Influence of Temperature on Estimation of Protein and Moisture in Wheat by Near-Infrared Reflectance[1]

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ABSTRACT

After 35 wheat samples (25 g) were ground successively on the Cyclotec grinder (recommended for near-infrared reflectance spectroscopy [NIRS] analysis), the temperature of the ground sample rose from room temperature (~22°C) to about 40°C. The influence of this rise in temperature on NIR analyses was investigated using NIRS instrumentation that was calibrated with samples at equilibrium room temperature. The instrument was calibrated with seven series of samples ranging in temperature from 45 to −10°C. This temperature range caused serious discrepancies in the analytical results. Samples at −10°C gave readings that were 1% higher than Kjeldahl, whereas the NIRS protein readings at 45°C were 0.5% lower than the true results when both series of samples were read at room temperature calibration. Discrepancies for samples of intermediate temperature fell between these extremes. Analysis verified that these discrepancies were not caused by changes in the moisture content incurred during grinding but were strictly a function of temperature change. The phenomenon appeared to be independent of algorithm. A simple, practicable, inexpensive procedure was developed for correction of the results to ambient room temperature in grain elevators.

The temperature of hard red spring (HRS) wheat delivered to grain elevators in Canada and the northern United States varies from −40 to 40°C, depending on the time of year the wheat is delivered. To obtain the most satisfactory results for protein testing by near-infrared reflectance spectroscopy (NIRS), instrument manufacturers and other workers (Hunt et al 1978, Williams 1975) have recommended that the wheat be ground before analysis by a high-speed hammer mill fitted with a 1.00-mm screen. The Cyclotec sample mill, which is used for NIRS protein testing of HRS wheat in Canada, operates at 12,000–13,000 rpm and forces the meal through a 1.00-mm screen. The air flow then passes through a plastic vane, and the resulting vortex forces the ground meal into the receiving jar. The grinding action is fairly severe and results in loss of moisture (Williams and Sigurdson 1975) and heating of the sample. Nevertheless, extensive studies have verified that the Cyclotec grinder is the most practical for use in NIRS analysis; it is cheaper and faster to operate than a hammer mill and gives excellent results. The present study was instigated to establish the changes in temperature of HRS wheat of different temperatures during grinding on the Cyclotec mill, as well as to study the effects of the temperature of the freshly ground sample on the accuracy of testing HRS wheat for protein and moisture.

MATERIALS AND METHODS

All wheat samples were ground on a Cyclotec grinder (U-D Corporation, Boulder, CO) fitted with a 1.0-mm screen. Kjeldahl protein was determined using the standard AACC procedure (1976). Moisture in whole grain was determined using the model 919 (Motomco) meter, and in the ground whole meal using the single-stage air-oven method (AACC 1976). Temperature of ground whole meals was measured using a thermistor thermometer fitted with a flat banjo type of probe.

Temperature Measurement and NIRS Analysis of the Freshly Ground Samples. Analysis was performed under conditions similar to those in a high throughput elevator. The samples were ground in the Cyclotec grinder and mixed thoroughly. Immediately after grinding and mixing, the sample cell of an NIR instrument was filled and the temperature probe inserted into the residual ground sample remaining in the grinder jar. The cell contents were made level, and the cell was capped, brushed clean, and placed into the instrument. Protein and moisture levels were then read. The result was converted to constant moisture basis. The temperature of the sample in the grinder jar was then recorded. This procedure was repeated for 50 samples and the temperature recorded for each sample.

To assess the rise in temperature caused by grinding very cold wheat, 12 samples of HRS wheat were placed outdoors during February 1978. The overnight temperature fell to −35° C. In the morning the cold samples were brought inside singly in an insulated container at intervals of 5–6 min, ground, and analyzed. This simulated the rate of arrival of railcars at a terminal elevator. This study was repeated on successive days. On both nights the temperature of the samples dropped to about −35° C. Using a standard room temperature calibration, the protein content of each sample of wheat was determined by NIR analysis at the different temperature levels obtained in the samples as the grinder became heated.

Calibration at Different Temperature Levels. A series of samples (100) was selected to provide a uniform distribution of protein (9.8–17.6%) and of moisture (10–18% in the whole wheat). Ground samples of 50 g were obtained by grinding 2 × 25-g subsamples through the Cyclotec grinder and thoroughly mixing the combined whole meals. The ground material was stored in cans, taped to restrict moisture loss, and tested in duplicate for protein by Kjeldahl, and for moisture by the air-oven method. Fifty of the samples covering the entire protein and moisture ranges were then used to calibrate a Neotec model GQA 31EL NIR analyzer, using two math treatments, delta log 1/R, and normalized delta log 1/R (delta log 1/R divided by delta log 1/R) (AACC 1976). The remaining 50 samples, which also encompassed the full moisture and protein ranges, were analyzed as "unknowns." This calibration is referred to as the room temperature (RT) calibration. The ambient temperature was about 22°C (72°F). Temperature changes in the ground samples were attained by overnight equilibration of the same samples in well-taped cans at the required temperatures, using a refrigerator or an oven. Use of the taped metal storage containers kept moisture changes to a minimum. Further calibrations were performed at −10, 5, 30, 35, 40, and 45°C with reciprocal analysis of unknowns on the respective calibrations.

Instrument/Temperature Interactions. The influence of temperature of the ground sample was extended to the DICKEY-john Grain Analysis Computer model III (GAC III) and the Technicon InfraAlyzer model 2.5 to assess the influence of temperature on different instruments, as distinct from different algorithms within an instrument.

Spectral Studies of Temperature Effects. The spectral traces of wheat, wheat protein, and water were run at 30 and 10°C. The difference of 20°C was sufficient to display any existing differences in spectra. Spectra of wheat at 10°C were subtracted by

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computer from spectra run at 30° C. Condensation of free water on the surface of the cell made preparation of NIR traces of samples at very low temperatures impracticable.

RESULTS AND DISCUSSION

Differences in Spectra of Wheat

Spectra run at different temperatures are illustrated in Figs. 1 and 2. These were obtained by computerized data manipulation that allowed us to subtract the spectrum of a sample read at 10° C from that of the same sample, having been elevated to 30° C at the time of recording. As a result, areas of maximum difference from zero reflectance indicate areas where measurements of the log 1/R signal are at their maximum sensitivity to temperature fluctuations. Similarly, areas of the spectrum where the spectra are close to or at zero indicate wavelengths of least sensitivity to temperature. The wavelengths used to measure protein by any algorithm using the log 1/R or a derivative thereof by commercial instruments are 2.14–2.19 μm (2,140–2,190 nm). Figure 1 illustrates the NIR spectrum of wheat gluten between 1,000 and 2,500 nm. At 2,180 nm, the log 1/R temperature difference curve is almost zero.

Theoretically, an instrument that makes a measurement for protein at 2,180 nm is relatively insensitive to temperature fluctuations. Conversely an instrument that takes a measurement from about 2,145 through 2,160, around 2,165, or upwards from about 2,190 may become sensitive to temperature fluctuations. The main wavelengths used by commercial NIR instruments are indicated in Table I. The Neotec Grain Quality Analyzer uses a wavelength around 2,163 for one of the wavelengths in protein measurement, and because of the optical system and method of wavelength or pulse-point selection, the actual wavelength varies slightly among instruments. Thus the instrument is fairly sensitive to temperature. Correlations between optical data and temperature are summarized in Table II. High correlations occurred between the GQA 31EL protein optical density and temperature for original and normalized mathematics (matrix board 3 on the GQA 31EL used the algorithm delta log 1/R divided by delta log $1/R_1$). Optical data for oil and water channels were not highly correlated with temperature, suggesting that the temperature effect would not be so pronounced with those constituents. The sensitivity appeared quite small in the magnitude of the log 1/R signal, but differences in spectra between wheat samples of different protein content are also relatively small. The errors observed in protein by the GQA were about 0.1% protein for every 5° C fluctuation in temperature and could be accounted for by the sensitivity to temperature indicated by the spectra in the wavelength area used to make the measurement. Small differences in the actual wavelength used in different instruments accounts for differences in relative sensitivity between instruments. Sensitivity of the Technicon InfraAlyzer and DICKEY-john GAC III is lower because these instruments use a simpler log 1/R algorithm with discreet wavelengths and no delta treatment. Different sensitivities at the individual wavelengths tend to compensate for each other. The 2,179-nm wavelength itself is only slightly less sensitive to fluctuations in temperature. In measurement of moisture, none of the commercial instruments tested displayed excessive sensitivity to temperature. The spectra at 10 and 30° C indicated minimum sensitivity at about 1,870 and 1,975 nm and maximum sensitivity at 1,922 nm. The GQA uses wavelengths at about 1,932 and 1,869 nm for the measurement of moisture, and the InfraAlyzer and GAC III use the 1,940 wavelength. The wavelengths used by all commercial instruments appear to be in areas where wheat spectra show high sensitivity to temperature (Fig. 2). Variations in log 1/R due to

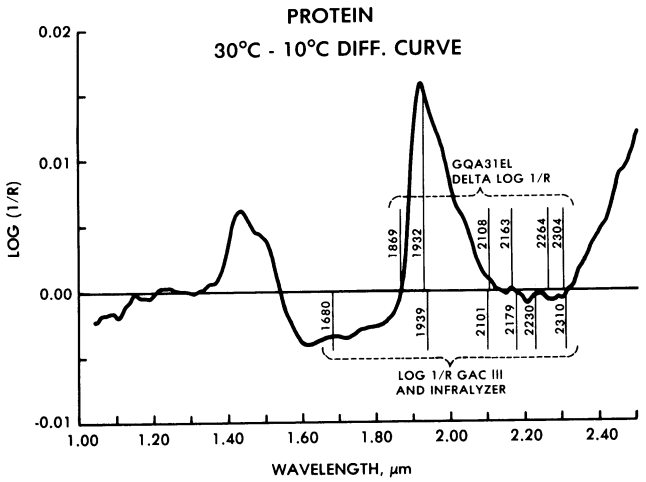

Fig. 1. Spectrum of difference between wheat proteins at different temperatures.

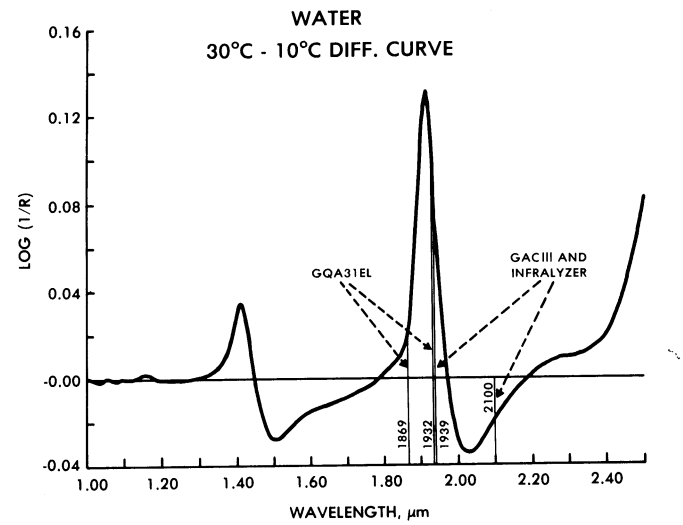

Fig. 2. Spectrum of difference between moisture levels at different temperatures.

TABLE I
Wavelengths Used by Commercial Near-Infrared Reflectance Instruments in the Measurement of Protein and Moisture[a]

Grain	Neotec Quality Analyzer 31EL	InfraAlyzer and DICKEY-john Grain Analysis Computer
2304	Delta log 1/R	2310
2264		2230
2163	Delta log 1/R	2179
2108		2101
1932	Delta log 1/R	1939
1869		1680

[a] All wavelengths in nanometers.

TABLE II
Correlation and Regression Statistics Between Log 1/R Sign of Grain Quality Analyzer 31EL and 101 with Sample Temperature

Comparison	r[a]	b	c
C_1:temp.[b]	−0.242	−0.0056	−44.72
C_2:temp.	0.967	0.0551	43.47
C_3:temp.	0.308	0.00874	−65.55
C_1/C_2:temp.[c]	0.932	0.0369	32.74

[a] r = coefficient of correlation, b = coefficient of regression (slope), and c = intercept.

[b] C_1, C_2, and C_3 are the optical delta log 1/R signals of the GQA 31EL for, respectively, the oil, protein, and moisture channels.

[c] C_1/C_2 is the normalized delta log 1/R divided by delta log $1/R_1$ algorithm used on matrix board 3 of the GQA 31EL (and also more recently on the GQA 101 analyzer). In this case C_1 is the primary protein wavelength and C_2 is the normalizing wavelength.

moisture are considerably larger than those caused by variations in protein (Norris and Williams 1977). The disturbances in log 1/R spectra caused by temperature at the wavelengths of measurement relative to the much larger variations caused by actual differences in moisture appeared to render the instruments less sensitive to temperature in the measurement of moisture. This is the reverse of the effect observed with the GQA 31 with regard to protein measurement, where the small differences in the magnitude of the log 1/R signal caused by temperature at sensitive wavelengths are much larger in proportion to the differences in log 1/R caused by the protein itself and are apparently not completely compensated for by the respective regression coefficients.

The sensitivity to temperature in protein measurement can be corrected by calibration at different temperatures. The success of a calibration depends on the relationship between the wavelengths at which measurements are made and on the magnitude of the regression coefficients. If wavelengths with low temperature sensitivity are used, sensitivity to other variables such as moisture content may be increased. As a precaution against this, optimization of wavelengths to both temperature and moisture (and other variables if necessary) can be verified by computerized spectrophotometer by simulation before transposition to an actual instrument.

Temperature Measurement of Ground Samples

Sequential Grinding at Room Temperature. The results of sequential grinding are summarized in Table III. Temperature of the first ground sample rose to 26° C. Thereafter, the temperature of the samples rose progressively until 20–25 samples were ground, when the temperature leveled off at about 40° C. If a sound-suppression cabinet was used with the grinder, the temperatures rose more abruptly to a maximum of 45° C. The temperature of the ground samples rose by about 1° C per sample until 20–25 samples had been ground, after which the temperature stabilized. The implications of these figures in an elevator operation are that when truckloads or railcar loads are being unloaded into the elevator, the temperature of the ground samples will likely rise to 5–25° C above ambient. In routine testing operations in which samples are being continuously ground and analyzed, the temperature of most of the ground samples are from 20–25° C above ambient.

Sequential Grinding of Ultracold Samples. The grinding of very cold wheat at intervals calculated to simulate an elevator operation resulted in elevation of the temperature of ground wheat to ambient (22° C) or up to 4–5° C above ambient (Table IV). The temperature of wheat ground in the Cyclotic grinder cannot be lower than ambient room temperature, regardless of how cold the wheat is before grinding. Analysis of cold, Cyclotec-ground wheat is unnecessary unless samples of ground wheat have been stored in a refrigerator.

NIR Determination of Protein and Moisture Contents of Cyclotec-Ground Wheat at Different Temperatures. Samples of wheat were ground in sequence and analyzed on the GQA model 31EL NIR instrument, calibrated for normal analysis of HRS wheat. The entire experiment was duplicated on successive days. The results are summarized in Table V. In each case, the apparent protein content of the wheat fell progressively as the temperature rose. On the other hand the moisture values remained fairly constant, showing that the phenomenon was not caused by progressive changes in the moisture status of the samples. For any given sample, the apparent protein result could vary as much as 0.5%, depending on when the sample was analyzed during a given day. Similarly, if the results of testing a given sample are compared with a repeat analysis of the same sample, a significant discrepancy might occur. Although the reason for the discrepancies is likely to be assigned to instrument or grinder variability, the discrepancies can arise simply as a result of the time of day that the respective analyses were performed.

Calibration at Different Temperature Levels. Results of this study are contained in Tables VI–IX. Calibration of the GQA 31EL with ground wheat of different temperatures, followed by intercept adjustment and analysis of unknowns at the corresponding temperatures gave very consistent results (Table VI). However, analysis of wheat of different temperatures on a single calibration, as room temperature calibration (22° C), showed discrepancies in apparent protein results that were highly significant (Table VII). The converse was true when room temperature samples were analyzed on calibrations generated from samples of different temperatures and the protein results varied in the opposite direction (Table VIII). Moisture results were not affected, and the variance displayed in moisture results was not abnormal. Both delta log 1/R and the normalized algorithm (delta log 1/R divided by delta log $1/R_1$) protein data showed the same trend, with protein results falling as the temperature of the ground sample rose. The

TABLE III
Temperature of Hard Red Spring Whole Meal After Grinding in Cyclotec Grinder Equipped with a Sound-Suppression Cabinet[a]

Sample No.	Without Cabinet (° C)	With Cabinet (° C)
1	26	26
6	31	34
16	37	36
26	38	41
36	39	44
46	40	45

[a] All samples at room temperature at commencement.

TABLE IV
Temperature of Hard Red Spring Whole Meal after Grinding in Cyclotec Grinder[a]

Sample No.	Chilled Temperature (° C)	Room Temperature (° C)
1	23	27
2	26	30
3	26	32
4	27	33
5	27	34
6	29	34
12	35	37

[a] Subsamples chilled to −35° C or at room temperature at commencement. Sound cabinet not used.

TABLE V
Mean Protein and Moisture Contents of Wheat Samples Sequentially Ground to Produce Whole Meals of Different Temperatures[a]

Temperature (° C)	NIR[b] Protein (OM[c])	NIR Moisture	NIR Protein (NM)	Kjeldahl Protein	Oven Moisture
23	13.5	11.2	13.4	13.4	11.1
25	13.4	11.2	13.2	13.4	11.1
28	13.4	11.2	13.3	13.4	11.1
31	13.2	11.1	13.2	13.4	11.1
35	13.1	11.3	13.0	13.4	11.1
39	13.0	11.1	12.9	13.4	11.1

[a] Neotec Grain Quality Analyzer 31EL data.
[b] NIR = near-infrared reflectance.
[c] OM = original mathematical treatment (delta log 1/R). NM = normalized mathematical treatment (delta log 1/R divided by delta log $1/R_1$).

TABLE VI
Analysis of Hard Red Spring Wheat of Different Temperatures at Individual Temperature Calibrations

	Calibrations (° C)						
	−10	5	22 (RT)	30	35	40	45
Protein OM[a]	13.30	13.31	13.26	13.30	13.31	13.29	13.31
Protein NM	13.31	13.31	13.25	13.31	13.31	13.29	13.30
Moisture	11.66	11.65	11.66	11.68	11.67	11.66	11.64

[a] OM = original mathematical treatment (delta log 1/R). NM = normalized treatment (delta log 1/R divided by delta log $1/R_1$).

mean C values (delta log 1/R) for the respective series showed that the trend in protein was matched by a corresponding increase in C_2 values as the temperature progressively rose, whereas no such trend was apparent in the C_1 and C_3 values (Table IX). The discrepancies between apparent protein were regressed against the actual Kjeldahl protein (Williams and Thompson 1978). Correlation and regression coefficients for the various temperature series for the most part were not statistically significant, and correlation coefficients did not exceed 0.47. This indicated that the temperature effects were generally not associated with significant slope changes, and the apparent discrepancies could be corrected by means of intercept adjustment, or, more conveniently, by the application of correction factors since the temperature of individual ground samples would be expected to vary according to the workload.

A multiple calibration was generated using samples from all temperature ranges. When the constants thereby generated were used to compute protein in six series of 12 samples representing different temperature regimes, no significant improvement in accuracy was observed. This is in contrast to other types of variables such as particle size, moisture, growing location, and season, which can be resolved by means of multiple calibrations, based on samples representing all of the variance likely to be encountered.

Application of Temperature Study to Other Instruments. A set of 30 samples was read on a Neotec GQA 31EL using old and normalized math, and also on a DICKEY-john GAC III (Table X).

TABLE VII
Analysis of Hard Red Spring Wheat of Different Temperatures on Constant (Room Temperature) Calibration

	Calibrations (°C)						
	−10	5	22	30	35	40	45
Protein OM[a]	14.03	13.71	13.26	13.20	13.05	12.94	12.80
Protein NM	14.11	13.78	13.25	13.42	13.22	13.09	12.89
Moisture	11.51	11.42	11.66	11.16	11.32	11.39	11.44

[a] OM = original mathematical treatment (delta log 1/R). NM = normalized treatment (delta log 1/R divided by delta log $1/R_1$).

TABLE VIII
Analysis of Samples of Constant (Room) Temperature on Calibrations Made with Samples of Different Temperatures

	Calibrations (°C)							
	−10	5	22	30	35	40	45	Kjeldahl
Protein OM[a]	12.38	12.77	13.07	13.25	13.37	13.45	13.63	13.26
Protein NM	12.35	12.74	13.15	13.04	13.24	13.33	13.49	13.26

[a] OM = original mathematical treatment (delta log 1/R). NM = normalized treatment (delta log 1/R divided by delta log $1/R_1$).

TABLE IX
Delta Log 1/R Values for Grain Quality Analyzer 31EL at Different Temperatures

	Calibration (°C)						
	−10	5	22	30	35	40	45
C_1[a]	−44.82	−44.31	−44.27	−45.00	−45.04	−44.95	−45.01
C_2	43.11	43.52	44.88	45.21	45.78	46.04	46.26
C_3	−65.76	−64.63	−65.06	−64.27	−64.72	−64.79	−64.87
λ_1/λ_2	32.40	33.07	34.14	33.80	34.20	34.48	34.71

[a] C_1, C_2, and C_3 are the optical data obtained on the GQA 31EL for, respectively, the oil, protein, and moisture channels. λ_1/λ_2 is the normalized optical signal of the GQA 31EL. This consists of dividing the optical signal at a primary wavelength (C_1) by a second signal at a dividing or normalizing wavelength (C_1). This algorithm is employed by the GQA 31EL on only one of its three matrix boards, and is an effective method of correcting for excessive particle size and moisture variance in the samples (Norris and Williams 1977, Williams and Norris 1982).

The samples were allowed to reach equilibrium at five different temperature levels before the individual series were read. Protein level fell progressively as the temperature rose, but the magnitude of change was much lower in the GAC III. Samples of the same series were also read on a Technicon InfraAlyzer model 2.5, after allowing the ground samples to come to equilibrium at three different temperatures; facilities at the Vancouver location did not permit the use of more than three temperatures. However, the same trend was apparent, and the apparent drop in protein was of the same magnitude as with the GAC III (Table X). Discrepancies in protein were regressed against temperature levels for the GQA and GAC, and the regression equations used to construct a chart for protein correction at different temperatures (Table XI). Correction for the errors incurred by the temperature factor is applied as follows. The sample is ground and the sample thoroughly mixed. The sample cell of the NIR instrument is loaded and the temperature probe of a thermistor thermometer inserted in the residual sample remaining in the grinder receptacle. The sample cell is then cleaned and the protein and moisture read. The protein result is corrected to the desired moisture level using the NIR protein and moisture figures. The time since the temperature probe is inserted is usually about 30 sec. The temperature is stabilized after 5–9 sec, and the appropriate correction can be applied directly, either from a chart, or by an incremental readout fitted directly to the thermistor display.

CONCLUSIONS

A significant inverse relationship has been demonstrated between the temperature of the ground sample and the apparent protein content, as determined by commercial near-infrared instruments. The effect is believed to be caused by a combination of the wavelengths at which protein measurements are made, and the mathematical treatment employed and is more pronounced in instruments which employ a delta log 1/R algorithm. The problem can be corrected by at least three possible methods. First, the wavelengths used in measurement can be selected by a computerized spectrophotometer to provide minimum sensitivity to temperature. This is the most satisfactory method and has recently been successfully used by Neotec/Pacific Scientific to

TABLE X
Changes in Apparent Protein at Different Temperatures as Tested by Different Instruments[a]

	Temperature (°C)				
Instrument	−10	5	22	40	45
GQA OM[b]	.58	.45	.04	−.35	−.46
GQA NM	.46	.39	−.05	−.42	−.54
GAC III	.10	.19	−.03	−.14	−.33
InfraAlyzer 2.5	···	.17	.03	−.18	···

[a] Differences from Kjeldahl.
[b] GQA = Grain Quality Analyzer. OM = original mathematical treatment (delta log 1/R). NM = normalized treatment (delta log 1/R divided by delta log $1/R_1$).

TABLE XI
Chart for Correction for Discrepancies in Protein at Different Temperatures

	Temperature of Ground Whole Meals (°C)								
Instrument	−10	0	10	20	25	30	35	40	45
GQA 31EL[a,b]	−.6	−.4	−.2	0	.1	.2	.3	.4	.5
GAC III[c] / InfraAlyzer	−.2	−.1	0	0	.1	.1	.2	.2	.2

[a] Figures represent increments of protein to be added to observed near-infrared reflectance protein at the respective temperatures.
[b] Applies to Grain Quality Analyzer 31EL with original and normalized mathematical treatments.
[c] GAC = Grain Analysis Computer.

improve their GQA series of instruments. Second, the temperature of the samples can be adjusted to coincide with the temperature of the samples with which the instrument was calibrated. This can cause difficulties in situations of high throughput, where sample temperatures rise to levels from which the cooling time can add significantly to the time per test. Third, the result can be adjusted by an increment that compensates for the change in result caused by temperature. Because this can be achieved simply and inexpensively by construction of a correction chart, the last approach appears to be the most practicable for use with existing commercial instruments. A system can be used to compensate for temperature whereby an increment of 0.1% protein is added for every five samples analyzed consecutively by means of the GQA 31EL, and 0.1% for every 10–15 samples analyzed consecutively by the GAC III or the InfraAlyzer.

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