APPLICATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY TO ANALYSIS OF CEREAL GRAINS AND OILSEEDS[1]

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ABSTRACT

Infrared reflectance spectroscopy (IRS) was first introduced in 1971 for rapid on-the-spot analyses of soybeans for oil, protein, and moisture. The equipment utilizes the principle of infrared reflectance spectroscopy, together with a unique multiple-wavelength filter system, which facilitates scanning of the near infrared area of the spectrum. Calibration against known standards is necessary. IRS equipment has been calibrated for analyses of a variety of materials, including hard red spring wheat (HRS), hard and soft wheat flours, vital wheat gluten, barley, oats, rapeseed, and soybeans. Calibration depends upon statistical principles, using either a multiple regression or simultaneous equation-type program. In either case, computer facilities are necessary. Accuracy of analyses, as measured by the standard error of estimate, is about ±0.22% protein and ±0.16% moisture in the case of red spring wheat, giving a coefficient of variability of 1.5% in each case. Accuracy of analyses of other cereals, oilseeds, and legumes for oil, protein, and moisture varies, but coefficients of variability are usually between 1–5%. Analytical precision as measured by check sample analyses is comparable with that of standard laboratory methods. Technical difficulties which may be encountered with calibration and operation of equipment are discussed. The most important factor influencing the accuracy and precision of analysis with IRS equipment is mean particle size of ground sample presented to the instrument, which is, in turn, influenced mainly by method of grinding. Other factors which are discussed include mixing of sample, type of grain, constitution of grain, growing environment of grain, including seasonal effect, and factors associated with instrument. Method of sample preparation is of fundamental importance to successful use of IRS instrumentation. Both accuracy and precision of analyses may be affected by method of sample preparation and also by method of presentation to instrument. Errors of over 2% protein have been encountered in analyses of HRS wheat when the instrument was used to analyze a sample which had been prepared by a system for which instrument had not been calibrated. Method of grinding a sample of grain affects both mean particle size of sample and type of particles produced. This, in turn, influences penetration and reflectance of light from sample presented to sensing unit. Extent of packing inside presentation cell may also influence analytical results.

During 1971 infrared reflectance spectroscopy (IRS) was introduced to the grain industry as a means of rapid analysis for oil, protein, and moisture (1). IRS instruments utilizing the original principle developed by Karl F. Norris, U.S. Department of Agriculture, Beltsville, Md., were engineered by two U.S. companies. Both instruments were claimed to be capable of complete analysis for protein, oil, and moisture—within a few seconds—with an accuracy equivalent to standard laboratory procedures. No reagents were involved, and no fumes or waste products were generated, other than the dust involved from presentation of the sample. During the early months of 1972, one of the first instruments available was acquired by the Canadian Grain Commission for investigational purposes. A second instrument was purchased later that year for a comparative

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study. The present communication summarizes earlier results obtained with this equipment. These data were presented at the 58th Annual Meeting, AACC, St. Louis, Mo., October 1973, and since that date, both companies have introduced several improvements to the engineering and circuitry of their respective instruments (which are now incorporated in the current models). However, regarding results of application of IRS to analysis of a variety of grains, accuracy and precision of analysis, and difficulties encountered remain basically unaltered, in the experience of the author, even in light of the more up-to-date instrumentation available.

METHODS

Standard Analytical Methods

Basic operating principle and methods employed to utilize the principle have been referred to by Trevis (2), and also in more detail, in the operators' manuals issued by the respective companies, which also include details of method of calibration of each instrument. Standard analytical procedures used throughout the investigations were as follows: total nitrogen was determined by the Kjeldahl method, as employed by the Canadian Grain Commission (3). Moisture was determined by the AACC single-stage air oven method using a 2.0-g sample with a heating period of 65 min at 135°C ± 1°C (4). Oil was determined by overnight extraction, using anhydrous diethyl ether and Goldfisch extraction equipment. Previous to extraction, pulverized samples were dried overnight under vacuum in an oven at 100°C. Wheat was pulverized in a Hobart Model 2040 coffee grinder, except where otherwise stated. Barley and oats were reduced in the Model CSM-2 Cyclone grinder using a 1.0-mm screen; soybeans were pulverized in a Holowick spiral burr mill, and all other seeds were ground in the Krups Model 75 coffee grinder. For calibration purposes, "as is" analytical results were used, since this is the form in which the equipment "sees" material, and sensing channels react accordingly to constituents present. In case of oilseeds, standard analyses are usually carried out on vacuum-dried samples, and this is the state in which materials were used for calibration and assessment of the IRS equipment. IRS instruments used included the Neotec Model 1 Grain Quality Analyzer and the Technicon/Dickey-john InfraAlyzer.

Statistical Analysis

Although IRS appears destined to become the first major breakthrough in routine analysis of cereal grains since the Kjeldahl test, its application depends as heavily upon mathematical statistics as it does upon the phenomenon of infrared radiation. In addition to calculations performed by the analog circuitry of the equipment itself, the method of arriving at calibration constants involves use of multiple linear regression analysis, whereas statistics found to be of most use in assessment of performance of equipment were: standard error of estimate (SEE); standard error of a single test (SEST); root mean square difference (RMSD); standard error of difference (SED); and coefficient of variability (CV). The formulas for calculation of these statistics are listed in Table I. A description of the use of these statistical concepts may be found in standard statistical texts such as Steel and Torrie (5), or Snedecor and Cochrane (6).

Standard Error of a Single Test—SEST—is commonly used to assess day-to-day precision, or reproducibility of laboratory analysis. This is given by calculation of standard deviation of results of analysis of a check sample or samples. Significance of SEST lies in the fact that for each "fresh" test carried out on a check sample, there is one chance in three that the result will deviate from the overall mean by an amount equivalent to the magnitude of the SEST or greater. There is one chance in five that the result will differ from the mean by a magnitude of 1.25 × SEST; and one chance in twenty that the result will differ from the mean by twice the SEST. This aspect is covered in more detail elsewhere (7) and applies to many statistical treatments of factors associated with normally distributed populations.

Standard Error of Estimate—SEE—gives standard deviation of y (calc) from true value of y, provided that x is held constant. In order to interpret this statistic, the operator should be aware of the fact that the SEE statistic indicates a theoretical value *before the actual analysis* based upon linear regression. This statistic gives an indication of accuracy of analysis which may be anticipated when actual analysis is carried out using values realized by the calibration.

The SEE statistic generally underestimates the error observed in subsequent testing by IRS equipment. Unless standard analytical procedures followed in

TABLE I

A. Standard Error of a Single Test—SEST

$$SEST = \sqrt{\frac{\Sigma x^2-(\Sigma x)^2/N}{N-1}}$$

B. Standard Error of Estimate—SEE

$$SEE = \sqrt{\frac{\Sigma y^2-(\Sigma xy)^2/\Sigma x^2}{N-2}}$$

C. Root Mean Square Difference—RMSD

$$RMSD = \sqrt{\frac{\Sigma(x-y)^2}{N-1}}$$

D. Standard Error of Difference—SED

$$SED = \sqrt{\frac{\Sigma(x-y)^2-[\Sigma(x-y)]^2/N}{N-1}}$$

E. Coefficient of Variability—CV

$$CV = \sqrt{\frac{SE^a \times 100}{x}}$$

[a]SE = Standard error.

analyzing material for calibration of equipment are of the highest degree of accuracy, the SEE, per se, may be of limited value in appraising the application of IRS to actual analysis.

Root Mean Square Difference—RMSD—may be applied to the results obtained by application of the instrumentation to actual analysis of material. This statistic enables operator to compare overall accuracy of results obtained by IRS with those obtained by standard analytical procedures. The RMSD statistic incorporates total error involved in both IRS and standard analyses and will almost invariably be greater than the SEE statistic.

Standard Error of Difference—SED—should be calculated at the same time as RMSD. This statistic takes into account the sign of differences between IRS and standard analyses. If differences which occur between the two sets of analytical data are biased mainly in one direction, the $[\Sigma(x-y)]^2/N$ value will be of a magnitude which will cause the SED to differ significantly from the RMSD. If differences between IRS and standard results are equally dispersed above and below results of standard analysis, the $[\Sigma(x-y)]^2/N$ value will be very small or even disappear completely, which will mean that the SED value will be similar or equal to that of the RMSD.

Where differences do occur between two sets of analytical data, the RMSD gives an estimate of magnitude of errors, whereas the SED statistic indicates the distribution of the error. As a rough guideline, the square root of the difference between the RMSD and SED values will be of the same order of magnitude as the difference between the mean IRS and standard analytical results. Some typical examples of this are given in Table II. In order to give a clear indication of the analytical accuracy and its reproducibility throughout a series of samples, it is sufficient to quote the SED statistic together with the comparative mean results for each series.

Coefficient of Variability—CV—may be used to relate the SEST, SEE, and SED statistics to the mean results of standard analysis of the samples used in various investigations. For accurate analysis of grain and related products, the CV should not exceed 2.5%.

RESULTS

Accuracy. The accuracy of IRS testing of wheat, wheat flour, and some other grains is illustrated in Table III. In most cases, the accuracy was found to be satisfactory for routine analysis. There was a tendency for analytical accuracy to deteriorate somewhat in crops which were high in fiber, such as barley and oats. The dehulling of oats led to a marked improvement in the accuracy attained.

Precision. The precision, or day-to-day reproducibility, is indicated in Table IV. IRS equipment was found to be capable of a degree of precision equivalent to that of standard laboratory practices. Both precision and accuracy of analysis were affected by method of sample preparation and by procedure of loading sample into cell. Performance of the instrument on a permanent check cell indicated that electronics of the equipment, per se, were capable of a high degree of reproducibility, provided that errors induced by sample preparation and presentation were minimized. Tables V and VI serve to verify precision of performance of IRS equipment. Duplicate readings in Table V refer to results

TABLE II
Relation between RMSD-SED Values and Kjeldahl (A) and IRS (B) Protein Values

RMSD-SED	A−B (Calc)	A−B (Obs)
0.007	0.084	0.05
0.015	0.120	0.08
0.038	0.195	0.16
0.074	0.272	0.24
0.138	0.372	0.35
0.264	0.514	0.50

TABLE III
Accuracy of IRS Analysis as Compared to Standard Analytical Procedures[a]

	IRS	Std.	SED	IRS	Std.	SED	IRS	Std.	SED
	Barley			Oats			Wheat (HRS)		
Oil	...	...	...	5.91	6.04	0.36	...	...	...
Protein	12.52	12.50	0.35	12.40	12.38	0.47	13.61	13.63	0.218
Moisture	7.11	7.08	0.12	7.47	7.50	0.17	9.0	8.8	0.280
	Soybean			Rapeseed			Flour (all types)		
Oil	17.5	17.5	0.21	44.9	45.7	0.56	...	...	...
Protein	41.7	41.6	0.50	24.5	24.2	0.75	11.01	11.04	0.220
Moisture	6.3	6.5	0.15	5.1	4.8	0.22	13.0	13.0	0.300

[a]Results on "as is" moisture basis.

TABLE IV
Reproducibility of IRS and Standard Analysis of Hard Red Spring Wheat[a]
(Standard error of a single test carried out daily)

		IRS		Standard Analysis	
Comments	Constituent	SEST	CV	SEST	CV
Fresh grind for each test	Protein	0.168	1.2	0.178	1.3
	Moisture	0.371	3.3	0.256	2.2
	Oil	...	...	0.87	2.3
Single grind for all tests	Protein	0.135	0.9	0.098	0.7
(Minimizes error due to	Moisture	0.117	1.0	0.069	0.6
grinding)	Oil	...	...	0.24	0.6
Permanent check cell	Protein	0.065	0.5	...	...
(Minimizes errors due to	Moisture	0.051	0.5	...	...
grinding and loading cell)	Oil	...	...	...	...

[a]Protein and moisture data taken from wheat analyses; oil data refer to rapeseed analyses.

TABLE V
Standard Error of Duplicate Readings[a]

Constituent	IRS		Standard Analysis	
	SEST %	CV %	SEST %	CV %
Protein	0.23	1.6	0.08	0.6
Moisture	0.09	1.0	0.07	0.7
Oil	0.17	0.4	0.21	0.7

[a]Protein and moisture data were taken from analysis of HRS wheat ground on Cyclone grinder; oil data were accumulated from analysis of rapeseed, ground on Krups Model 75 grinder.

TABLE VI
Day-to-Day Reproducibility of GQA "C" Values and InfraAlyzer Log Values[a]

C Value	Mean Result	Standard Deviation	CV	Log Value	Mean Result	Standard Deviation	CV
1	41.7	0.51	1.2	1	1029	18	1.8
2	−21.7	0.25	1.2	2	690	15	2.2
3	71.1	0.54	0.8	3	645	14	2.1
				4	644	17	2.6
				5	520	11	2.1
				6	155	11	7.0

[a]Analysis of HRS wheat check sample over a period of several weeks.

TABLE VII
Time Per Test for Ground Samples[a,b]

No. of Readings	No. of Sample Cells[c]	Time sec
2	1	102
1	1	90
2	2	82
1	2	62

[a]Add 1.5 min to time, to allow for grinding in case of whole grains.
[b]Data are means for 24 consecutive tests.
[c]With two sample cells, one is being loaded while other is in process of analysis.

obtained by rotating the sample cell through 180° between successive readings without reloading the cell. Differences between duplicate protein readings were rather greater than duplicate Kjeldahl tests, but the moisture and oil channels showed a high degree of duplication. The C values and log values indicated in Table VI were recorded from the day-to-day analysis of a sample of freshly ground wheat using the GQA and the Technicon InfraAlyzer over a period of several weeks. In order to ensure the best results from the equipment over a long period, it is advisable to record these data. In the event of failure, the operator will then be able to tell at a glance whether rectification of the faults has materially affected the sensing system. Under ordinary conditions, both the "log" and the C values are very reproducible, and in our experience there is little or no indication of the "aging" effect which was noticeable during our earliest experiences with the equipment.

Time per test. The GQA is claimed to be capable of testing a sample of grain for oil, protein, and moisture in 10 sec. This does not take into consideration unavoidable time necessary for selection of sample, mixing and loading cell, recording the result and emptying and cleaning the cell. Table VII summarizes time per test under a number of conditions. These times are mean times for two separate operators who each carried out 24 successive tests by each system. In our experience, it does not appear likely that an operator could complete tests consistently in less than 1.5 min. Time for a single test using the InfraAlyzer is a few seconds longer. If sample requires grinding, time should be extended a further minute. However, an overall testing period of 2.5 min still constitutes one of the most rapid and simplest testing systems. The testing procedure takes slightly longer using the InfraAlyzer, because of a longer period of internal selfcalibration.

Factors which Influence Performance of IRS Equipment

When compared to standard methods of analysis, there are relatively few factors that exert an influence on the accuracy of performance of IRS instrumentation. For example, there are over 30 factors which may affect the accuracy of the Kjeldahl test for protein, while the Udy dye absorption test for protein lies at the mercy of about 20 sources of error (7). The factors which influence IRS analysis may be subdivided into factors associated with material under investigation, those which stem from the instrumentation itself, and other factors, including operator error.

I. *Factors associated with the material under investigation.*—Accurate calibration is absolutely essential to the successful applications of IRS equipment to a testing program. Calibration is influenced by number, composition, and nature of samples selected for calibration; by the method of sample preparation, by the light source, by the settings of the C potentiometers (in the case of the GQA), and by the accuracy of the standard laboratory analyses against which the equipment is to be calibrated. Most of the factors to be discussed affect both the GQA and the InfraAlyzer in a similar manner. For the sake of brevity, the following comments will be limited mainly to observations made using the GQA. At a later date, a comprehensive comparison of the available IRS instrumentation will be submitted.

A. Sample Selection

Number of samples and concentration range. The instruments should be calibrated over a working range of composition with respect to constituents to be assayed. It is not necessary or advisable to include samples which represent extremes in concentration. Table VIII indicates that provided an adequate range of composition is available, extension of the range does not greatly improve accuracy of subsequent testing. In case of the GQA, inclusion of material containing extremes of composition may result in K values which are too large to be accommodated by K potentiometers. In general for this instrument, computed values for K_0 should be less than ±30, while values for K_1, K_2, and K_3 should not exceed ±98. If any K values exceed these limits this usually implies that the particular channel to which the K value relates is operating at or above its maximum capacity. The K values can usually be accommodated by: a) reducing the concentration range of the samples used in calibration; b) adjusting the C potentiometers; c) electronic modification.

Material used in calibration may be selected to represent a given range of concentration, or taken at random. Furthermore, the number of samples used in a calibration may vary. Use of standard error of estimate (SEE) statistic may be misleading in this area. SEE is based upon correlation between calculated results and actual standard laboratory results. If a small number of samples, such as 10–12, is used in calibration SEE may be quite small, which implies that an accurate assay may be obtained with regard to constituent under investigation. Table IX indicates the SEE, RMSD, and SED data for two series of samples. The first series was chosen at random, whereas the second series was specifically selected to represent a given range of protein. The SEE data indicate that the smaller the number of samples used in calibration, the lower error is likely to be. This is the case whether samples are selected to represent a given range of protein, or taken at random. However, the RMSD data indicate a trend in the opposite direction. Table IX also shows that the trend in RMSD values is supported by the RMSD-SED figures and also by the mean differences between GQA and Kjeldahl values for different numbers and types of samples. The data in this table suggest that: a) the selection of samples to represent a given range of protein

TABLE VIII
Influence of Sample Range on Efficiency of Protein Calibration
(N = 34)

Range in Protein %	SEE	RMSD[a]	SED[a]	RMSD-SED
12–14	0.165	0.482	0.223	0.159
12–16	0.194	0.276	0.221	0.055
10–16	0.134	0.250	0.161	0.029
10–18	0.193	0.228	0.151	0.075

[a]RMSD and SED between GQA and Kjeldahl results.

content generally leads to more precise calibration; b) about 30 samples are necessary. A larger number will not lead to any great improvement in calibration, provided that the samples are selected to cover a sufficient working range. The samples should be selected so as to give as even distribution as possible throughout this range.

A further implication—c) is that if calibration material is to be taken at random, at least 40 samples are likely to be necessary.

The SEE statistic may be misleading in another area dealing with calibration. Suppose a series of samples is prepared from blends of *e.g.*, a high- and a low-protein sample of wheat, so that about 20–30 samples are prepared with a regular gradient of protein. If these samples are then used for calibration, a very low standard error of estimate will result—usually of the order of 0.10% or lower—and there will be a linear variation in all three series of C values. However, this calibration is only satisfactory for estimation of protein in one of the "blend" series or "family" and analysis of extraneous samples may lead to errors of as much as 2% protein or higher. The reason for this lies principally in the fact that IRS equipment is highly "statistical" and programming of the analog computer depends upon multiple linear regression or some similar concept. Each sample from a normal series "appears" to be different to the sensing channels and an accurate calibration will resolve these differences in such a manner that programming of the computer will enable the instrument to translate interactions received from all three channels into terms of protein or other constituents for which the instrument is calibrated. Use of blended series will bias the sensing channels and final calibration so that the instrument will only respond satisfactorily to material which either belongs to the series or possesses a similar reflectance spectrum to the series. For all practical purposes, calibration has been completed with two samples—the highest and lowest of the blend series. Blend-type calibration may be suitable for the analysis of material which is very uniform in nature; but in most cases, grain samples represent a variety of locations and even seasons. It is unlikely to lead to accurate analysis of this type of material, and is not to be recommended.

The K values themselves should remain stable once they are set into either instrument. They should not change by more than 1 digit in the third column over

TABLE IX
Influence of Sample Selection on Efficiency of IRS Protein Calibration

	Random Selection of Samples					Material Selected to Represent Working Range				
N	SEE	RMSD	SED	RMSD -SED	Kjeldahl -IRS	SEE	RMSD	SED	RMSD -SED	Kjeldahl -IRS
10	0.114	0.370	0.131	0.184	0.27	0.119	0.402	0.256	0.190	0.37
20	0.140	0.390	0.201	0.168	0.30	0.176	0.382	0.275	0.157	0.31
30	0.175	0.403	0.256	0.166	0.30	0.183	0.325	0.257	0.105	0.01
40	0.211	0.260	0.192	0.021	0.04	0.211	0.260	0.192	0.021	0.04

an indefinite period of time. However, K values are regression coefficients, and small differences in the values may lead to disproportionate changes in the results obtained subsequently. In our experience, immediately following the setting or adjustment of K values in an instrument, it is advisable to allow a double-zero calibration before commencing analytical operations. The first reading, taken immediately after switching from the "K-CAL" mode, is suspect.

B. Sample Preparation

Method of sample preparation is of fundamental importance to the successful use of IRS instrumentation. Both accuracy and precision of analyses may be affected by method of sample preparation and also by method of presentation to the instrument. Errors of up to 4% protein have been encountered in analysis of HRS wheat which had been prepared by a system for which the instrument had not been calibrated. The method of grinding a sample of grain affects both mean particle size of sample, type of particles produced, and consequently, surface presented to the instrument. This in turn influences reflectance of light received by the sensing unit. Density of packing inside the presentation cell may also influence analytical results.

Material to be used in calibration of the equipment must be prepared in exactly the same manner as that which will be involved in subsequent analytical

TABLE X
Influence of Sample Preparation of Wheat on Sensing Unit of GQA

Grinder	C_1	C_2	C_3
A	39.0	−11.8	64.9
B	33.0	− 8.9	53.5
C	28.7	− 6.8	50.5

TABLE XI
Mean Protein Content of Wheat Ground in Eight Different Grinders

Grinder	Single Calibration for all Analyses	Individual Calibration for Each Grinder Series
A	13.8	14.2
B	13.5	14.0
C	14.1	14.2
D	15.3	14.1
E	14.0	14.2
F	13.6	14.0
G	14.1	14.0
H	14.3	14.1
Mean GQA	14.1	14.1
Kjeldahl	14.2	14.2

operations. Table X illustrates influence of three different types of grinder on reaction of sensing system of a GQA to ground wheat surface, as indicated by C values. Changes of this order of magnitude may lead to serious errors in subsequent analysis unless the instrument is calibrated for the type of grinder employed. Table XI gives typical data for mean protein contents of a series of wheat samples analyzed on a GQA after preparations involving eight different types of grinder with a single overall calibration and individual calibrations. The individual calibration revealed marked improvement in results. A similar comparison was carried out using an InfraAlyzer which had been calibrated using wheat ground in a Model CSM-2 Cyclone grinder. The results of the analysis for protein of wheat ground on a Krups coffee grinder deviated from the standard Kjeldahl results by up to nearly 3%.

The adequacy of a sample preparation system may be verified by the operator by a comparison of the SED with the RMSD statistics for material analyzed by IRS and standard analysis. A wide discrepancy between the SED and RMSD indicates that efficiency of calibration may be improved, which can often be achieved simply by repeating calibration. If there is good agreement between the two statistics but the SED value remains high in proportion to the concentration, this indicates that analytical error is likely to continue to be high, and will probably not be improved by further recalibration. The remedy for this is to change sample preparation technique, or, in the case of the GQA, to adjust the C potentiometers. In some cases, it may be necessary to carry out electronic modification to the circuitry. In situations where the SED remains unaccountably high in proportion to the mean concentration, it may be concluded that the IRS approach is not suitable for analysis of the material or constituent under investigation.

The influence of grinding action is particularly significant and a set of samples of red spring wheat ground on seven different grinders was found to possess reflectance spectra which indicated wide differences in absolute reflectance, although the general patterns of the spectra were similar. The reflectance spectra were originally determined by Dr. Karl Norris of the U.S. Department of Agriculture, Beltsville, Md., using specialized equipment. The exercise is to be reported more fully. Size, shape, and uniformity of particles are all affected by the grinding process. All of these characteristics will influence the reflectance spectrum and physical depth of penetrable layer presented to the sensing system, and as a result, the reaction of the sensing unit to the sample surface. Accurate analyses with IRS may be achieved on samples ground by a wide variety of grinders, provided that the instrument is carefully calibrated for the individual grinder. Sample preparation is not without effect upon standard methods of analyses. It remains a mystery that new, expensive, and sophisticated pieces of equipment for measurement of various constituents in a wide range of commodities appear on the market continually, yet the area of sample preparation remains practically unexplored. Oilseeds, in particular, constitute a serious problem in size reduction, and particle size and uniformity exert significant influences on completeness and reproducibility of extractions, as well as on sampling error.

C. Sample Presentation

Mixing the sample. Ground samples must be thoroughly mixed prior to

loading the cell in order to achieve a satisfactory degree of precision. Figure 1 illustrates effect of progressive mixing of the sample on standard error between duplicate readings. About 15 mixes (one "mix" consists of a "turn" with a spatula) are sufficient to ensure adequate uniformity. The uniformity of the sample may be remarkably affected by the grinding procedure. Very finely ground wheat samples (mean particle size about 150 μ) may show a larger discrepancy between duplicate readings, *i.e.*, on one-cell loading, rotated through 180° between readings, than between the values obtained for duplicate grinds. This is due in part to stratification of sample during loading, and partly to degree of packing at surface which affects penetrability of light, and as a result, the "sampling area" from which this signal is received.

Sample size. Provided that surface presented to sensing unit is uniform and unbroken, sample size is unimportant. However, a study on wheat revealed that about 5 g was optimum for both speed and reproducibility of analyses. This could be reduced, if necessary, by modification of the sample cell and/or focusing of the light beam, but for most applications, sample size does not constitute a significant problem.

D. The Nature of the Sample

Type of grain. In common with standard analytical practices and many other instruments, IRS performance may be affected by type of material presented to the instrument. To date, the best results in our laboratory have been obtained with soybeans, HRS wheat and ground rapeseed, in that order, followed by hard spring wheat flour, peas, oat groats, soft wheat flour, barley, ground whole oats, sunflower, safflower, and unground rapeseed. Composition of material is also a factor which affects the efficiency of analysis. For example, if a GQA is calibrated for analyses of wheat, flour, barley, or oats, a change to rapeseed may involve adjustment of the C_1 potentiometer. The high concentration of oil (about 40%) will tend to "saturate" the C_1 channel and the C_1 values will exceed 100.

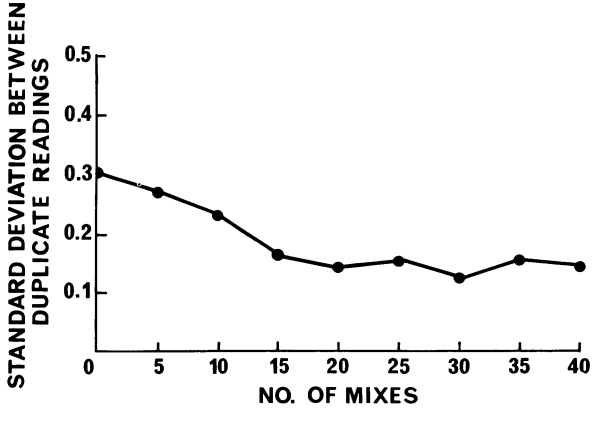

Fig. 1. Influence of mixing sample upon precision of duplicate readings on IRS equipment.

Similarly, vital wheat gluten, with 75 to 80% protein and virtually no oil, will necessitate adjustment to the protein channel. Conversely, soybeans contain 20% oil and 45% protein but may be handled satisfactorily with no necessity for modification. The principal reason for this is that the absolute protein "signal" is much lower than that of oil and the sensing and amplification circuitry is capable of accommodating higher concentrations of protein. Different types of wheat require separate calibrations. For example, HRS wheat could not be analyzed satisfactorily on an instrument which had been calibrated for soft white spring or even hard red winter wheat. The principal reason for this lies in the differences in mean particle size of ground wheat of different kernel texture. Table XII illustrates the mean particle size of several different types of ground wheat, ground on two grinders—the Hobart Model 2040 and the Cyclone grinder Model CSM-2.

Sensitivity of the instrument, or rather, the sensing head of the instrument, to oil, protein, and/or moisture contents of any particular type of material depends mainly upon two factors: a) the sample surface presented to the instrument, which is influenced mainly by physical texture of grain, and by method of sample preparation; and b) the actual wavelength at which instrument is hard-wired in near infrared area of spectrum for respective constituents. The surface area "scanned" by sensing unit varies inversely as the mean particle size of material. Consequently, the physical amount of sample which is involved in generation of the signal to the sensing unit will be affected by any factor which influences grain texture and particle size of the pulverized grain. Wavelength selection is achieved by means of a reference filter for the respective constituents. Small differences in the energy of electromagnetic radiations of a constituent in the material under investigation may result in small shifts in optimum wavelength points at which measurements are taken. These shifts may in turn result in significant losses in the

TABLE XII
Mean Particle Size of Eight Types of Wheat Ground on Two Different Types of Grinder

Wheat	Hobart 2040	Cyclone 1.0-mm Screen
Amber Durum	350	210
HRS	304	186
HRW	316	183
HWS	303	173
SRS	266	211
SRW	270	209
SWS	272	214
SWW	272	184
Mean	294	196
Standard Deviation	29.6	16.3
Standard Deviation between duplicates	11.6	12.9

efficiency of the sensing unit and, therefore, of the IRS instrument as an analytical tool. It is anticipated that future instruments will possess facilities to enable the operator to attenuate the wavelength selection according to specific requirements of material to be examined. Such a modification would greatly increase both accuracy of equipment and its adaptability to analysis of different materials and constituents.[2]

Influence of season and location. Physical texture of a grain such as wheat may be materially affected by changes induced to the chemical constitution of grain as a result of soil fertility and weather conditions during maturation of the grain. Changes to physical texture of grain may be expected to influence the manner in which grain reduces during the grinding stage of sample preparation, which may in turn affect the surface presented to instrument. Accuracy of analysis may be affected if the assumption is made that a single calibration is adequate for analysis of grain for any season and/or location, without loss of accuracy. In order to investigate this aspect, wheat samples were drawn from 12 shipping "blocks" selected to represent replicated areas of similar soil type across the Canadian Prairie, where wheat is grown over an area of approximately 900×400 miles. This exercise is incomplete but indications to date are that the accuracy of calibration may be affected by both season, and to a lesser extent, area where the wheat is grown. Influence of season is of greater significance and it appears that recalibration of IRS equipment may become a seasonal chore, at least for certain crops.

Miscellaneous factors affecting nature of grain. This category encompasses factors such as grain size, disease, immaturity, frost, etc., all of which may be expected to affect texture, particularly of caryopsis-type grains. Even protein content of this type of grain may influence manner in which grains reduce during grinding, and, therefore, of the surface presented to sensing head. In a study of effect of protein content upon calibration and subsequent analysis of HRS wheat, two series of calibrations were run: one using a high-protein range of wheat; while the second series was lower. It was found that calibration factors for lower protein series of wheat were suitable for analysis of wheat of up to 17% protein, whereas the K values obtained from high protein calibration gave rise to fairly large discrepancies when calibration was used for analysis of wheat of protein contents of 11% or less.

Interferences by constituents other than oil, protein and moisture. There is evidence that performance of the GQA may be affected by external interferences by constituents such as starch and some fiber components. For example, accuracy of analysis of hard wheat flour is significantly superior to that of soft wheat flour. In view of the highly cellular nature of the more granular hard wheat flour, soft wheat flour may present more than 10 times as much starch at the surface presented to sensing head. This aspect of the program is still under investigation.

II. *Factors associated with the instrument.*—

A. *GQA "C" channel settings.* Reaction of the GQA sensing unit to sample surface is relayed to analog computer through three "C" channels which are

[2]Since the original presentation of this manuscript, a new instrument has been introduced, the Neotec Model 41, which incorporates this feature.

respectively wired to react to wavelengths specific to oil, protein, and moisture. Voltage flow through the "C" channels is controlled by standard 10-turn potentiometers. These potentiometers are present at the assembly plant prior to shipping and settings are usually adequate for analysis of material for which the instrument is purchased. If the "C" value for any constituent approaches 100, this is an indication that that channel is approaching saturation, and the instrument will lose response if "C" channels are overloaded. This can be avoided by turning the "C" potentiometers clockwise, which tends to optimize sensitivity of the channel to the respective constituent. Having been set prior to calibration for a particular type of material, a "C" potentiometer affects the response of that channel and also of the entire instrument to constituents present in material under investigation.

In the case of the InfraAlyzer, saturation is indicated by log values in excess of 2000. This situation can be resolved by changing the diode setting for the log calibration number, which will increase the capacity of the channels to be analyzed using a particular one of 3 grain boards.

B. *Cell covers.* The special glass cell covers of both the GQA and InfraAlyzer sample cells are completely interchangeable and replacement of glass does not involve recalibration.

C. *Teflon® standard plates.* These plates should be wiped daily with a suitable type of tissue. Grain dust may accumulate on the Teflon, which will modify internal zeroing of the instrument to the extent that "drift" in results may occur. To date, there has been no evidence that dust accumulates at any other points in sufficient amounts to influence accuracy of analysis. Air filters should be cleaned once a week, to prevent excessive heat buildup within the instruments.

D. *Warm-up time.* Unless equipment is allowed to "warm up" for at least 1 hr prior to use, the results may be suspect.

III. *Factors associated with operator.*

Provided that operators are instructed in uniform mixing of sample and loading the cell, there is no appreciable operator error in application of calibrated IRS equipment.

In conclusion, introduction of IRS instrumentation represents an entirely new concept for rapid routine analysis of cereal grains and oilseeds for oil, protein, and moisture.

Accuracy and precision of analysis are influenced by factors associated with calibration; sample preparation, and presentation; by factors which influence nature of grain, and by factors associated with the instrument itself. Influences of some of the above factors upon standard laboratory practices are frequently overlooked. As a result, appearance of the IRS concept upon the analytical scene may serve to focus attention anew upon the fundamental importance of sampling, sample preparation, and accurate standardization of laboratory practices.

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Literature Cited

1. ROSENTHAL, R. D. Introducing: The Grain Quality Analyzer. Annual meeting, Kans. Ass. Wheat Growers, Hutchinson, Kans., Dec. 1971.
2. TREVIS, J. E. Seven automated instruments. Cereal Sci. Today 19: 182 (1974).
3. WILLIAMS, P. C. Large-scale Kjeldahl testing - Semi-automation of an ancient system. Lab. Prac. 1975 (in press).
4. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC. The Association: St. Paul, Minn. (1962).
5. STEEL, R. G. D., and TORRIE, J. H. Principles and procedures of statistics. McGraw-Hill: New York, N.Y. (1960).
6. SNEDECOR, G. W., and COCHRANE, W. G. Statistical methods (6th ed.). Iowa State University Press: Ames, Iowa (1967).
7. WILLIAMS, P. C. Errors in protein testing and their consequences. Cereal Sci. Today 19: 280 (1974).

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