (SS.EXE of SpectraScope, Ver. 2.2 (8/94), Ocean Optics, Inc., Dunedin, Fla.) ran the ADC expansion board with a sampling frequency of 75 kHz. Raw data and scope mode (file extensions *sam* and *ssm*, respectively) files were used for analysis. The settings were adjusted for a long CCD sensor integration period (32768/2*ADC rate), unity gain, and settings corrected for dark to obtain a response signal approximately 50% of full scale (2000 of 4096 bits).

Spectral Scans

An average of 10 individual scans taken in succession and collected in approximately five seconds were recorded for each scan. Three recorded scans were taken of each sample and averaged to give the sample spectrum, $S(\lambda)$.

A reference spectrum, $S_{REF}(\lambda)$, was necessary for making comparisons between the master and slave spectrophotometers. Obtaining a consistent reference spectrum required a constant medium. A Teflon sheet with a thickness of 0.1 cm placed on a Teflon block 10 cm long was chosen. The reference spectrum for Teflon was similar to that of milk.

The dark spectral scan, $S_D(\lambda)$, was measured by placing the probe in a dark environment. The dark scan measured instrument error and light cross-over in the fiber bundle and was treated as an offset error. Diffuse reflectance, $DR(\lambda)$, was calculated as:

$$DR(\lambda) = [S(\lambda) - S_D(\lambda)]/[S_{REF}(\lambda) - S_D(\lambda)]$$

The measured diffuse reflectance scans between light wavelengths of 320 and 1000 nm were averaged into sixty-nine 10 nm bands to reduce the number of data. Tungsten halogen bulbs have decreased emission intensity below 350 nm (de Galan, 1971), which resulted in low reflectance energy below 350 nm. Therefore data below 350 nm was eliminated from scans. Diffuse reflectance data from 350 nm to 1000 nm was analyzed.

Analysis of variance was performed on the diffuse reflectance data to determine the effects of milk pH, fat concentration, protein concentration, and calcium addition on diffuse reflectance. Linear regression with MAXR selection (SAS, 1988) was performed to determine if selected wavelengths could be used to indicate milk pH.

Results and Discussion

A typical plot of diffuse reflectance for milk at various pH levels is shown in figure 2. Diffuse reflectance appeared to decrease in a quadratic fashion for light wavelengths between 350 and 520 nm and between 950 and 990 nm as pH increased. However, diffuse reflectance appeared to decrease linearly with increasing pH levels at wavelengths between 550 and 850 nm.

Diffuse reflectance profiles for milk samples were averaged over each replication as shown in figure 3. The consistent difference in diffuse reflectance between the replications was attributed to length of time to condense milk. Because of difficulties with the condenser, the time required to condense milk for Rep 1 took 14 h as compared to 2.5 h for Rep 2. This observed drop in diffuse reflectance with heat treatment is consistent with the findings of Walstra and Jenness (1984) who reported that diffuse reflectance decreased with a mild heat treatment and that a more severe treatment resulted in Maillard browning reactions. Consequently, the significant

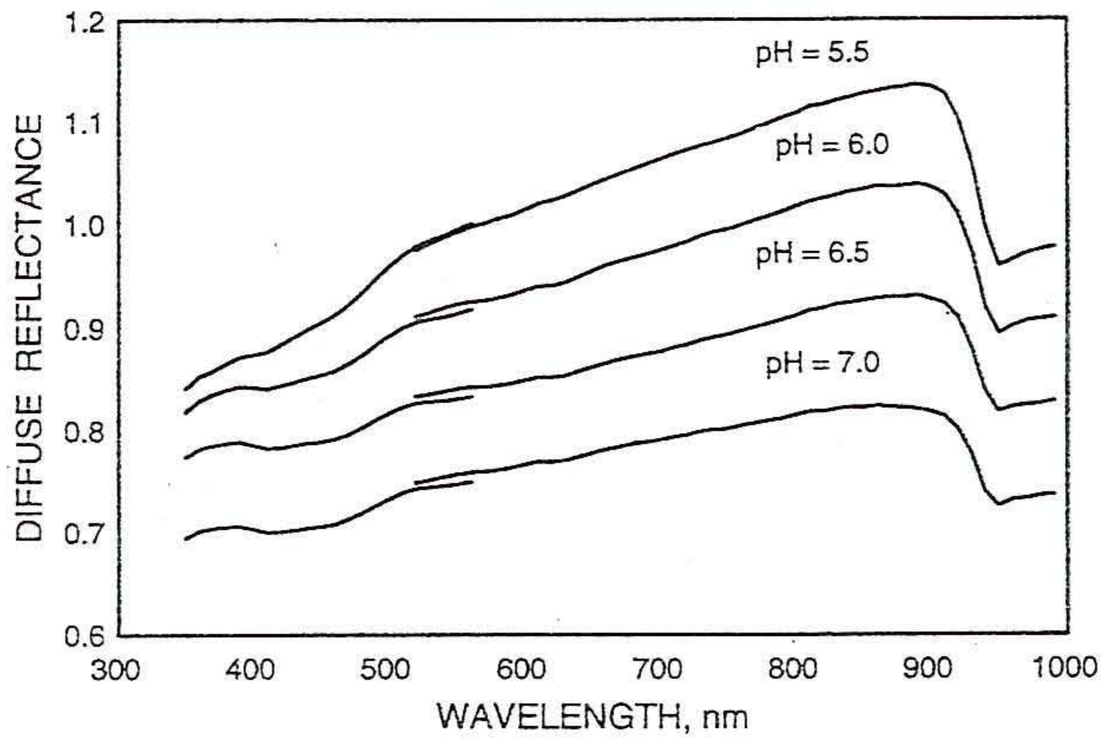

Figure 2–Typical plot of spectrums at pH levels 5.5, 6.0, 6.5, and 7.0. Fat, protein, and calcium at 1.0%, 5.3%, and 0.03 mmol, respectively.

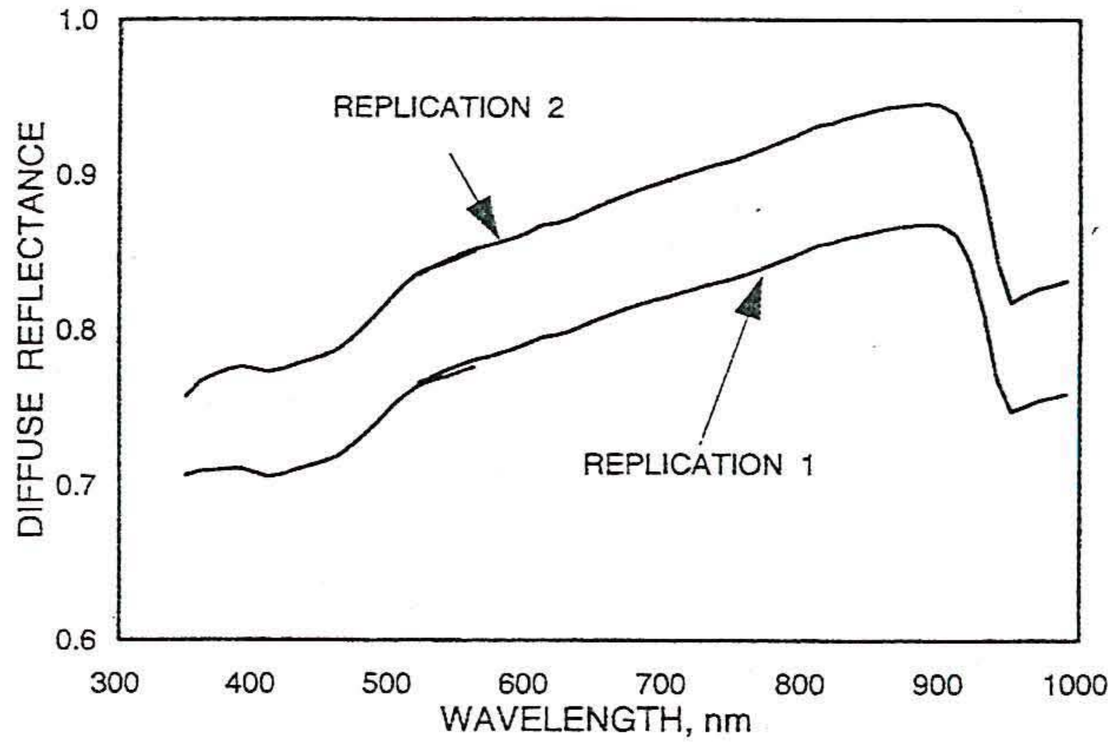

Figure 3–Plot of diffuse reflectance spectrums averaged for each replication. Replication 1 had a condensing time of 14 h and replication 2 had a condensing time of 2.5 h.

replication effect in the statistical analyses that follow is attributed to the different condensation times.

It was believed that the mixing required during heating of the sample and pH adjustment may have resulted in some fat deposition on the probe and fat agglomeration in the milk. The result was some reflectance profiles higher than normal (outliers). Thirty of the 144 diffuse reflectance scans were judged to be outliers and removed from the data set. Criteria for outlier determination included a diffuse reflectance at 900 nm which was 1.25 to 2.5 times greater than typical scans (fig. 2). Twenty-seven outliers were at 4% fat and one outlier was at 1% fat concentrations. When the probe was removed, fat globules had collected over the probe, causing a distortion in the scan. Two other scans had an unusually shaped diffuse reflectance spectrum and were considered outliers.

Analysis of Variance

Average diffuse reflectance (ADR) was calculated by averaging the diffuse reflectance, $DR(\lambda)$, spectrum between 350 and 990 nm. The general linear model procedure (PROC GLM) in the SAS statistical program (SAS,1988) was used to analyze ADR to examine the effects of treatment factors, their two-way interactions, main effect of replication (condensing time), and its first and second order interactions with fat concentration, protein concentration, calcium addition, and target pH. The main plot (fat*protein), sub-plot (calcium), and sub-sub plot (target pH) errors were pooled. From preliminary results, it was observed that the differences caused by three-, four-, and five-way interactions were insignificant and therefore the errors were pooled.

Milk condensation time (replication) had a large effect on diffuse reflectance over the spectrum tested as shown in figure 3. Each line is the average of all tests in replication 1 and 2, respectively. An analysis of variance was conducted on the data with time to condense (replication) as a covariant to determine if the same effects were observed irrespective of replication. Results of the analysis of variance are listed in table 2. Main effects of fat, protein, target pH and replication were highly significant ($p < 0.01$). Thus, milk fat, protein content, and pH affect the diffuse reflectance of milk in the wavelength range of 350 to

Table 2. Analysis of variance and mean squares for 2 × 3 factorial split-split-plot design for dependent variable average diffuse reflectance, ADR (analyzed with replication as a covariant)

Source	df	Mean Square
Model	32	0.0431**
Replication	1	0.0965**
Whole plot		
Fat	1	0.3668**
Protein	2	0.1195**
Fat × protein	2	0.00603**
Split plot		
Calcium	2	0.00033 ns
Fat × calcium	2	0.00019 ns
Protein × calcium	4	0.00331*
Split-split plot		
Target pH	3	0.1184**
Calcium × target pH	6	0.00110 ns
Fat × target pH	3	0.00373*
Protein × target pH	6	0.00283*
Experimental error	81	0.00111
Total (less 30 outliers)	113	
R^2 0.939		
CV 4.04 %		

** $p < 0.01$.
* $p < 0.05$.
ns not significant at the 0.05 level.
NOTE: Three-, four-, and five-way interactions assumed to be insignificant in explaining variance of ADR.

990 nm. The two-way interaction of fat × protein was highly significant ($p < 0.01$). The two-way interactions of fat × target pH, protein × target pH and protein × calcium were also significant ($p < 0.05$). Note, however, that all interactions except protein × calcium are insignificant when using observed pH, fat, and protein concentrations instead of target values, as described in next section.

Analysis of Interactions

Effects of significant factors and interactions were explained using plots of averages, least square means, and observed pH instead of target pH levels. Least square means were used because the data set was unbalanced as a result of removing outliers. Interactions were determined to be insignificant by regressing ADR against observed values and using a t-test to compare slopes (Aaron, 1993). Figure 4 illustrates the interaction of protein × observed pH to be insignificant, as determined by nearly parallel slopes. Similarly, two-way interactions between fat × observed pH and fat × protein can be shown to be insignificant using observed values rather than class levels. The two-way interaction of protein × calcium was significant ($p < 0.05$) as shown in table 2. Thus, calcium and protein interacted to produce a detectable effect on diffuse reflectance.

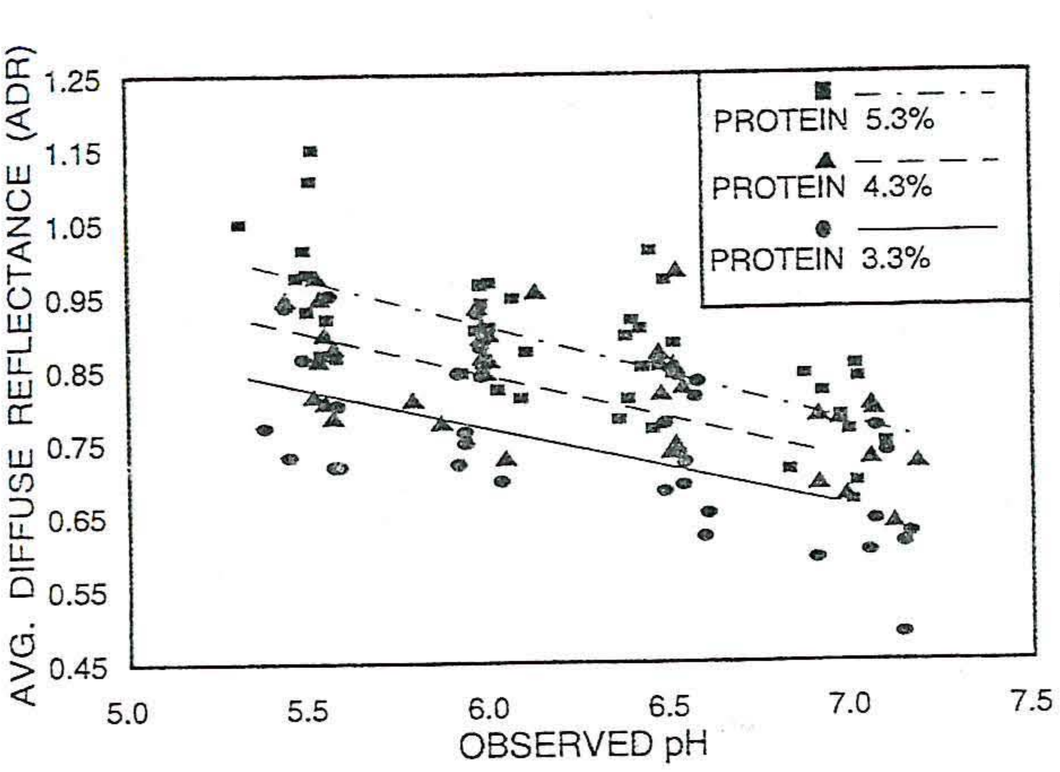

Figure 4–Plot of average diffuse reflectance (ADR) as a function of observed pH with linear regression lines for each protein level.

Model Development

Several linear models were tested with the objective of predicting milk pH with as few selected reflectance parameters and as few milk composition characteristics as possible. Model development was performed using a MAXR option in the PROC REG procedure (SAS, 1988). Regression analysis was performed with the observed pH as the dependent variable and one of the following groups of regressors: (1) protein, fat, calcium, replication, and spectrum parameters; or (2) replication and spectrum parameters. Spectrum parameters include diffuse reflectance [$DR(\lambda)$], quadratic diffuse reflectance terms {$DRQ(\lambda) = [DR(\lambda)]^2$}, and reflectance ratios [$RR(\lambda_i/\lambda_j) = DR(\lambda_i)/DR(\lambda_j)$].

Summary results of model development are presented in table 3. Obtaining a model in which fat and protein concentrations and condensing time were not required was preferable. Models which used replication (or condensing time) with linear, quadratic, or reflectance ratio terms (Linear Model 1, Quadratic Model 1, Reflectance Ratio Models 1 and 2) had an R^2 value (regression fit) between 0.34 and 0.44 and SEPs (standard error of prediction) between 0.46 and 0.42 pH units (Lochte, 1995). A fifth model, Combined Model 1, in which a quadratic term, DRQ (350), and reflectance ratio, RR (560/760), were used without replication improved R^2 and SEP to 0.51 and 0.40 pH units, respectively. This was an improvement over Linear Model 1, Quadratic Model 1, and Reflectance Ratio Models 1 and 2. Combined Model 1 suggests that only three LEDs in the wavelength range of 350, 560, and 760 would be sufficient to support a pH monitoring device, if fat and protein concentrations and pretreatment are consistent.

Models which included fat and protein as independent parameters in the regression had higher R^2 values. Combined Model 2 (table 3) had an R^2 of 0.74 and an SEP of 0.29 pH units which include terms for replication (condensing time), fat, protein, DRQ(360), and RR(390/410). Plots of predicted pH and residual pH for this model versus observed pH are shown in figures 5a and 5b, respectively.

All models tested tended to over-predict at lower pH levels and to underpredict at higher pH levels. Over-fitting of data was apparent in all models as additional terms were added.

Conclusions

Diffuse reflectance of milk varied with pH, fat and protein concentration, milk pretreatment, and the interaction of calcium and protein. Diffuse reflectance increased linearly with protein and fat and decreased with pH. A significant difference in diffuse reflectance was

Table 3. Summary of pH prediction models considered

General Model: $pH = \beta_0 + \beta_1 \times Rep + \beta_2 \times Fat + \beta_3 \times Protein + \beta_4 \times A + \beta_5 \times B + \beta_6 \times C + \beta_7 \times D + \varepsilon^*$

Model	R^2	SEP	β_0 (se)	Rep β_1 (se)	Fat β_2 (se)	Protein β_3 (se)	A β_4 (se)	B β_5 (se)	C β_6 (se)	D β_7 (se)
Linear Model 1							DR(350)†			
	0.425	0.428	9.40 (0.368)	0.225 (0.085)			−4.77 (0.527)			
Linear Model 2							DR(500)			
	0.721	0.301	8.80 (0.224)	0.382 (0.061)	0.222 (0.025)	0.431 (0.043)	−6.99 (0.418)			
Quadratic Model 1							DRQ(350)‡			
	0.437	0.423	7.75 (0.203)	0.247 (0.084)			−3.47 (0.374)			
Quadratic Model 2							DRQ(470)			
	0.718	0.302	6.21 (0.172)	0.420 (0.063)	0.203 (0.024)	0.419 (0.043)	−4.88 (0.294)			
Reflectance Ratio Model 1						RR(390/410)§		RR(360/460)		
	0.339	0.459	−201 (30.4)					248 (37.2)	−43.0 (7.36)	
Reflectance Ratio Model 2						RR(390/410)		RR(350/460)	RR(620/990)	
	0.407	0.436	−164.4 (22.9)					171 (26.5)	−18.3 (4.23)	15.6 (3.75)
Combined Model 1							DRQ(350)	RR(560/760)		
	0.508	0.395	−0.270^{ns} (2.00)				−3.75 (0.375)	8.71 (2.14)		
Combined Model 2							DRQ(360)	RR(390/410)		
	0.744	0.287	−31.3 (6.52)	0.466 (0.061)	0.199 (0.024)	0.402 (0.040)	−5.11 (0.345)	37.3 (6.36)		

* Variable A is for linear or quadratic diffuse reflectance terms. Variables B, C, and D are for reflectance ratio terms.
† DR(λ) – linear term of diffuse reflectance at wavelength λ.
‡ DRQ(λ) – quadratic term of diffuse reflectance at wavelength λ.
§ RR (λ_i/λ_j) – reflectance ratio of diffuse reflectance at wavelength λ_i over diffuse reflectance at wavelength λ_j.
SEP standard error of prediction (pH units).
ns coefficient not significant at p = 0.05 level.
se standard error.

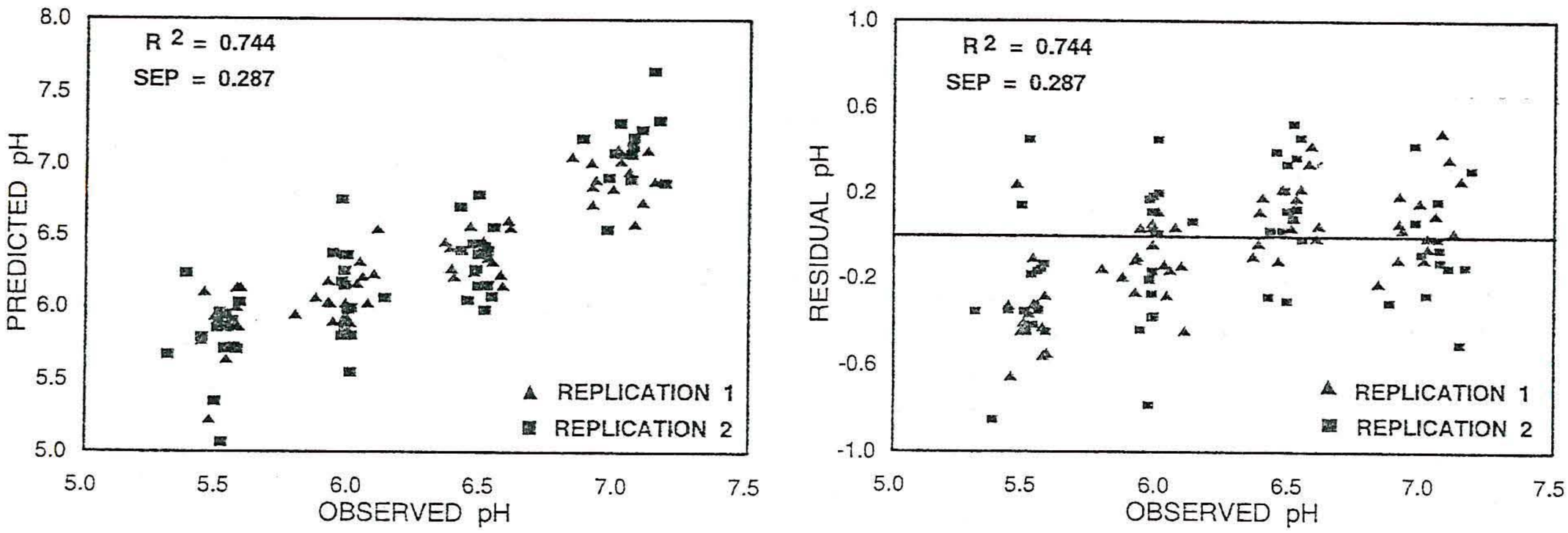

Figure 5–Plot of (a) predicted pH, and (b) residual pH vs observed pH for Combined Model 2: $pH = \beta_0 + \beta_1 \times$ replication $+ \beta_2 \times$ fat $+ \beta_3 \times$ protein $+ \beta_4 \times$ DRQ(360) $+ \beta_5 \times$ RR(390/410) $+ \varepsilon$.

measured for different condensing times, suggesting that milk pre-treatments such as separation, homogenization, storage, condenser temperature and time, and thermal treatment affect diffuse reflectance. These results suggest that the measurement of diffuse reflectance to predict pH has limited potential. However, for processes such as vat culture of cottage cheese and yogurt where protein, fat, milk pretreatment, and calcium are constant, the use of diffuse reflectance as an indicator of pH change has potential and should be investigated further.

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REFERENCES

Aaron, D. K. 1993. Personal Communication. Dept. of Animal Science, University of Kentucky, Lexington, Ky.

Ashworth, U. S. 1969. Turbimetric methods for measuring fat content of homogenized milk. *J. Dairy Sci.* 52:262-263.

Ben-Gera, I., and K. H. Norris. 1968. Influence of fat concentration on the absorption spectrum of milk in the near-infrared region. *Israel J. Agric. Res.* 18(3):117-124.

Brunner, J. R. 1976. *Characteristics of edible fluids of animal origin: Milk. Principles of Food Science. Part I. Food Chemistry*, ed. O. R. Fennema. New York, N.Y.: Marcel Dekker Inc.

Casal, H. L., U. Kohler, and H. H. Mantsch. 1988. Structural and conformational changes of β-lactoglobulin B: An infrared spectroscopic study of the effect of pH and temperature. *Biochim. Biophys. Acta* 957:11-20.

de Galan, L. 1971. *Analytical Spectrometry*. London, England: Adam Hilger Ltd.

Flux, D. S., J. A. Raven, and I. K. Gray. 1982. Accuracy of the Milko-Tester over a wide range of milkfat concentrations. *New Zealand J. Dairy Sci.* 17:15-25.

Fox, P. F. 1987. *Cheese: Chemistry, Physics and Microbiology,* Vol. 1. London, England: Elsevier Applied Science.

Goulden, J. D. S. 1957. Diffuse reflexion spectra of dairy products in the near infra-red region. *J. Dairy Res.* 24:242-251.

Goulden, J. D. S., and P. Sherman. 1962. A simple spectroturbidimetric method for the determination of the fat content of homogenized ice-cream mixes. *J. Dairy Res.* 29:47-53.

Gruen, L. C., and Z. J. Tao. 1985. Time-dependent ultraviolet absorption changes in proteins at high pH. *Int. J. Peptide Protein Res.* 26:381-389.

Haugaard, G. 1966. Photometric determination of fat in milk. *J. Dairy Sci.* 49:1185-1189.

Jeunet, R., and R. Grappin. 1970. A note on the relationship between the refractive index of milk fat and the precision of determination of fat by means of the Milko-tester. *Lait.* 50:654-657.

Kamishikiryo-Yamashita, H., Y. Oritani, H. Takamura, and T. Matoba. 1994. Protein content in milk by near-infrared spectroscopy. *J. Food Sci.* 59(2):313-315.

Kuaye, A. Y. 1994. An ultraviolet spectrophotometric method to determine milk protein content in alkaline medium. *Food Chem.* 49:207-211.

Lochte, K. R. 1995. *Effects of Milk pH on Diffuse Reflectance.* Unpub. M.S. thesis. Lexington, Ky.: University of Kentucky.

Morr, C. V. 1967. Effect of oxalate and urea upon ultra centrifugation properties of raw and heated skim milk casein micelles. *J. Dairy Sci.* 50:1744-1751.

Payne, F. A., C. L. Hicks, S. Madangopal, and S. A. Shearer. 1993. Fiber optic sensor for predicting the cutting time of coagulating milk for cheese production. *Transactions of the ASAE* 36(3): 841-847.

Richardson, G. H. 1985. *Standard Methods for the Examination of Dairy Products*. New York, N.Y.: Am. Public Health Assoc.

SAS®. 1988. Rel. 6.03 Ed. *SAS/STAT ® User's Guide.* Cary, N.C.: SAS Institute Inc.

Schmidt, D. G. 1982. Association of caseins and casein micelle structure. In *Developments in Dairy Chemistry*, Vol. 1:61-82, ed. P. F. Fox. Essex, England: Applied Science Pub.

Slattery, C. W., and R. Evard. 1973. A model for the formation and structure of casein micelles from subunits of variable composition. *Biochim. Biophys. Acta* 317:529-538.

Tamime, A. Y., and R. K. Robinson. 1985. *Yoghurt Science and Technology*. New York, N.Y.: Pergamon Press.

Tanford, C. 1968. Protein denaturation. In *Advances in Protein Chemistry,* Vol. 23:121-282, eds. C. B. Anfinsen, M. L. Anson, J. T. Edsall, and F. M. Richards. New York, N.Y.: Academic Press.

Van Hooydonk, A. C. M., H. G. Hagedoorn, and I. J. Boerrigter. 1986. pH-induced physico-chemical changes of casein micelles in milk and their effect on renneting. 1. Effect of acidification on physico-chemical properties. *Neth. Milk Dairy J.* 40:281-296.

Visser, J., A. Minihan, P. Smits, S. B. Tjan, and I. Heertje. 1986. Effects of pH and temperature on the milk salt system. *Neth. Milk Dairy J.* 40:351-368.

Walstra, P. 1967. Turbidimetric method for milk fat determination. *J. Dairy Sci.* 50:1839-1840.

Walstra, P. and R. Jenness. 1984. *Dairy Chemistry and Physics*. New York, N.Y.: John Wiley & Sons.

Wong, N. P., R. Jenness, M. Keeney, and E. H. Marth. 1988. 3rd Ed. *Fundamentals of Dairy Chemistry*. New York, N.Y.: Van Nostrand Reinhold Co.

NOMENCLATURE

ADR	Average diffuse reflectance
Ca	Calcium addition (mmol/g protein)
DR (λ)	Diffuse reflectance term at wavelength λ
DRQ (λ)	Quadratic diffuse reflectance term at wavelength λ
Rep	Replication
RR (λ_i/λ_j)	Reflectance ratio of diffuse reflectance at wavelength λ_i divided by diffuse reflectance at wavelength λ_j
S	Average of three scans for each sample
S_D	Average of three scans for dark spectrums
S_{REF}	Average of three scans for reference spectrums
β_0	Predicted pH intercept for general model.
β_1, β_2, and β_3	Coefficients for the significant variables; replication, fat, and protein, respectively, in model determination
β_4	Coefficients for significant linear or quadratic diffuse reflectance terms in model determination
β_5, β_6, and β_7	Coefficients for significant reflectance ratios in model determination
λ, λ_i, and λ_j	Wavelengths (nm)