Determination of Starch and Energy in Feed Grains by Near-Infrared Reflectance Spectroscopy†

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Near-infrared reflectance spectroscopy (NIRS) has been applied to the determination of starch, protein, and energy in feed grains with a Pacific Scientific research composition analyzer, Model 6250, a Pacific Scientific feed-quality analyzer, Model FQA 51A, and a DICKEY-john grain analysis computer, Model GAC III. The first or second derivative of the log $(1/R)$ algorithm in normal or quotient version gave the best accuracy of analysis by RCA 6250 and FQA 51A. The GAC III uses the log $(1/R)$ algorithm. Accuracies for protein and energy analyses were similar to those obtained by the RCA 6250 or the FQA 51A.

It is estimated that up to half of the grains grown in Canada are used for animal feed. The grains consumed most frequently are barley, feed wheat, and maize (corn). The primary nutritional value of grain depends on its protein and energy content. A knowledge of these would greatly improve the efficiency of feed formulation. Conventional chemical methods are not only expensive but also time-consuming. Moreover, no standard method has been established for the determination of starch in grains.

Near-infrared reflectance spectroscopy (NIRS) is a rapid, economical, and nondisruptive method. It provides the simultaneous determination of several constituents and is widely used for the analysis of many commodities in food and feed industries. In grains, determination has been reported of protein (Williams, 1975; Tkachuk, 1981; Williams et al., 1983; Williams and Cordeiro, 1985), moisture (Williams, 1975; Williams et al., 1983; Downey, 1985), amino acids (Bruinsma and Rubenthaler, 1978; Williams et al., 1984), hardness (Saurer, 1978; Williams, 1979; Williams and Sobering, 1986; Downey et al., 1986; Gaines et al., 1987), and oil (Hymowitz, 1974; Williams, 1975; Hilliard and Daynard, 1976). Nevertheless, little work has been reported on the NIRS analysis of starch and energy in feed grains. Hilliard and Daynard (1976) attempted to determine starch content in corn using a Neotec grain-quality analyzer (GQA) but were unable to get a satisfactory calibration. Baker (1985) reported the successful determination of starch in snack foods. Davies et al. (1985) were able to obtain satisfactory results for the determination of starch in pea flour, while Osborne (1983) determined the starch content of air-dried bread. Krischenko (1984) reports the determination of starch in barley and wheat. These appear to be the only references to applications of NIRS to starch determination.

The purpose of the present study was to investigate the application of NIRS analysis to determine the starch, protein, and gross energy values in Canadian feed grains.

MATERIALS AND METHODS

Canada feed wheat, barley (six-row and two-row), and corn were obtained from the Canadian Grain Commission Inspection Division. Termamyl Novo 132 kNu/g) was provided by Dr. A. W. MacGregor, Grain Research Laboratory. Amyloglucosidase from *Aspergillus niger* (E.C. 3.2.1.3), hexokinase/glucose-6-phosphate dehydrogenase, and phosphate buffer/NADP/ATP were purchased from Boehringer Mannheim Canada (Dorval, Quebec). All other chemicals were of analytical reagent grade.

Sample Preparation. Grains were ground on a Udy Cyclone grinder fitted with a 1.0-mm screen for NIRS, moisture, oil, and protein analyses. To determine the starch content and gross energy, samples were further ground on the Udy Cyclone grinder with use of a 0.5-mm screen.

Moisture was determined according to the single-stage air-oven method (AACC Method 44-15A; AACC, 1981) by heating at 130 °C for 65 min and protein by the Kjeldahl method (AACC Method 46-12; AACC, 1983). Wheat protein was reported as N × 5.7, and barley and corn proteins were reported as N × 6.5. Oil analysis of corn samples was performed by overnight extraction using anhydrous diethyl ether and Goldfisch extraction equipment.

The moisture levels ranged from 8.8 to 12.1% for barley samples, from 10.2 to 12.7% for wheat samples, and from 10.4% to 13.3% for corn samples.

Starch Determination. Starch was hydrolyzed to glucose according to the procedure suggested by Salomonsson et al. (1984). Duplicate samples of 50 mg were weighed into 35-mL Pyrex glass tubes with screw caps containing tightly fitting Teflon washers. Samples were extracted with 20 mL of 80% EtOH for 30 min in a boiling water bath with constant shaking. The tubes were then centrifuged at 2000*g* for 10 min, and the supernatant was removed with a pipet. A 25-mL portion of sodium acetate buffer (0.156 M, pH 5.0) and 100 μL of thermostable α-amylase (Termamyl) were added and the tubes incubated for 30 min in a boiling water bath with constant shaking. After cooling to 60 °C, the contents of each tube were incubated with 100 μL of amyloglucosidase (from *A. niger*) and kept capped at 60 °C overnight in a water bath with constant shaking.

After this incubation, the test tubes were centrifuged at 2000*g* for 10 min, and 5 mL of the supernatant was transferred into a volumetric flask and diluted to 100 mL with distilled water. The amount of glucose released by the hydrolysis of starch was determined by the hexokinase method of Beutler and Bergmeyer (1984). A 4-mL portion of the hexokinase working solution was added to 1 mL of the diluted sample solution and the resultant solution mixed on a vortex mixer. Absorbance was measured at 340 nm. The starch content of the solution was calculated from the calibration curve obtained from the standard solutions of glucose. The glucose content was multiplied by the factor 0.9 for the calculation of starch.

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Gross Energy Determination. Gross energy (cal/g) was determined with use of an adiabatic bomb calorimeter (Parr Instrument Co., IL). One gram of sample was made into a pellet with use of a pellet press (Parr Instrument). An electric wire was attached to the pellet and placed inside the bomb. Oxygen pressure of 30 atm was added to the bomb. After the sample material was ignited, the change in temperature of the water was measured. The hydrothermal equivalent of the calorimeter was determined by means of benzoic acid. Values were corrected by subtracting the oxidation of the fuse wire and the acid production from the combustion, which was measured by titration with sodium carbonate standard.

NIRS Analysis. NIRS analysis was performed on a research composition analyzer (RCA), Model 6250 (Pacific Scientific Ltd.), a feed-quality analyzer, Model 51A (Pacific Scientific), and a DICKEY-john grain analysis computer, Model GAC III. With the RCA 6250, the samples were scanned between 1100 and 2500 nm. A reference scan on a ceramic standard was carried out between each sample scan. To reduce the effect of instrument noise, each spectrum was recorded after the average reading of 50 scans per sample was computed and the data were recorded as log $(1/R)$ (apparent reflectance). Multiple linear regression analysis was performed by the dedicated Northstar computer on a calibration set of samples. Mathematical treatments investigated included log $(1/R)$ and the first and second derivatives thereof, in normal and quotient versions (Norris and Williams, 1984). Calibration equations were then tested on a unique set of prediction samples.

The FQA 51A, was interfaced to the RCA 6250 Northstar computer system. Spectra of the samples were recorded with use of the FQA 51A optical system, which carried six narrow band-pass tilting filters, scanning the range from about 1600 to 2360 nm. The RCA 6250 Northstar computer was used to select pulse points (wavelengths) for the FQA 51A. The first derivative (segment size of 10 points, gap size of 29 points) and second derivative (segment size of 20 points, gap size of 20) of the log $(1/R)$ were tested. These derivative parameters are factory installed. The selected pulse points were entered into the FQA 51A, and the instrument was calibrated by its own autocalibration capabilities by reading the calibration samples. Prediction samples were then analyzed from the calibration produced by autocalibration. The FQA 51A was tested further by optimizing derivative size and predicting composition and energy with use of the FQA 51A interfaced to the computer.

For the DICKEY-john grain analysis computer III, the raw log $(1/R)$ signals were taken at wavelengths of 2310, 2230, 2180, 2100, 1940, and 1680 nm. These data were regressed against the chemical data for starch, protein, gross energy, and oil (in the case of corn) using stepwise multiple linear regression. Calibration constants were set into the GAC III, which was then used to analyze the prediction samples.

Accuracy was expressed in terms of the standard error of performance (SEP), which is the standard deviation of differences between NIRS and standard chemical analysis. The mean of the deviations ($\bar{d}$) (or bias) and the coefficients of correlation (r) between NIRS and standard analysis were also reported. In practice, biases are always set to zero before analysis and slope corrections made where necessary. For the purpose of this paper, the biases obtained after first run verification are reported to illustrate the magnitude of bias that can be anticipated.

RESULTS AND DISCUSSION

Starch Analysis. For starch analysis, it was necessary to grind the grains with a 0.5-mm screen in order to minimize the separation of the light bran and heavy endosperm particles (Aman and Hesselman, 1984). In this study, it was found that starch values were about 4% higher in samples ground with a 0.5-mm screen than in samples ground with a 1.0-mm screen. Commercial wheat starch and starches prepared from soft white wheat flour and hard wheat four were analyzed with the present method. Recoveries were 100 ± 1.4% for all samples. A preliminary experiment showed that the content of low molecular weight sugars in barley samples were 0.4–2.5%. It was considered necessary to extract the low molecular weight sugars with 80% EtOH prior to the starch analysis, as suggested by Aman and Hesselman (1984).

In order to confirm the completeness of the starch content determination, the residue and the supernatant were retreated with 100 μL of thermostable α-amylase and then 100 μL of amyloglucosidase. The same procedure was used to determine any change in starch content in the supernatant. Results showed that the starch content in the supernatant was increased only slightly (0–0.24%) with the treatment. Accordingly, the method used in this study was considered to provide quantitative recoveries of the starch from the feed grains. Reproducibility was about ±1.2% (CV = 2%); i.e., the method for starch determination was found to be both accurate and precise.

Composition of Grain Samples. Samples for both calibration and prediction series were selected to represent a uniform distribution of composition across the range. The ranges in concentration, mean, and standard deviation for barley, wheat, and corn samples used in the calibration and the prediction are presented in Tables I–III.

Although there was little difference in energy values between barley and wheat samples, starch and protein contents of wheat samples were higher than those of barley samples. This was attributed to the higher amount of fiber content in barley samples relative to that of the wheat samples.

Corn samples showed the highest starch content and energy values, while protein content was the lowest. The oil contents of corn samples obtained in this study were in agreement with literature data (Candlish, 1974; Simmonds, 1978; Williams, 1981). Barley and wheat are known to contain about 2% oil (dry basis) (Candlish, 1974; Simmonds, 1978; Williams, 1981). The higher average energy values of corn were believed to be due to the higher oil content of corn compared to the barley or wheat samples.

Table I. Composition of Barley Samples[a]

	calibration (n = 38)			analysis (n = 15)		
	starch, %	protein, %	energy, cal/g	starch, %	protein, %	energy, cal/g
high	62.2	15.5	4199	60.8	15.4	4196
low	52.2	8.7	4054	51.4	8.7	4035
mean	57.0	11.8	4105	57.5	11.7	4107
std dev	2.5	2.1	37.6	2.9	2.3	52.6

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

Table II. Composition of Wheat Samples[a]

	calibration (n = 26)			analysis (n = 15)		
	starch, %	protein, %	energy, cal/g	starch, %	protein, %	energy, cal/g
high	68.1	18.6	4155	65.7	18.4	4136
low	54.0	8.9	3964	53.9	8.7	3961
mean	60.9	13.6	4053	60.4	13.9	4068
std dev	3.4	2.7	54.5	3.3	3.0	60.5

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

Table III. Composition of Corn Samples[a]

	calibration (n = 39)				analysis (n = 16)			
	starch, %	protein, %	fat, %	energy, cal/g	starch, %	protein, %	fat, %	energy, cal/g
high	68.4	12.0	4.4	4212	67.6	11.1	4.3	4234
low	62.0	7.0	3.2	4034	63.3	7.2	3.3	4077
mean	65.3	9.1	3.9	4135	65.6	9.1	3.8	4143
std dev	1.5	1.3	0.3	47.1	1.2	1.2	0.3	43.1

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

Table IV. Relationships between Starch, Protein, Fat, and Energy Values in Barley, Wheat, and Corn

component	r		
	calibration	analysis	overall
	Barley		
starch/protein	−0.80	−0.86	−0.82
starch/energy	−0.69	−0.82	−0.73
protein/energy	0.84	0.95	0.87
	Wheat		
starch/protein	−0.84	−0.96	−0.88
starch/energy	−0.67	−0.74	−0.69
protein/energy	0.89	0.82	0.86
	Corn		
starch/protein	−0.60	−0.77	−0.63
starch/energy	−0.36	−0.71	−0.43
protein/energy	0.82	0.88	0.83
fat/protein	−0.35	0.09	−0.29
fat/starch	0.20	0.06	0.16
fat/energy	−0.03	0.04	−0.03

Table V. Main Absorbance Bands for Protein, Starch, and Oil (nm)

protein (wheat gluten)		oil (corn)		starch (wheat)	
1144	1804	1162	1932	1202	1914
1186	1826	1212	1960	1270	1974
1238	1856	1390	2008	1362	2048
1280	1918	1416	2048	1432	2060
1308	1934	1488	2120	1580	2096
1366	1980	1540	2144	1702	2132
1398	2054	1612	2178	1750	2200
1418	2108	1662	2208	1826	2282
1458	2168	1712	2270	1872	2424
1494	2188	1724	2306		2446
1532	2270	1762	2346		
1578	2306	1814	2390		
1594	2346	1840	2428		
1628	2384	1898	2460		
1692	2416		2478		
1736	2464				

Wheat samples revealed the widest ranges in concentration for all the constituents, whereas corn samples showed the narrowest ranges for starch and protein contents, while the oil content also had a low range (3.2–4.6%). In particular, the ranges in starch concentration in corn samples were only 6.4% and 4.3% for the calibration and prediction samples, respectively. These were about half of the ranges found in barley or wheat.

Relationships between starch, protein, and energy values in barley, wheat, and corn (including fat data) are given in Table IV. In all the samples, the starch and protein contents as well as the starch and energy values were negatively correlated with each other, while the protein and energy values showed strong positive correlations.

The negative correlation between the starch and the protein contents was in agreement with findings by other workers (Shia and Slinkard, 1977). The positive correlation between the protein and energy values was attributed to the higher variance in protein content among the samples, relative to starch, in conjunction with the higher energy value of protein (5.65 cal/g) compared to that of starch (4.1 cal/g). The oil content of the corn samples was not correlated with energy or any of the other constituents, due to the very low variance in oil content among samples (SD = 0.3).

Mathematical Treatments and Wavelengths. Table V gives the main absorbance bands of protein (wheat gluten), starch, and oil. Analysis involving several mathematical treatments revealed that the first- or second-derivatized log $(1/R)$ algorithm using the normal or quotient version gave slightly better results than the raw log $(1/R)$ for the analysis of feed grains. It has been reported that mean particle size, particle size distribution, and bulk density affect the packing characteristics of ground grain and the nature of the surface presented to the instrument. This influences the penetration of radiation into the sample and the reflectance of light from the samples (Williams, 1975; Williams and Thompson, 1978). Errors caused by variations in mean particle size, particle size distribution, bulk density, and moisture in feed grains can be reduced by taking the first or second derivatives of the log $(1/R)$ and by using the quotient version (Williams and Norris, 1983; Norris and Williams, 1984).

Tables VI and VII summarize the mathematical treatments and wavelengths most successful in analysis of feed wheat, barley, and corn, with the RCA 6250 and the FQA 51A. The first derivative of the log $(1/R)$ (D10D) gave the best results for the determination of starch and energy in barley, energy in wheat, and oil in corn with the FQA 51A and for the determination of protein in wheat and corn and energy in wheat with the RCA 6250. For all other determinations, the second-derivative treatment (D20D) was best. The quotient form of D20D gave the best results for the determination of starch and energy in barley and corn with the RCA 6250. The quotient form of D10D gave the best results for the determination of protein in corn and energy of wheat with the RCA 6250. When the derivative size was optimized for the FQA 51A, the results improved in some cases, showing that the factory-installed algorithms were not optimum for that constituent/commodity. This was particularly apparent with corn, wherein the accuracy of prediction of all constituents was markedly improved with optimized derivatives. The optimization in some cases led to use of wavelengths that conformed more closely to wavelengths commonly associated with constituents. For example, the 1725-nm band used for oil in corn after optimization is a strong oil band, while the 1694- and 2311-nm bands used for energy are, respectively, protein and oil bands.

Derivative mathematics involves setting the form of smoothing (called the "segment") and of the derivative itself. The derivative size is sometimes referred to as the "gap" (Norris and Williams, 1984). In the present study, optimum segment size varied from 5 to 20 wavelength points, while gap size ranged from 5 to 40 points. This illustrates the importance of optimization of the algorithm when derivative mathematics is used in NIRS analysis.

Wavelength points selected by computerized instruments for the determination of constituents with derivatized data may not coincide with the literature values for wavelenths associated with the constituents. This is due partly to the nature of the derivative and partly to interactions between constituents that may result in higher correlations between optical signal and concentration of specific constituents at wavelength points other than the "classical" bands. It is important to visualize all constituents not as flat two-dimensional formulas but as dynamic arrangements of atoms in space that spend most of their time in patterns conforming to, for example, the proposed helical form of protein and starch molecules, linear forms of cellulose molecules, and other atomic/molecular arrangements. In a highly complex substance such as grain, whose major constituents are starch, protein, water, fiber, and oil, the combination of electrostatic and electromagnetic forces caused by the juxtapo-

Table VI. Math Type and Wavelengths (nm) Used for Analysis by RCA 6250

		barley			wheat			corn		
		1	2	3	1	2	3	1	2	3
starch	math type	D20D 5/10[a]			D20D 10/25			D20D 5/15		
	λ	1988	2382/1644[b]		2158			2094/2472		
protein	math type	D20D 5/40			D10D 5/25			D10D 5/35		
	λ	2178	2434	1900	1664	2226	2122	2128/2442		
energy	math type	D20D 5/15			D10D 10/10			D20D 5/10		
	λ	2160/2172			1770/2214			2058/2476		
fat	math type							D20D 5/35		
	λ							2390	2386	1894

[a] 5/10 = 5 wavelength points (10-nm) segment size, 10 points (20-nm) derivative size, etc. [b] Quotient algorithm.

Table VII. Math Type and Pulse Points (Wavelengths, nm) Used for Analysis by FQA 51A

		barley				wheat				corn			
		1	2	3	4	1	2	3	4	1	2	3	4
		A. Factory Algorithms											
starch	math type	D10D 10/29				D20D 20/0					D20D 20/0		
	pp (λ)	387 (2116)				386 (2114)				385 (2113)			
protein	math type	D20D 20/0				D20D 20/0					D20D 20/0		
	pp (λ)	396 (2128)				405 (2139)				385 (2113)			
energy	math type	D10D 10/29				D10D 10/29					D20D 20/0		
	pp (λ)	387 (2116)	316 (2063)			203 (1738)	343 (2082)	650 (2305)		158 (1704)			
fat	math type										D10D 10/29		
	pp (λ)									573 (2288)	197 (1734)		
		B. Optimized Algorithms											
starch	math type	no improvement				D20D 10/5					D20D 5/5		
	pp (λ)					385 (2100)	318 (1998)	503 (2286)		329 (2007)	454 (2182)	574 (2292)	540 (2270)
protein	math type	no improvement				D20D (10/10)					D10D 5/5		
	pp (λ)					409 (2133)	492 (2208)	426 (2154)	470 (2194)	385 (2100)	455 (2182)	90 (1663)	439 (2167)
energy	math type	D20D 10/10				D20D 5/25					D20D 5/25		
	pp (λ)	388 (2105)				397 (2117)	556 (2181)	396 (2116)	359 (2026)	156/615 (1694/2311)			
oil	math type										D20D 5/10		
	pp (λ)									192 (1725)			

sition of atoms such as carbon, oxygen, nitrogen, and hydrogen with inorganic elements in several forms, which differ widely in electronic structure and behavior, can cause considerable variation in the spacial relationships of the constituents. This is complicated still further by variation in the water content, which affects the degree of hydrogen bonding between and within molecules.

In the case of the first-derivative algorithm, the wavelength points selected are usually on the shoulders rather than at the "peaks" of absorption bands. The particular wavelengths correspond to the highest coefficient of correlation between the optical signal and the reference method data for the sample set used for the selection. Differences between wavelengths actually selected for NIRS analysis occur mainly as a result of interactions between the major constituents of the material under investigation. When two constituents are interrelated either positively or negatively, the wavelength points selected may conform to recognized bands for either constituent; for example, the two most significant wavelengths used for the determination of proteins in fixed-filter instruments such as the DICKEY-john GAC III are 2100 and 2180 nm. The 2100-nm band is a very wide band associated with starch. It actually consists of at least two bands at 2060 and 2096 nm. A strong band at 2100 nm is not noticeable in dried starch and is likely a water band. The 2180-nm band has long been associated with the determination of protein. It has been assigned (Law and Tkachuk, 1977) to a combination of N–H deformation and C–H stretching (amide II). It is not uncommon for bands close to either 2100 or 2180 nm to be selected for the determination of protein or starch, with the log $(1/R)$ or second-derivative algorithms.

In the analysis of barley by the FQA 51A the first derivative of the log $(1/R)$ gave the best prediction of starch, with a primary wavelength that conformed most closely to a starch band. The second derivative of the log $(1/R)$ gave the best prediction of protein, while the first derivative gave the best prediction of energy, with the same primary wavelength as in starch prediction and a secondary wavelength identifiable with a strong protein band. In the case of wheat, the second-derivative treatment gave the closest predictions of starch and protein, while the second derivative was best for prediction of gross energy. Bands identifiable with both starch and protein figured in the prediction of both constituents. Turning to corn, the first derivative of log $(1/R)$ was most successful in the prediction of oil, while the second derivative gave the most accurate results for prediction of all other constituents. Two wavelength points were necessary for the prediction of oil, both of which were close to oil bands. The main band used in the prediction of energy was close to a prominent protein band at 1692 nm. In view of the low range in oil (only a little more than 1%), the coefficient of correlation between NIRS and extraction values for oil was very significant. After optimization of deriv-

Table VIII. Calibration Coefficients Used for Analysis by DICKEY-john GAC III

	barley			wheat			corn			
	starch	protein	energy	starch	protein	energy	starch	protein	energy	fat
K_0	0.11910	0.47611	0.37979	-3.1013	0.09548	0.28595	-0.75345	0.08166	0.24190	0.43493
K_1	0.29499	-0.33714	-0.52618	-0.46188	-0.38496	-0.33408	0.5923	-0.30287	-0.51152	-0.15848
K_2	-2.0260	1.8402	0.52259	0.93400	1.7192	0.26399	-1.1110	1.3417	0.62907	-0.31141
K_3	0.73932	-1.8732	-0.49979	4.2105	-1.1721	-0.30630	1.7037	-1.0343	-0.37282	-0.13702
K_4	0.58413	0.09300	-0.01203	-0.42047	-0.00905	-0.06456	-0.40956	0.01967	-0.03811	0.02334
K_5	0.71581	-0.35164	0.15935	-1.4648	-0.41161	0.21616	0.11719	-0.14192	0.02783	0.13813

Table IX. Accuracy of Analysis by RCA 6250

	barley			wheat			corn		
	r[a]	SEP[b]	$\bar{d}$[c]	r	SEP	$\bar{d}$	r	SEP	$\bar{d}$
starch	0.93	1.08%	-0.20%	0.98	0.63%	-0.31%	0.88	0.51%	0.04%
protein	0.997	0.185%	-0.055%	0.994	0.349%	-0.006%	0.997	0.087%	0.005%
energy	0.95	16.4 cal/g	-6.33 cal/g	0.92	23.8 cal/g	-17.0 cal/g	0.94	15.8 cal/g	0.16 cal/g
fat							0.87	0.13%	0.01%

[a] r = coefficient of correlation. [b] SEP = standard error of performance; i.e. standard deviation of the difference between NIRS and chemical results. [c] $\bar{d}$ = mean difference between NIRS and chemical results.

Table X. Accuracy of Analysis by FQA 51A

	barley			wheat			corn		
	r[a]	SEP[b]	$\bar{d}$[c]	r	SEP	$\bar{d}$	r	SEP	$\bar{d}$
				A. Factory Algorithms					
starch	0.93	1.36%	0.22%	0.97	1.00%	-0.31%	0.762	0.825%	-0.15%
protein	0.992	0.295%	-0.127%	0.986	0.529%	0.015%	0.992	0.160%	+0.160%
energy	0.90	23.7 cal/g	-5.36 cal/g	0.92	25.1 cal/g	-17.54 cal/g	0.802	26.03 cal/g	-17.54 cal/g
fat							0.765	0.185%	-0.02%
				B. Optimized Algorithms					
starch	no change			0.98	0.69%	0.41%	0.90	0.66%	0.10%
protein	no change			0.992	0.37%	0.07%	0.998	0.09%	-0.01%
energy	0.97	21.9	-4.41 cal/g	0.95	21.0	-19.8 cal/g	0.98	11.2	-0.65 cal/g
fat							0.91	0.13%	0.04%

[a] r = coefficient of correlation. [b] SEP = standard error of performance; i.e., standard deviation of the difference between NIRS and chemical results. [c] $\bar{d}$ = mean difference between NIRS and chemical results.

Table XI. Accuracy of Analysis by DICKEY-john GAC III

	barley			wheat			corn		
	r[a]	SEP[b]	$\bar{d}$[c]	r	SEP	$\bar{d}$	r	SEP	$\bar{d}$
starch	0.85	1.39%	0.09%	0.92	0.72%	0.26%	0.79	0.68%	0.29%
protein	0.993	0.278%	-0.172%	0.994	0.178%	0.017%	0.997	0.106%	0.082%
energy	0.95	24.0 cal/g	-5.45 cal/g	0.89	16.0 cal/g	-6.10 cal/g	0.93	17.9 cal/g	9.95 cal/g
fat							0.88	0.131%	0.072%

[a] r = coefficient of correlation. [b] SEP = standard error of performance; i.e., standard deviation of the difference between NIRS and chemical results. [c] $\bar{d}$ = mean difference between NIRS and chemical results.

ative format, the results obtained with the FQA 51A improved considerably to the extent that they were comparable with those of the RCA 6250.

Using the RCA 6250, the second derivative of the log $(1/R)$ gave the most accurate results for the prediction of starch in all grains, also protein and energy in barley, and energy and oil in corn. For the prediction of starch and protein, most of the prominent wavelengths were associated with protein bands. The equation for the prediction of oil employed oil bands, while protein and starch bands were employed in the prediction of energy in corn and barley. In the case of wheat, one of the bands used in the prediction of energy was an oil band. For the prediction of protein in wheat with the RCA 6250, the first derivative of log $(1/R)$ gave the best results.

Analysis by DICKEY-john GAC III. Calibration coefficients used for analysis by DICKEY-john GAC III are presented in Table VIII. High positive K_3 values, which are related to absorption at the starch band (2100 nm), were associated with starch determination in all grains. For the protein determination, high positive K_2 values and negative K_3 values were used in all grains. This indicated that absorption at the protein band (2180 nm) was positively correlated and absorption at the starch band (2100 nm) negatively correlated with protein content. As in protein determination, it was found that, in general, positive K_2 values and negative K_3 values were used for energy analysis, consistent with the positive correlations between protein and energy and negative correlation between starch and energy wet chemistry values. These observations were consistent with results obtained in this study. For corn oil, the calibration coefficient of K_0 was the highest, confirming that absorption at 2310 nm (oil band) was the most closely related to oil determination.

Accuracy of Analysis. Accuracy of analysis by RCA 6250, FQA 51A, and DICKEY-john GAC III is shown in Tables IX–XI, respectively.

In general, high correlation coefficients and low standard errors of performance (SEP) values were obtained for all the constituents in feed grains used in this study with the RCA 6250, FQA 51A, or the DICKEY-john GAC

III. The correlation coefficients for starch analysis in all grains by DICKEY-john GAC III were a little lower than those from the RCA 6250 or the FQA 51A. However, accuracies of predictions for protein and energy were excellent for all the samples with the three NIRS instruments, indicating a high degree of predictability.

In starch analysis, correlation coefficients for wheat were the highest and those for corn samples the lowest with use of all three NIRS instruments. This was attributed to the fact that the range in concentration for wheat starch was the widest and that for corn starch the narrowest. Starch analysis statistics were slightly superior to data reported in the literature for starch analysis in the commodities referred to earlier.

The accuracies for determination of protein were all excellent. The use of the three NIRS instruments for the analysis of energy gave similar results in wheat and barley, but a lower coefficient of correlation with corn, even though the coefficient of variability (0.6) was very good. Accuracy of analysis for corn oil was acceptable in spite of the narrow range in concentration. The corn results for most constituents all improved when the derivative was optimized. On the basis of results from the present study, it was concluded that NIRS can be applied in the feed industry for the rapid and accurate determination of the starch, protein, and energy values. The best overall results were obtained with the RCA 6250. Despite the extra expense of this type of instrument, the versatility of wavelength and mathematical treatment available indicates that such equipment could be justified in an operation that utilizes a very wide diversity of raw materials.

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LITERATURE CITED

Aman, P.; Hesselman, K. Analysis of Starch and Other Main Constituents of Cereal Grains. *Swed. J. Agric. Res.* **1984**, *14*, 135–139.

American Association of Cereal Chemists. *Approved Methods of the AACC*; AACC: St. Paul, MN, Oct 1975 (revised Oct 1981); Method 44-15A.

American Association of Cereal Chemists. *Approved Methods of the AACC*; AACC: St. Paul, MN, Oct 1976 (revised Nov 1983); Method 46-12.

Baker, D. The Determination of Fiber, Starch, and Total Carbohydrate in Snack Foods by Near-infrared Reflectance Spectroscopy. *Cereal Foods World* **1985**, *30*, 389–392.

Beutler, H. O.; Bergmeyer, H. U. Starch. In *Methods of Enzyme Analysis*, 3rd ed.; Verlag Chemie: Weinheim, FRG, 1984; Vol. 6, pp 2–10.

Bruinsma, B. L.; Rubenthaler, G. Lysin Estimation in Cereals by Near-infrared Reflectance. *Corp. Sci.* **1978**, *18*, 1039.

Candlish, E. Animal Feeding. Special Report, The Canadian Wheat Board, 1974.

Davies, A. M. C.; Coxon, D. T.; Gavrel, G. M.; Wright, D. J. Determination of Starch and Lipid in Pea Flour by Near-infrared Reflectance Analysis. The Effect of Pea Genotype on Starch and Lipid Content. *J. Sci. Food Agric.* **1985**, *36*, 49–54.

Downey, G. Estimation of Moisture in Undried Wheat and Barley by Near-infrared Reflectance. *J. Sci. Food Agric.* **1985**, *36*, 951–958.

Downey, G.; Byrne, S.; Dwyer, E. Wheat Trading in the Republic of Ireland: The Utility of a Hardness Index Derived by Near-infrared Relfectance Spectroscopy. *J. Sci. Food Agric.* **1986**, *37*, 762–766.

Gaines, C. S.; Miller, R. E.; Donelson, J. R.; Bean, M. M. Optimizing Grinder Type and Methods of Expressing Wheatmeal Particle Size for Wheat Texture (Hardness of Softness) Measurement and Near-infrared Reflectance Spectroscopy. *Cereal Chem.* **1987**, *64*, 46–49.

Hilliard, J. H.; Daynard, T. B. Measurement of Protein and Oil in Grains and Soybeans with Reflected Near-infrared Light. *Can. Inst. Food Sci. Technol.* **1976**, *9*, 11–14.

Hymowitz, T.; Dudley, J. W.; Collins, F. E.; Brown, C. M. Estimation of Protein and Oil Concentration in Corn, Soybean and Oat Seed by Near-infrared Light Reflectance. *Crop Sci.* **1974**, *14*, 713–715.

Krischenko, V. P. Use of Near-infrared Reflectance Spectroscopy in Investigations in the U.S.S.R. In *Near-infrared Technology in the Agriculture and Food Industries*; Williams, P. C., Norris, K. H., Eds.; The American Association of Cereal Chemists: St. Paul, MN, 1987.

Law, D. P.; Tkachuk, R. Near-infrared Diffuse Reflectance Spectra of Wheat and Wheat Components. *Cereal Chem.* **1977**, *54*, 256–265.

Norris, K. H.; Williams, P. C. Optimization of Mathematical Treatments of Raw Near-infrared Signal in the Measurement of Protein and Moisture in Hard Red Spring Wheat. I. Influence of Particle Size. *Cereal Chem.* **1984**, *61*, 158–165.

Osborne, B. G. Applications of NIR in the Baking Industry. *Anal. Proc. (London)* **1983**, *20*, 79–83.

Salomonsson, A.; Theander, O.; Westerlund, E. Chemical Characterization of Some Swedish Cereal Wholemeals and Bran Fractions. *Swed. J. Agric. Res.* **1984**, *14*, 111–117.

Saurer, W. Use of Infrared Reflectance Measurement for the Determination of Protein and Moisture Content and Kernel Hardness in Wheat. *Getreide, Mehl, Brot.* **1978**, *32*, 272–276 (in German).

Shia, G.; Slinkard, A. E. Relationship of Seed Shape and Cotyledon Colour to Percent Protein of Peas. *Corp. Sci.* **1977**, *17*, 183–184.

Simmonds, D. H. Structure, Composition and Biochemistry of Cereal Grains. In *Cereals '78: Better Nutrition for the World's Millions*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1978.

Tkachuk, R. Protein Analysis of Whole Wheat Kernels by Near-infrared Reflectance. *Cereal Foods World* **1981**, *26*, 584–587.

Williams, P. C. Application of Near-infrared Reflectance Spectroscopy to Analysis of Cereal Grains and Oilseeds. *Cereal Chem.* **1975**, *52*, 561–576.

Williams, P. C. Screening Wheat for Protein and Hardness by Near-infrared Reflectance Spectroscopy. *Cereal Chem.* **1979**, *56*, 169–172.

Williams, P. C. Analytical Tests for Grain Quality. In *CRC Handbook of Transportation and Marketing in Agriculture. Field Crops*; Finney, E. E., Jr., Ed.; CRC Press: Baton Rouge, LA, 1981; Vol. II.

Williams, P. C.; Thompson, B. N. Influence of Wholemeal Granularity on Analysis of HRS Wheat for Protein and Moisture for Near-infrared Reflectance Spectroscopy (NIRS). *Cereal Chem.* **1978**, *55*, 1014–1037.

Williams, P. C.; Norris, K. H. Effect of Mutual Interactions on the Estimation of Protein and Moisture in Wheat. *Cereal Chem.* **1983**, *60*, 202–207.

Williams, P. C.; Sobering, D. C. Attempts at Standardization of Hardness Testing of Wheat. II. The Near-infrared Reflectance Method. *Cereal Foods World* **1986**, *31*, 417–420.

Williams, P. C.; Cordeiro, H. M. Effect of Calibration Practice on Correction of Errors Induced in Near-infrared Protein-testing of Hard Red Spring Wheat by Growing Season. *J. Agric. Sci.* **1985**, *104*, 113–123.

Williams, P. C.; Norris, K. H.; Zarowsky, W. S. Influence of Temperature on Estimation of Protein and Moisture in Wheat by Near-infrared Reflectance. *Cereal Chem.* **1978**, *59*, 473–477.

Williams, P. C.; Norris, K. H.; Gehrke, C. W.; Bernstein, K. Comparison of Near-infrared Methods for Measuring Protein and Moisture in Wheat. *Cereal Foods World* **1983**, *28*, 149-152.

Williams, P. C.; Preston, K. R.; Norris, K. H.; Starkey, P. M. Determination of Amino Acids in Wheat and Barley by Near-infrared Reflectance Spectroscopy. *J. Food Sci.* **1984**, *49*, 17-20.

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Inhibitory Effect of Phenolics on Carotene Bleaching in Vegetables

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Lipoxygenase is known to be involved in the indirect oxidation of carotene, and some phenolic compounds are known to prevent lipoxygenase activity. Inhibitory potencies of major phenolics in vegetables on carotene oxidation were studied by using both a model system and various vegetables. Catechin and epicatechin were the highest and *p*-coumaric and ferulic acids were the lowest in inhibitory efficacy. In general, in a model system flavans showed the highest inhibitory effect followed by flavonols and acidic phenolic compounds. The concentration of phenolics in vegetables appeared to have a high correlation with the inhibitory effect on carotene bleaching, in general, but spinach, which contains the highest concentration of phenolics among vegetables studied, did not show the same level of inhibitory potencies. This may be due to the fact that spinach phenolics consist mainly of methylated flavones and *p*-coumaric derivatives, compounds that have low inhibitory effects.

A recent report showed that the apparent increase in vitamin A value in canned peas as compared to fresh peas was not due to an actual increase but rather to the loss of carotenoids in fresh peas during analysis (Edwards and Lee, 1986). The endogenous enzyme system in raw vegetables was shown to have carotene-oxidizing activity (Booth, 1960). Lipoxygenase oxidizes fatty acids and produces peroxides that oxidize carotene by a secondary or coupled reaction (Walsh and Hauge, 1953). The addition of antioxidants, such as pyrogallol, during the analysis for carotene resulted in higher carotene values in fresh vegetables (Ueno et al., 1982). Certain phenolic compounds are known to prevent lipoxygenase activity (Rhee and Watts, 1966; King and Klein, 1987; Takahama, 1985). Lipoxygenase activity and phenolic content vary greatly among vegetables, with the result that carotene bleaching is affected (Lee and Smith, 1988). Little work, however, has been done to determine the effect of different phenolic groups on carotene bleaching by lipoxygenase in vegetables. In this study, the carotene bleaching effect of several major phenolics was investigated by using both a model system and several different vegetables.

MATERIALS AND METHODS

Materials. Spinach, broccoli, carrots, green beans, and peas were obtained from a local market. The β-carotene (1% CWS) was a gift from Hoffmann-LaRoche Chemical Co. The phenolic standards, (+)-catechin, (−)-epicatechin, phloretin glucoside, phloretin, quercetin, rutin, chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid, linoleic acid, lipoxygenase, and Tween 80 were purchased from Sigma Chemical Co. Procyanidin B_2 was isolated from grapes (Lee and Jaworski, 1987).

Table I. Relative Inhibitory Effects (%) of Various Phenolics on Carotene Bleaching by Lipoxygenase

phenolics	concn 1.25 mM	2.5 mM	5 mM
(+)-catechin	45.0	75.2	96.4
(−)-epicatechin	33.4	61.6	97.0
procyannidin B_2	29.0	*a*	*a*
phloretin glucoside	7.8	10.9	17.0
phloretin	33.4[b]		
rutin	10.9[b]		
quercetin	11.2[b]		
chlorogenic acid	8.8	14.1	23.2
caffeic acid	9.2	15.9	26.6
p-coumaric acid	2.4	5.4	11.4
ferulic acid	2.0	5.0	8.8

[a] Precipitation took place. [b] Saturated solution, <0.02 mM.

Inhibitory Effect of Phenolics in Model Systems. All phenolic compounds, except for quercetin, rutin, and phloretin, were individually dissolved in pH 7 McIlvaine buffer at concentrations of 2.5, 5, and 10 mM. Quercetin, rutin, and phloretin were made to only one concentration (saturated, <0.2 mM) due to their poor solubilities. β-Carotene (840 μg/mL) was dissolved in McIlvaine's buffer at pH 7; linoleic acid (1.2 mg/mL) was dissolved in the same buffer except that it contained 800 μg/mL Tween 80. Lipoxygenase (1 mg/mL, 126 500 units/mg) was dissolved in distilled water.

Fifteen microliters of lipoxygenase solution was added to a solution containing 0.5 mL of β-carotene, 0.5 mL of linoleic acid,

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