

NIR Transmittance Estimation of Free Lipid Content and Its Glycolipid and Digalactosyldiglyceride Contents Using Wheat Flour Lipid Extracts¹

J. B. Ohm^{2,3} and O. K. Chung²⁻⁵

ABSTRACT

Cereal Chem. 77(5):556–559

Free lipids (FL) in 72 hard winter wheat flours were extracted and dissolved in hexane. Absorbance (log 1/T) values were measured using a scanning spectrophotometer with a 2-mm cuvette, and used to develop calibration models for estimating flour FL content and glycolipid (GL) and digalactosyldiglyceride (DGDG) contents. Fifty-one calibration samples were selected based on the cutoff point of 0.3 of Mahalanobis distance, and the remaining twenty-one samples were used for validation. The best

model for the estimation of FL content showed coefficients of determination (R^2) of 0.95 for the calibration set and $R^2 = 0.89$ for the validation set. Glycolipid contents could be estimated by a model which had $R^2 = 0.87$ for the calibration set and $R^2 = 0.89$ for the validation set. For DGDG, the best model showed $R^2 = 0.94$ for the calibration set and $R^2 = 0.88$ for the validation set.

Free lipids (FL) in wheat flour have been recognized as important quality factors in breadmaking (Chung et al 1978, Chung et al 1980a,b; Chung and Ohm 1997). The glycolipids (GL) of FL have a significant linear correlation with bread loaf volume and a curvilinear relationship with mixing time (Chung et al 1982). Although lipids are a minor component of flour, the significant relationship with baking properties emphasizes the importance of lipid analysis for flour quality evaluation. However, safety and environmental impact are concerns in lipid analysis which usually requires large amounts of toxic, flammable or carcinogenic organic solvents. The tedious and time-consuming procedure involved in lipid analysis has also impeded the routine determination of flour lipid contents and compositions in wheat breeding programs or in marketing channels.

Norris (1964) first applied near-infrared (NIR) spectroscopy to the determination of moisture. Recently, the technique has been used to analyze the biochemical constituents of numerous agricultural products. In an earlier study, Holman and Edmondson (1956) measured and reported the NIR absorption spectra of several fatty acids and other lipids at 900–3,000 nm, pointing out the possible use of NIR in lipid analysis. Goddu and Delker (1960) summarized spectral absorption bands and the correlation of spectra and structure in the NIR region. Law and Tkachuk (1977) reported the identification of NIR bands for wheat lipids and other contributing groups. However, Simmons (1985) reported that the direct determination of flour GL by NIR spectroscopy has not been satisfactory, probably because of the low concentration of flour lipids. Simmons (1985) tried to determine flour GL content using a filter disk technique in which a paper disk was coated with the extracted lipids and then analyzed.

Recently, NIR technology has achieved great advances in instrumentation and data processing (Martin 1992) that offer great potential for determining the lipid contents of flours. Unlike oilseed or corn grains, the lipid content in wheat flour is quite low and unable to be measured with high accuracy directly from flour using NIR technology. The main objective of this research was to determine FL, GL, and digalactosyldiglyceride (DGDG) contents in flour by NIR transmittance spectroscopy using a liquid cuvette. Hexane and petroleum ether were used for dissolving FL instead of carbon tetrachloride because they are less toxic (Sax and Lewis 1988).

MATERIALS AND METHODS

Materials

Straight-grade flours were obtained by milling 72 wheat samples. Winter wheat cultivars used in this study were Arlin, Cimmaron, Discovery, Karl, Karl 92, Newton, Scout 66, TAM 107, TAM 200, Tomahawk, Voyager, and 2163 harvested in Ellis, Franklin, Greeley, Labette, Stafford, and Thomas Counties in Kansas in 1993. All organic solvents and reagents used in this experiment were chromatographic and analytical grade, respectively. Quality data for these wheat samples were previously reported (Ohm et al 1998, Ohm and Chung 1999a).

Extraction and Fractionation of FL

FL were extracted from flour with petroleum ether using a Soxhlet system (Chung et al 1980a). Flour samples (12.5 g, db) were extracted for 16 hr at a solvent condensation rate of two to three drops per second. After petroleum ether was reduced to ≈ 3 mL by a rotary evaporator, the remaining material was dried under a nitrogen gas stream. The FL was fractionated by column fractionation, and the GL fractions were analyzed using HPLC according to methods of Ohm and Chung (1999b). A solid-phase extraction (SPE) system using a prepacked column (Mega Bond Elut Bonded Phase SI 1GRM, Varian) with a vacuum manifold (Supelco Inc., Bellefonte, PA) was used to fractionate FL into nonpolar lipid (NL), GL, and phospholipid (PL) fractions. After the columns were conditioned with 5 mL of chloroform, flour FL were applied in 2 mL of chloroform. The NL, GL, and PL fractions were separated by successive elution with 10 mL of a chloroform-acetone mixture (4:1, v/v), followed by 15 mL of an acetone and methanol mixture (9:1, v/v), and 10 mL of methanol, respectively. The flow rate of the SPE was adjusted to 0.7 mL/min by applying a 2.5-in. (6.35 mmHg) vacuum using a vacuum manifold. The gravimetric recovery of fractionated lipids was 95–100%. After the solvent was evaporated to ≈ 5 mL with a rotary evaporator, the separated lipid fractions were dried under a nitrogen stream. The purity of fractionated lipids was determined by thin-layer chromatography. Fractionated lipids (≈ 100 μ g) were applied to thin-layer chromatography plates (G 20

¹ Cooperative investigations, USDA, Agricultural Research Service, and the Department of Grain Science and Industry, Kansas State University. Contribution 98-480-J from the Kansas Agricultural Experiment Station, Manhattan, KS 66506. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

² Graduate research assistant and professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.

³ Present address: USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS 66502.

⁴ Supervisory research chemist, USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS 66502.

⁵ Corresponding author. Phone: 785-776-2703. Fax: 785-537-5534. E-mail: okchung@usgmrl.ksu.edu

× 20) that were developed in a mixture of chloroform, methanol, and water (65:25:4, v/v). Lipids were detected by spraying the plate with 0.2% α -naphthol in ethanol followed by a light spray of 95% sulfuric acid. After the plate was heated to 120°C, GL were identified as dark gray purple spots (data not shown).

HPLC of GL Fraction

HPLC of GL fractions was performed according the method developed by Ohm and Chung (1999b). A liquid chromatograph (HP 1090, Hewlett-Packard, Palo Alto, CA) was used with a short analytical column (HP Hypersil 5 μ m, 100 × 4.6 mm, i.d.). The GL fractions obtained by SPE separation were diluted to 1 mL with hexane, and 20 μ L of diluted solution was injected.

Monogalactosyldiglycerides (MGDG) and DGDG were separated using a gradient of hexane, isopropanol, and water. An evaporative light-scattering detector (ELSD) (Varex MK III, Deerfield, IL) was used to measure GL. The drift tube temperature was adjusted to 110°C with an exhaust temperature of 45°C. The nitrogen gas flow rate was set at 2.00 standard L/min. Calibration equations were derived using 30, 50, 70, 90, and 110 μ g of standard MGDG and DGDG, based on peak areas obtained from ELSD. The accuracy and precision of the reference methods were reported previously (Ohm and Chung 1999b).

NIR Scanning

The dried FL were diluted to 5.0 mL using hexane, and spectra of FL-hexane solutions were collected using a scanning spectrophotometer (6500 NIRSystems, Inc., Silver Springs, MD). Log (1/T) value of each FL-hexane solution was measured at 400–2,498 nm at 8-nm intervals using a 2-mm liquid cuvette. The collected spectra were smoothed by four-point fast Fourier transformation. All analyses were duplicated and the average spectrum of the duplicates was used for calibration, cross validation, and validation.

TABLE I

Mean ($n = 72$), Standard Deviation, and Range for Free Lipid Content of Flour (mg/10 g flour)

Free Lipids ^a	Mean	Standard Deviation	Range
Unfractionated FL	99.8	7.0	86.1–115.0
Nonpolar lipids	76.2	5.5	65.8–88.9
Glycolipids	14.1	2.5	8.3–19.2
MGDG	2.5	0.7	0.5–4.3
DGDG	6.0	1.3	2.7–9.3
Phospholipids	6.6	1.3	3.5–9.8
Polar lipids	20.7	3.4	12.9–27.1

^a FL= free lipids, MGDG = monogalactosyldiglycerides; DGDG = digalactosyldiglycerides; and polar lipids = glycolipids + phospholipids.

Selection of Samples for Calibration

Samples for calibration were selected using SELECT software (Version 3.0 Infracsoft International, NIRSystems). The spectra were derivatized using math treatment 1, 10, 5, 1, in which the first 1 is the order of derivatives, 10 is the gap data points over which the derivative was taken, 5 is the number of data points used in performing average smoothing, and the second 1 is the number of smoothings taken. The math treatment was 1, 10, 5, 1 by default. The distance between the spectra of two samples was measured using principal components calculated from spectral data (Shenk and Westerhaus 1991a). Fifty-one calibration samples were selected based on the cutoff point of 0.3 of Mahalanobis distance that showed the best results in this experiment (data not shown).

Calibration and Validation

Calibration, cross validation, and validation were performed using routine operation and calibration software (Version 3.0 Infracsoft International, NIRSystems). Log (1/T) spectra were transformed mathematically by detrend and standard normal variate (SNV) transformation, multiplicative scattering correction (MSC), or weighted MSC (WMSC). The first and second derivatives of spectra also were taken with four-point (1, 4, 4, 1) and six-point gaps (2, 6, 4, 1) respectively, using the transformed spectra (Hruschka 1987) after moving the average through four points.

Stepwise multiple regression (SMR) and modified partial least square (MPLS) were used to develop calibration models, using the spectra of samples selected by the SELECT procedure (Shenk and Westerhaus 1991a). Cross validation was performed to eliminate outliers and to determine the optimum number of terms. Cross validation was repeated by default. Each time a different three-fourths

TABLE II

Range, Mean, and Standard Deviation of Free Lipid Content of Calibration and Validation Sets for Near-Infrared Analysis

Lipids and Set	n	Range	Mean	SD ^a	CV ^b (%)
Unfractionated free lipids (mg/10 g of flour, db)					
Calibration	51	86.1–115.0	100.2	7.5	7.5
Validation	21	87.6–110.1	98.9	5.7	5.8
Glycolipids (mg/10 g of flour, db)					
Calibration	51	8.3–19.2	14.1	2.6	18.4
Validation	21	9.3–18.4	14.4	2.5	17.4
Digalactosyldiglycerides (mg/10 g of flour, db)					
Calibration	51	3.1–9.3	6.0	1.2	20.0
Validation	21	2.7–7.4	6.1	1.3	21.3

^a Standard deviation.

^b Coefficient of variance.

TABLE III

Results of Near-Infrared (NIR) Calibration and Validation Sets for Free Lipid Contents

Calibration Method ^a	Spectra Transformation ^b	No. of Terms	Calibration			Validation		
			n	SEC ^c	SECV ^d	R^2	SEP ^e	r^2
Unfractionated free lipids (mg/10 g of flour, db)								
SMR	2nd derivative	6	49	1.69	1.85	0.95	2.20	0.90
MPLS	MSC	9	46	1.71	1.98	0.95	2.06	0.89
Glycolipids (mg/10 g of flour, db)								
SMR	Detrend and SNV 1st derivative	4	51	0.92	0.95	0.87	0.88	0.89
MPLS	MSC	9	46	0.49	0.68	0.97	0.92	0.86
Digalactosyldiglycerides (mg/10 g flour, db)								
SMR	Detrend and SNV 2nd derivative	3	49	0.37	0.43	0.89	0.55	0.86
MPLS	2nd derivative	6	47	0.28	0.48	0.94	0.48	0.88

^a SMR = stepwise multiple regression, MPLS = modified partial least square.

^b MSC = multiplicative scattering correction; SNV = standard normal variate; 1st derivative = 1, 4, 4, 1; 2nd derivative = 2, 6, 4, 1.

^c Standard error of calibration.

^d Standard error of cross validation.

^e Standard error of performance ($n = 21$).

of the calibration samples were used to calibrate and to predict the other one-fourth of the samples, allowing up to 12 terms. When outliers with large residuals (T value > 2.5 or H value > 10) were detected, they were removed, and then cross validation was performed again. The cycle of cross validation to eliminate outliers was done a maximum of two times. The optimum number of terms was determined in the final cross validation based on standard error of the cross validation. Validation was performed on the remaining 21 samples after selecting the calibration samples. The performance of the model was determined by the following statistics: standard error of calibration (SEC), standard error of cross validation ($SECV$), standard error of performance (SEP), coefficient of determination (R^2), and linear correlation coefficient (r) between reference values and values estimated by prediction models developed from NIR scans.

RESULTS AND DISCUSSION

Ranges of Flour FL

The means, ranges, and standard deviations of FL contents of 72 flours are summarized in Table I. Tweeten et al (1981) reported a range of 2.9–11.0 mg/10 g of flour (db) for the DGDG content

of flours that varied widely in baking quality. The DGDG content (2.7–9.3 mg/10 g of flour) in FL was slightly narrower in this experiment because flours from only released cultivars were analyzed.

Because NIR spectroscopy is calibrated on an empirical basis, the calibration sample set should be representative and structured to cover all the variations present (Shenk and Westerhaus 1991a,b). Sample sets for the calibration were selected using the NIR spectra of samples (Naes 1987; Shenk and Westerhaus 1991a,b). Among the 72 samples analyzed in this study, 51 samples were selected for the calibration set using the SELECT program. The remaining 21 samples were used as the validation set. The mean values, ranges, standard deviations, and coefficients of variance for the two sets are summarized in Table II. The variations in FL and lipid fractions were typical of variations found in hard winter wheat cultivars: Chung et al (1982) reported a mean of 94.0 mg/10 g of flour, a range of 83.4–108.7 mg/10 g of flour and a standard deviation of 2.1 for the FL of 23 hard winter wheat flours. Thus, even though the 21 samples were not independently collected samples, typical variation in FL content and composition justified their use as the validation set.

Calibration Models for FL Contents

The statistical evaluations of calibration and validation for total unfractionated FL are summarized in Table III. Both SMR and MPLS were effective as modeling methods for the estimation of FL contents. The model estimated by SMR with the second derivatives of spectra without scatter correction showed $R^2 = 0.95$ for the calibration. The model performance was satisfactory showing $r^2 = 0.90$ for the validation set (Fig. 1A). One model developed by MPLS using spectra transformed by MSC performed well, showing $R^2 = 0.95$ and 0.89 for the calibration and validation sets, respectively, and SEC of 1.71 and SEP of 2.06; $SECV = 1.98$ (Table III).

The models developed for free GL contents were less accurate than the models for FL contents (Table III). The best model among them was obtained by SMR using the first derivatives of spectra transformed by detrend and SNV. The model showed an $R^2 = 0.87$ and $SEC = 0.92$ and an $r^2 = 0.89$ and $SEP = 0.88$ for the validation set as shown in Table III and Fig. 1B. A model that was also developed by SMR from the spectra of $\log(1/T)$ without scatter correction performed well, showing an $R^2 = 0.87$ for both calibration and validation sets (data not shown). The models derived by MPLS usually had high R^2 and lower SEC and $SECV$ values for the calibration set, but performances of these models were not quite satisfactory due to the low r^2 and high SEP values for the validation set (data not shown). This result suggested that the developed models could not be robust. A model for GL estimated by MPLS from the spectra transformed by MSC performed well, showing $R^2 = 0.97$ and 0.86 for the calibration and validation sets, respectively, and $SEC = 0.49$, $SEP = 0.92$, and $SECV = 0.68$ (Table III). Both models in Table III were accurate enough to apply them for estimating the free GL contents for flour quality evaluation in breeding programs.

Models developed by MPLS estimated free DGDG contents more accurately than those developed by SMR (Table III). Based on the results of the validation set, the model developed by MPLS

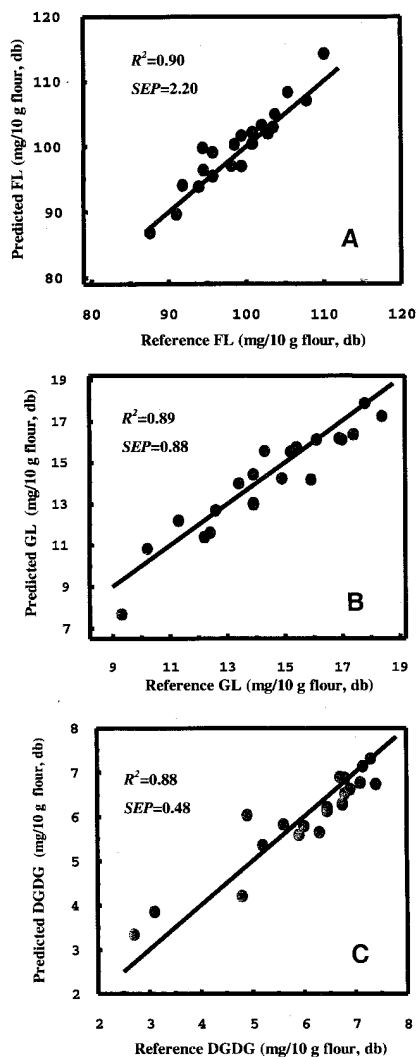


Fig. 1 Comparison of free lipid contents in hard winter wheat flours determined by prediction models of near-infrared transmittance spectroscopy and by reference methods on a validation set ($n = 21$). **A**, total free lipid contents (FL), calibrated by stepwise multiple regression with 2nd derivative; **B**, glycolipid content (GL), calibrated by stepwise multiple regression with detrend and standard normal variate and 1st derivative; **C**, digalactosyldiglyceride contents (DGDG), calibrated by modified partial least square with 2nd derivative.

TABLE IV
Wavelengths Included in Calibration Models by Stepwise Multiple Regression for Free Lipid Contents

Free Lipids ^a	Spectra Transformation ^b	Wavelengths (nm)
Unfractionated free lipids	2nd derivative	584, 840, 1868, 1876, 1,988, 2,484
Glycolipids	Detrend and SNV 1st derivative	1,540, 1,588, 1,724, 2,020
DGDG	Detrend and SNV 2nd derivative	1,388, 1,860, 2,164

^a DGDG = digalactosyldiglycerides.

^b SNV = standard normal variate.

using the second derivatives of spectra without any scattering correction gave the best results among the developed models. An MPLS model developed from the second derivatives of spectra showed an $R^2 = 0.94$ and $SEC = 0.28$ for the calibration set and $SECV = 0.48$ (Table III); the model showed good performance with $r^2 = 0.88$ and $SEP = 0.48$ for the validation set (Fig. 1C). Also, an MPLS model developed from the spectra of $\log(1/T)$ showed an $r^2 = 0.90$ and $SEP = 0.44$ for the validation set and an $R^2 = 0.87$, $SEC = 0.43$, and $SECV = 0.49$ for the calibration set (data not shown). The models developed by SMR using the second derivatives of spectra transformed by detrend and SNV performed well. However, this model also had a lower R^2 and higher SEC values for the calibration set when compared with those developed by MPLS. Shenk and Westerhaus (1991a) reported that the use of MPLS gave better or similar results when developing a model for the low concentration sample in comparison with SMR.

When calibrating estimation models, outliers were detected and deleted. No specific commonality was shown among outliers. The MPLS deleted samples as outliers, in addition to those deleted by the SMR procedures, and thus, MPLS calibration models showed higher R^2 values than those of SMR. We may speculate that one of reasons for outlier being deleted might be due to the unintended partial oxidation of lipids during experiments. The oxidation of lipids increased the weight of lipids and thus could cause overestimation of lipid contents.

The wavelengths selected by SMR are summarized in Table IV. These do not match the NIR bands of wheat lipids observed by Law and Tkachuk (1977). This might have been caused partly by the transformation of spectra by scattering correction and derivatization. Osborne (1988) also reported changes in NIR peaks and bands by spectral transformation. Interaction between chemical components might be another possible reason for the difference. Specifically, hexane was used to dissolve lipids in this experiment instead of carbon tetrachloride, which usually is applied to investigate lipid absorption bands, because of its transparency in the NIR region (Goddu and Delker 1960). The use of hexane might have changed the lipid peaks and bands of NIR spectra from those observed by other researchers. Hydrogen bonding has been known to influence the peaks in the NIR region (Martin 1992, Ciurczak 1995). The galactose groups in GL could contribute to select 1,540 and 1,588 nm that are in the range where carbohydrate could absorb (Law and Tkachuk 1977). Nonindicating wavelengths or neutral wavelengths also could be included in models to normalize the scattering effect (Hruschka 1987). The wavelengths $>2,200$ nm were not selected in calibration models of GL and DGDG developed by the SMR. This could be due to nonlinear relationships between lipid contents and transmittance of NIR $> 2,200$ nm that is caused by intense absorbance at that region. The NIR absorbance spectra becomes stronger as the wavelenths approach the mid-infrared region due to more appearance of combination bands. However, scattering correction of detrend and SNV would overcome the nonlinearity of the NIR region near the fundamental (mid-infrared) region (Barnes et al 1989). The calibration models for GL and DGDG were developed using NIR spectra transformed by detrend and SNV. Thus, nonlinearity of NIR $> 2,200$ nm could not be the reason that the SMR procedure selected only those wavelengths $<2,200$ nm for development of calibration models of GL and DGDG.

CONCLUSIONS

NIR transmission spectroscopy with a liquid cuvette of an FL solution in hexane can be used to quantify FL, GL, and DGDG contents in flour. Analysis time is short, and many samples can be

evaluated in a limited period. Therefore, this method will serve nicely in evaluating wheat flour quality in breeding programs. The method is also expected to improve safety by using less toxic and/or carcinogenic organic solvents.

LITERATURE CITED

- Barnes, R. J., Dhanoa, M. S., and Lister, S. J. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* 43:772-777.
- Chung, O. K., and Ohm, J. B. 1997. Wheat lipids as a quality determinant. Pages 83-100 in: *Proceedings of Wheat Quality Conference*. J. L. Steele and O. K. Chung, eds. Grain Industry Alliance: Manhattan, KS.
- Chung, O. K., Pomeranz, Y., and Finney, K. F. 1978. Wheat flour lipids in breadmaking. *Cereal Chem.* 55:598-618.
- Chung, O. K., Pomeranz, Y., Jacobs, R. M., and Howard, B. G. 1980a. Lipid extraction conditions to differentiate among hard red winter wheats that vary in breadmaking. *J. Food Sci.* 45:1168-1174.
- Chung, O. K., Pomeranz, Y., Shogren, M. D., and Finney, K. F. 1980b. Response to shortening addition and lipid removal in flours that vary in bread-making quality. *Cereal Chem.* 57:111-117.
- Chung, O. K., Pomeranz, Y., and Finney, K. F. 1982. Relation of polar lipid content to mixing requirement and loaf volume potential of hard red winter wheat flour. *Cereal Chem.* 59:14-20.
- Ciurczak, E. W. 1995. Use of near-infrared spectroscopy in cereal products. *Food Testing Anal.* (Oct-Nov):35-39.
- Goddu, R. F., and Delker, D. A. 1960. Spectra-structure correlations for the near-infrared region. *Anal. Chem.* 32:140-141.
- Holman, R. T., and Edmondson, P. R. 1956. Near-infrared spectra of fatty acids and some related substances. *Anal. Chem.* 28:1533-1538.
- Hruschka, W. R. 1987. Data analysis: Wavelength selection methods. Pages 35-55 in: *Near-Infrared Technology in the Agricultural and Food Industries*. P. Williams and K. Norris, eds. Am. Assoc. Cereal Chem. St. Paul, MN.
- Law, D. P., and Tkachuk, R. 1977. Near infrared diffuse reflectance spectra of wheat and wheat components. *Cereal Chem.* 54:256-265.
- Martin, K. A. 1992. Recent advances in near-infrared reflectance spectroscopy. *Appl. Spectrosc. Rev.* 27:325-383.
- Naes, T. 1987. The design of calibration in near infra-red reflectance analysis by clustering. *J. Chemometrics* 1:121-134.
- Norris, K. H. 1964. Simple spectroradiometer for 0.4 to 1.2-micron region. *Trans. ASAE* 7:240-242.
- Ohm, J. B., and Chung, O. K. 1999a. Gluten, pasting, and mixograph parameters of hard winter wheat flours in relation to bread-making. *Cereal Chem.* 76:606-613.
- Ohm, J. B., and Chung, O. K. 1999b. Estimation of free glycolipids in wheat flour by high performance liquid chromatography. *Cereal Chem.* 76:873-876.
- Ohm, J. B., Chung, O. K., and Deyoe, C. W. 1998. Single-kernel characteristics of hard winter wheats in relation to milling and baking quality. *Cereal Chem.* 75:156-161.
- Osborne, B. G. 1988. Comparative study of methods of linearization and scatter correction in near-infrared reflectance spectroscopy. *Analyst* 113:263-267.
- Sax, N. I., and Lewis, Sr., R. J. 1988. Pages 222, 600 in: *Hawley's Condensed Chemical Dictionary*. Van Nostrand Reinhold: New York.
- Shenk, J. S., and Westerhaus, M. O. 1991a. Population definition, sample selection, and calibration procedure for near infrared reflectance spectroscopy. *Crop Sci.* 31:469-474.
- Shenk, J. S., and Westerhaus, M. O. 1991b. Population structuring of near infrared spectra and modified partial least square regression. *Crop Sci.* 31:1548-1555.
- Simmons, C. T. 1985. The development and application of new infrared methods for determining the glycolipid fraction in hard red spring wheats. PhD dissertation. North Dakota State University: Fargo, ND.
- Tweeten, T. N., Wetzel, D. L., and Chung, O. K. 1981. Physicochemical characterization of galactosyldiglycerides and their quantitation in wheat flour lipids by high performance liquid chromatography. *J. Am. Oil Chem. Soc.* 58:664-672.

[Received September 30, 1999. Accepted May 15, 2000.]