Near-Infrared Reflectance Model for the Rapid Prediction of Total Fat in Cereal Foods

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AOAC method 996.01, used in cereal foods to determine total fat as defined by the U.S. Nutrition Labeling and Education Act (NLEA), is laborious and time-consuming and utilizes hazardous chemicals. Near-infrared (NIR) reflectance spectroscopy, a rapid and environmentally benign technique, was investigated as a potential method for the prediction of total fat using AOAC method 996.01 as the reference method. Near-infrared reflectance spectra (1104–2494 nm) of ground cereal products ($n = 72$) were obtained using a dispersive spectrometer, and total fat was determined according to AOAC method 996.01. Using multivariate analysis, a modified partial least-squares model was developed for total fat prediction. The model had a SECV of 1.12% (range = 0.5–43.2%) and a multiple coefficient of determination of 0.99. The model was tested with independent validation samples ($n = 36$); all samples were predicted within NLEA accuracy guidelines. The results indicate that NIR reflectance spectroscopy is an accurate means of determining the total fat content of diverse cereal products for nutrition labeling.

KEYWORDS: NIR; near-infrared; cereal foods; fat; lipid

INTRODUCTION

Fat is essential to a healthy diet and has important properties in food processing; however, a high intake of certain types of fat can lead to an increased risk of serious health conditions, especially when accompanied by obesity (*1–3*). Accurate nutrition labeling of foods such as cereal products can help consumers make informed food selection decisions and monitor the amount and type of fat in their diets. Thus, the U.S. Nutrition Labeling and Education Act (NLEA) of 1990 requires that all processed and packaged foods display the amount of total fat and saturated fat on the "nutrition facts" label (*4*). Total fat is defined by the NLEA as "total lipid fatty acids ... expressed as triglycerides" and is measured, in cereal foods, according to AOAC method 996.01 (*5, 6*). This method measures the total fat content of diverse cereal foods more accurately than gravimetric methods (*7*). AOAC method 996.01 involves the hydrolysis of fat components and subsequent extraction of previously bound and free lipids into ethyl and petroleum ethers, followed by evaporation of the ethers and saponification of the extract. The resulting fatty acids are esterified, and the quantity of individual fatty acid methyl esters is determined by capillary gas chromatography (GC).

AOAC method 996.01 is the method approved for U.S. nutrition labeling of total fat because of the complete and specific measurement of all fatty acids present in the food matrix. Earlier gravimetric methods (*8–10*) tended to over- or underestimate fatty acids in most cereal foods, depending on whether hydrolysis was included in the procedure (*7, 11*). These methods were, therefore, not acceptable for nutrition labeling purposes.

In contrast to earlier methods in which the lipid extract was determined gravimetrically, AOAC method 996.01 is arduous and time-consuming and requires the use of hazardous chemicals such as boron trifluoride and hydrochloric acid as well as diethyl and petroleum ethers (*5, 6*). An accurate technique that is rapid and does not require the use of hazardous chemicals would benefit consumers, industry, monitoring agencies, and the environment.

Near-infrared (NIR) reflectance spectroscopy is a rapid analytical technique that requires very little sample preparation, requires minimal labor, and does not require the use of chemicals or chemical disposal. These advantages parallel trends in industry toward reduction in bench time and consolidation of laborious procedures into one instrument (*12*). Near-infrared spectroscopy is used on a large scale in agriculture for the evaluation of cereal grain quality in the United States, Canada, and Europe (*12–14*) and has been applied extensively to foods (*14, 15*). The technique involves measuring sample absorptions in the NIR region of the electromagnetic spectrum (750–2500 nm). Absorption is affected by any property that affects bond strength such as the presence of aromatic groups, polar groups, and hydrogen bonding to neighboring atoms. A spectrum results from the reflected (or transmitted) energy at each wavelength

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and is usually expressed as log 1/reflectance (log $1/R$). The spectra of numerous samples can be modeled against an analyzed component and, then, using the model, the component can be predicted in new samples.

In addition to the assessment of cereal grain quality, NIR has been used to predict several nutritional components in a wide range of processed cereal products (*14*); however, studies on the prediction of fat content in cereal products have been limited. Osborne (*16*) used 44 cereal food samples (biscuits, breads, and breakfast cereals) to develop a single fat calibration and tested the calibration using 39 cereal food samples of similar types. Fat was measured gravimetrically after hydrolysis and solvent extraction of the sample, a method that has limitations for the analysis of total fat. Techniques involving acid hydrolysis, solvent extraction, and gravimetric analysis of lipid components can overestimate total fat content in cereal foods due to extraneous ether-soluble materials, which are included in the gravimetric measurement (*7, 11*). Kays et al. (*17*) used 45 representative cereal product samples in a modified partial least-squares model for the prediction of crude fat. NIR reflectance spectra were obtained using a dispersive monochromator, and crude fat was measured gravimetrically after Soxhlet extraction with petroleum ether (*10*). Again, the reference method has limitations in that the ether extraction—gravimetric analysis technique, in general, underestimates total fat (*18*) and is not accurate for the analysis of heat-treated cereal samples in which some lipids are not readily extracted by solvent alone (*7, 11*).

AOAC method 996.01 extracts lipid components more accurately than either of the two reference methods used for the NIR reflectance models described above and provides an analyte that meets the NLEA definition for total fat. The acid hydrolysis step of the procedure allows the release of bound lipids that are subsequently extracted. Although hydrolyzed, nonlipid components can be included in the extract, the specificity of the esterification and GC analysis in AOAC method 996.01 prevents nonlipid components being included in the measurement, allowing the measurement of both total and individual fatty acids. Consequently, AOAC method 996.01 is the approved method for the determination of total, saturated, unsaturated, and monounsaturated fat for nutrition labeling of cereal products.

It was proposed that a rapid NIR reflectance model could be developed that is sufficiently accurate for the prediction of total fat for nutrition labeling and monitoring using AOAC method 996.01 as the reference method. In contrast to previous models, this model will include measurement of all fatty acids in cereal products, as required by U.S. Nutrition labeling regulations. In addition, the range of fat content in the model developed will be considerably wider than in previous models, thereby reflecting the range of retail products available.

MATERIALS AND METHODS

Cereal Food Products. Four commercial retailers were the source of cereal samples for the study. Samples encompassed a wide range of fat contents, grain types, and processing methods and included breakfast cereals, crackers, cookies, granola bars, flours, snack chips, pastas, meal kits, sweet and savory snacks, and minimally processed grains. The oil ingredients present in the data set were vegetable shortening (partially hydrogenated soybean and or cottonseed oil); partially hydrogenated canola and sunflower oils; hydrogenated palm and coconut oils; canola, soybean, corn, sunflower, safflower, sesame, palm, and palm kernel oils; and butter. Grain types present in products utilized in the study were wheat, oats, corn, rice, millet, buckwheat, and multiple-grain mixtures including combinations of rye, barley, triticale, amaranth, and quinoa. Products contained a wide range of concentra-

tions for sugar, fat, dietary fiber, and protein and a variety of ingredients such as salt, cocoa, honey, dried vegetables, soy, dried fruits, nuts, spices, and herbs. Processing methods used in the formation of the products include baking, extrusion, milling, frying, and air puffing. Seventy-three products were purchased for calibration, and 36 were purchased, at a later date, for validation. The samples purchased for validation were not part of the calibration data set.

Sample Preparation and Handling. Cereal products with both a low fat (<10% fat) and low sugar (<20% sugar) content, based on nutrition label values, were dry milled to <500 μm with a Cyclotec 1093 cyclone mill (FOSS North America, Inc., Eden Prairie, MN). Low-fat, high-sugar (>20% sugar) products required the addition of liquid nitrogen to aid in grinding. High-fat (>10%) products were ground using a household coffee grinder (model BC G100WH, KitchenAid, St. Joseph, MI). Particle sizes of ground high-fat samples ranged from 180 to 1000 μm with the majority of particles <500 μm.

After grinding, samples were scanned to obtain NIR spectra and then stored overnight at −28 °C in low-density polyethylene bags. Total fat analysis (AOAC method 996.01) was performed the following day, and samples were subsequently stored at −70 °C in doubled low-density polyethylene bags to retard lipid oxidation. Milled cereal products were found to be stable when stored at −28 °C for at least 3 weeks; therefore, repeat sample analyses were performed within 3 weeks. Following total fat analysis, aliquots of fatty acid methyl esters in *n*-heptane were held overnight in Teflon-capped glass vials at −28 °C for repeat GC analysis the following day, if necessary.

Spectroscopic Analysis. To obtain NIR reflectance spectra, samples were scanned immediately after grinding in triplicate spectral analysis cells (internal diameter = 38 mm, depth = 9 mm) using a dispersive NIRSystems 6500 spectrometer (FOSS North America Inc.). The instrument was fitted with a spinning cup sampling device. Each cell was scanned 16 times, and the spectra were averaged and transformed to log($1/R$). After visual inspection, the spectra of the triplicate subsamples were averaged.

Reference Analysis. The total fat content of test samples and the National Institute of Standards and Technology (NIST, Gaithersburg, MD) SRM 1846 (infant formula) was determined according to AOAC method 996.01 (*5, 6, 19*). The standard reference material was analyzed along with each group of samples to check the accuracy of the reference procedure. A 2 g sample size is recommended for analysis, but sample size was reduced for samples containing >13% (based on the nutrition label declaration) total fat. Briefly, aliquots of each sample were weighed into duplicate Mojonnier tubes, the internal standard, tri-tridecanoin (T-3882, Sigma, St. Louis, MO) in chloroform was added, and the sample was then digested with hot 8 N HCl. The hydrolyzed fat components were extracted into diethyl and petroleum ethers while still in the Mojonnier tube (to minimize transfer loss). The diethyl ether and petroleum ether were then evaporated and the extracts saponified and esterified. The fatty acid methyl esters formed were analyzed in parallel with a fatty acid methyl ester standard (KEL-FIM-FAME-5 Metreya, Inc., Pleasant Gap, PA) using a Hewlett-Packard 5890 series II gas chromatograph (Agilent Technologies Inc., Palo Alto, CA) fitted with an autosampler and a Restek Rtx-2330 capillary column (10% cyanopropylphenyl—90% biscyanopropyl polysiloxane, 30 m × 0.25 mm internal diameter × 0.2 μm film thickness (Restek Corp., Bellefonte, PA). System settings for the gas chromatograph were an injector temperature of 250 °C and a detector temperature of 275 °C with gas flows of 34 mL/min for hydrogen and 300 mL/min for air with a split ratio of 100:1. Helium was the carrier gas with a linear velocity of 21 cm/s at 175 °C. The temperature program was an initial temperature of 120 °C for 4 min, which was then increased 5 °C/min until a final temperature of 230 °C was reached and held for 5 min. Total fat was calculated as the sum of individual fatty acids expressed as triglycerides.

Sample dry matter was determined using a forced-air oven (105 °C) according to AOAC method 935.29 (*20*). Reference values for total fat were expressed on a dry weight basis.

Calibration Development. The NIR reflectance model was developed using a commercial spectral analysis program (ISI40 NIRS3 version 4.01 and WINISI software, FOSS North America Inc.). Preprocessing of the spectral data (1104—2494 nm) for 73 samples

consisted of a normal multiplicative scatter correction (to partially remove scattering interferences due to particle size) followed by second-derivative processing with a gap (interval between the points subtracted) of 8 nm and a smoothing interval (width of the boxcar smooth) of 8 nm. The data were centered on the mean spectrum and mean reference value using modified partial least-squares (PLS) regression. The PLS regression used to develop the model is modified in that the reference values and reflectance data are scaled at each wavelength to have a standard deviation of 1.0 before each PLS regression term (*21*). One sample was eliminated from the calibration set, because of a high Mahalanobis distance (9.9). The sample was a baked cookie with a high fruit content and, unlike most samples, had a paste-like consistency after milling. The preprocessing methods chosen for the model were optimum to obtain minimum error following cross-validation (*22*) (20 cross-validation groups). The optimum number of PLS regression terms for the calibration was also determined by cross-validation and was the number of factors that gave the minimum error between predicted and reference values (standard error of cross validation, SECV) (*22*).

The modified PLS regression model was tested using independent validation samples ($n = 36$). Statistics used to assess the model were standard error of performance (SEP, not bias corrected), coefficient of determination (r^2), slope, and intercept of the linear regression of NIR-predicted versus AOAC-determined values (*23*). The ratio of deviation to performance or RPD (the standard deviation of the reference values divided by the SEP) was also calculated (*23*). An RPD of ≥ 5.0 generally indicates the model is suitable for quality control, an RPD of ≥ 6.5 indicates suitability for process control, and an RPD of ≥ 8.1 generally indicates suitability for any application.

Accuracy of the Model for U.S. Nutrition Labeling. NLEA regulations (*4*) for reporting total fat state that the product is not in compliance if "the nutrient content of the composite is greater than 20% in excess of the value for that nutrient declared on the food label". This is "provided that no regulatory action be based on a determination of a nutrient value that falls above this level by a factor less than the variability generally recognized for the analytical method used in that food at the level involved". For the nutrition label, amounts of fat per serving "shall be expressed to the nearest 0.5 g increment below 5 g and to the nearest gram increment above 5 g. If the serving contains less than 0.5 g, the content shall be expressed as zero".

If the regulation is applied to the NIR-predicted values obtained for total fat (the values that would appear on the nutrition label) for the validation samples, the NIR predictions would be in compliance with the NLEA as long as the predictions did not underestimate the actual total fat content of the cereal products by >20%. For the purposes of estimating compliance for the model, we assume that the AOAC-determined values for total fat content are the actual or real values. Thus, the error of the NIR prediction for total fat for each cereal sample was calculated as follows:

$$\text{NIR prediction error } (\%) = 100(p - a)/a$$

where p = NIR-predicted total fat and a = AOAC-derived total fat,

The prediction error was determined using AOAC- and NIR-derived values for both percent total fat and grams of fat per serving (using the serving size as stated on the product label). Negative values for prediction error indicate underestimation of the AOAC value by NIR. If the NIR prediction underestimates the AOAC value by >20%, the NIR prediction will not meet NLEA requirements, that is, unless the error or variability of the analytical method is greater than the amount by which the NIR prediction underestimates the AOAC value.

RESULTS

Reference Data. The ranges for total fat content in the calibration and validation data sets, using AOAC method 996.01, were 0.48−43.17 and 2.06−35.71%, respectively. The pooled standard deviation of the repeatability of the reference method (*24*) was 0.33% total fat. The distribution of grain types with range, mean, and standard deviation for total fat for each grain type in the calibration and validation data sets is given in **Tables 1** and **2**, respectively.

Table 1. Range, Mean, and Standard Deviation (SD) of Total Fat (Percent), Expressed as Triglycerides, in Cereal Food Products in the Calibration Data Set

cereal grain	n^a	range	mean	SD
wheat	28	2.0–42.9	17.3	10.6
oats	6	4.8–23.4	13.0	7.9
corn	7	0.5–38.1	20.7	13.8
rice	6	1.4–25.8	11.6	10.1
multiple[b]	25	2.3–43.2	15.5	9.9

[a] n = number of samples. [b] Cereal grains present in multiple-grain products followed by the number of products in which they occur (in parentheses) are wheat (19), oats (18), corn (8), rice (15), rye (7), barley (10), millet (1), triticale (3), buckwheat (2), amaranth (1), and quinoa (1).

Table 2. Range, Mean, and Standard Deviation (SD) of Total Fat (Percent), Expressed as Triglycerides, in Cereal Food Products in the Independent Validation Data Set

cereal grain	n^a	range	mean	SD
wheat	10	2.1–32.5	19.0	8.6
oats	5	6.9–27.0	17.5	7.4
corn	4	21.4–35.7	25.9	6.8
millet	1	4.3		
buckwheat	1	5.9		
multiple[b]	15	2.7–32.5	15.6	9.1

[a] n = number of samples. [b] Cereal grains present in multiple-grain products followed by the number of products in which they occur (in parentheses) are wheat (13), oats (12), corn (4), rice (8), rye (3), barley (6), millet (3), triticale (2), buckwheat (1), amaranth (0), and quinoa (0).

Spectra of Cereal Products. The spectra of cereal food samples had bands attributed to absorption by CH groups in oil at 1162, 1212, 1728, 1760, 2308, and 2346 nm. Other predominant absorption bands were observed at 1434 nm for the O−H stretch in crystallized sugar, at 1936 nm for the O−H stretch plus O−H deformation combination band in water, and at 2104 nm for the O−H deformation plus C−O stretch combination in starch (*15*, *25*). These bands are typical in spectra of processed cereal food products. Peak intensities at 1162, 1212, 1728, 1760, 2308, and 2346 nm were observed to be systematically greater as the fat content of the samples increased (**Figure 1**).

NIR Reflectance Model for Total Fat. A NIR reflectance model was developed for the prediction of total fat using modified PLS regression. Four factors were used for the model and gave the minimum in cross-validation error between predicted and reference values. The SECV between predicted and reference values for the modified PLS model was 1.12% (range = 0.5−43.2%) with an R^2 of 0.99 (**Table 3**; **Figure 2A**). When independent validation samples were predicted with the model, the linear regression of the NIR-predicted values against the AOAC reference values for the model gave an equation of $y = 1.00x + 0.12$. The intercept and slope were not significantly different from 0.00 and 1.00 ($p > 0.05$), respectively, the SEP was 1.07% (range = 2.1−35.7%), the r^2 was 0.99, and the RPD was 8.4 (**Table 3**; **Figure 2B**).

PLS Loadings. The NIR model, using four factors, explained 98.8% of the spectral variation. The modified PLS loading for factor 1 had high variation in the the regions of 1212, 1386, 1728, 2304, and 2346 nm (**Figure 3**). These wavelengths are attributed to absorption by C−H stretch second overtone at 1212 nm; $2 \times$ C−H stretch plus bend at 1386 nm; C−H first overtone asymmetric stretch at 1728 nm with symmetric stretch at 1760 nm; and the CH_2 asymmetric stretch plus CH_2 deformation
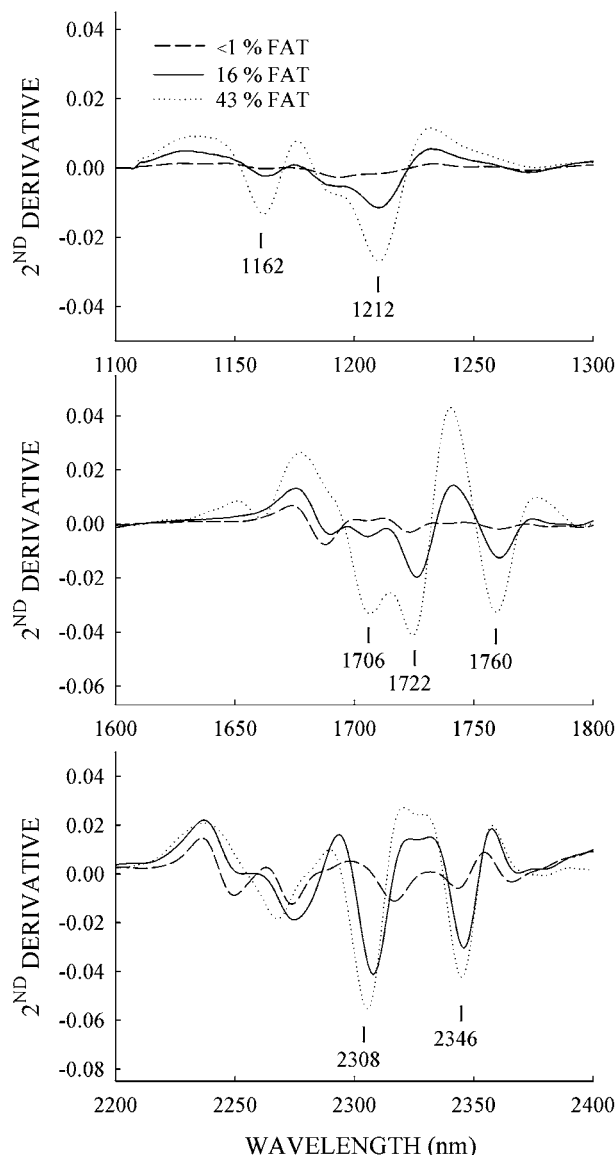
NIR Prediction of Total Fat in Cereal Products

J. Agric. Food Chem., Vol. 53, No. 5, 2005 **1553**

Figure 1. Second-derivative NIR spectra of milled cereal products having low (<1%), medium (16%), and high (43%) total fat content.

Figure 2. AOAC-determined total fat (percent) versus NIR-predicted total fat (percent) for cereal food products in the calibration (**A**) and independent validation data sets (**B**).

combination at 2308 nm with CH_2 symmetric stretch plus CH_2 deformation combination at 2346 nm (*15*, *25*). In the second loading (not shown) the greatest variation was at 1434 nm, which is attributed to absorption by O−H groups in carbohydrate or possibly water (not shown). However, the major influences in the model appear to be from conformations in oil observed in the first factor.

Accuracy of the Model for U.S. Nutrition Labeling. Applicability of the model was determined for U.S. nutrition labeling, and it was found that all of the samples were predicted within the accuracy required by nutrition labeling regulations
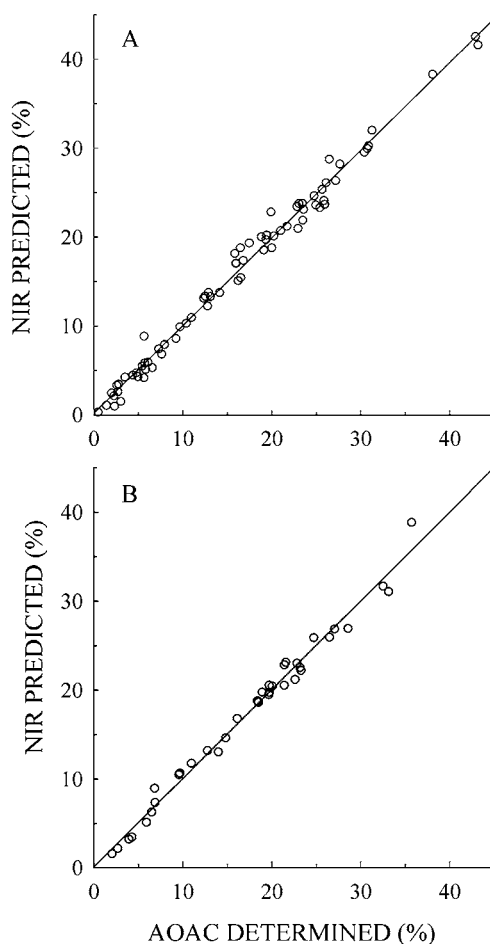
(**Figure 4**) (*4*). Although the NIR error (percent) was identical whether total fat was expressed as a percent of the sample or as grams per serving of the sample, the sequence of products, when sorted by fat content, differed as serving size declared on the nutrition label varied appreciably by product (range = 10−58 g/serving). As expected, the samples with the lowest fat content had the most error. Only one sample, a whole grain puffed cereal, was underestimated by >20% (23%) by the NIR model. However, the sample had a very low fat content of 0.31 g of fat/serving as determined by the AOAC method and 0.24 g of fat/serving as predicted by the NIR model. The label declaration for <0.5 g/serving is zero (*4*); therefore, the AOAC and NIR determinations would be in agreement for this sample after rounding. Only one cereal product sample was overestimated by the NIR model by >20% (31%). This sample also had a low fat content of 0.68 and 0.89 g/serving as determined by the AOAC method and predicted by the NIR model,

Table 3. Statistics for the Near-Infrared Prediction of Total Fat (Percent) in Cereal Food Products[a]

		calibration					validation						
method	n	mean	SD	SECV	R[2]	n	mean	SD	SEP	r[2]	bias	slope	RPD
AOAC	72	16.19	10.39			36	17.39	9.02					
NIR	72	16.18	10.31	1.12	0.99	36	17.47	9.07	1.07	0.99	−0.08	0.99	8.43

[a] AOAC method 996.01 (AOAC); near-infrared spectroscopy (NIR); number of samples (*n*); standard deviation (SD); standard error of cross-validation (SECV); multiple coefficient of determination (R^2); standard error of performance (SEP); coefficient of determination (r^2); ratio of the standard deviation of the AOAC values to the SEP (RPD).
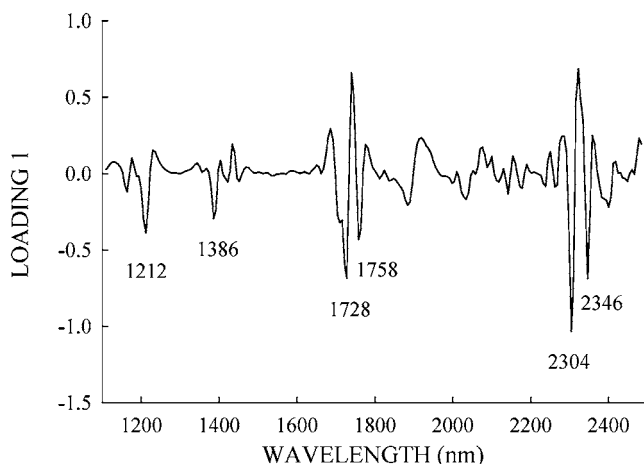
Figure 3. Loading spectrum for the first modified partial least-squares model to predict total fat in cereal food products.
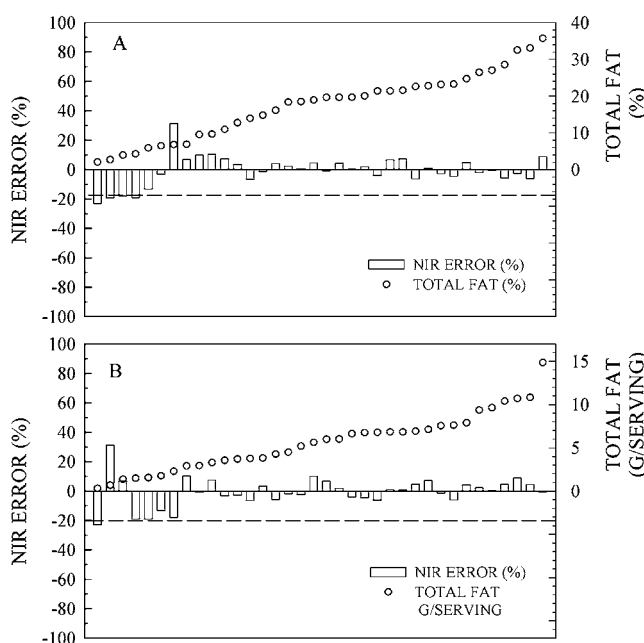

Figure 4. Accuracy of the NIR model to predict total fat in cereal food products for U.S. nutrition labeling. Bars indicate the percent error of the NIR predictions compared to AOAC-determined total fat, for individual validation samples. The NLEA limit of error is −20%. Circles represent AOAC-determined total fat calculated as percent (**A**) and grams per serving (**B**) for corresponding validation samples.

respectively. According to nutrition labeling regulations, reasonable overstatements of total fat on the label "are acceptable within good manufacturing practice" (*4*).

DISCUSSION

A NIR model for the prediction of total fat in cereal foods has been developed using a calibration data set designed to be robust for the range of products available in the marketplace. The set included a wide range of grain types, ingredients, and processing methods. A broad range of fat and sugar contents and a variety of other ingredients, such as salt, cocoa, honey, vegetables, soy, fruits, nuts, spices, and herbs were also included. The model was tested using an independent set of validation samples, which also contained a wide range of fat, sugar, and fiber contents and a wide variety of flavors, spices, and other ingredients and additives. Linear regression of the NIR-predicted

values versus the AOAC values for total fat in the validation data set gave a low SEP and bias accompanied by a coefficient of determination and slope very close to 1. These results together with the high RPD indicate excellent potential for the precise determination of total fat by NIR throughout the range of fat contents for most applications, including quality and process control (*23*). When assessed by U.S. nutrition labeling standards, predictions for samples were found to be within the accuracy required by the NLEA (*4*). Thus, the model has excellent potential for nutrition labeling and monitoring purposes for total fat.

In addition to total fat, the NLEA requires a declaration of saturated fat in processed, packaged foods. Saturated fat is currently measured according to AOAC method 996.01; thus, a NIR method is required for saturated fat. Because of the precision of the reference method for low saturated fat samples, NIR calibrations for saturated fat are not yet at the accuracy required by the NLEA and will be the subject of future work.

The range in total fat content in the validation samples in the current study is broad, but not as broad as that in the calibration data set. This is because additional commercial samples in the 36−43% range were not available at the four retail stores used as the source of samples. In fact, cereal products with >36% total fat content are rare. Although the accuracy of the model has not been tested in the 36−43% range, the high multiple coefficient of determination and low SECV for the model indicate good potential for predicting total fat accurately at these levels.

Interpretation of the loading plot for the first modified PLS factor indicated that alkyl chains in lipids are the most important in the development of the model. Influences from water or carbohydrate are not seen in the first factor but are present in the factor two loading, which is of lesser importance. Therefore, influences for the model are predominantly from functional groups present in oil with little interference from other constituents.

After a NIR reflectance model for total fat prediction in cereal products is constructed, all that is needed by the operator, food processor, or agency is to grind and pack the sample into spectral analysis cells and scan with the NIR spectrometer. If the scanning is done in triplicate, the time involved in packing the sample measurement cells and scanning is <10 min per product. In comparison, the traditional method of analysis, AOAC method 996.01, can take up to 10 h for hydrolysis of the sample and extraction and esterification of fatty acids, plus additional time for GC analysis. Therefore, NIR spectroscopy has excellent potential as a rapid and environmentally benign technique for the prediction of total fat for the food industry.

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NIR Prediction of Total Fat in Cereal Products

J. Agric. Food Chem., Vol. 53, No. 5, 2005 **1555**

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