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Near-Infrared (NIR) Prediction of *trans*-Fatty Acids in Ground Cereal Foods

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Near-infrared (NIR) reflectance spectroscopy was evaluated as a rapid method for prediction of *trans*-fatty acid content in ground cereal products without the need for oil extraction. NIR spectra (400−2498 nm) of ground cereal products were obtained with a dispersive NIR spectrometer and correlated to *trans*- and *cis*-fatty acid content determined by a modification of AOAC Method 996.01. Partial least-squares regression and Marten's uncertainty test were applied to calculate models for prediction of *trans*-fatty acids using spectral regions affected by lipid absorption. The best model ($n = 84$) for *trans*-fat prediction used the 700−2498 nm region and second-derivative processing of spectra. When used to predict test samples ($n = 27$) the model had an RPD of 4.8 with a standard error of performance of 0.70% (range of 0.05−11.74%) and r^2 of 0.97. Optimum models for *cis*-fatty acids were developed with the 1100−2498 and 700−2498 nm ranges and had an RPD of 4.0. Regression coefficients indicated that useful absorbance for prediction of *trans*- and *cis*-fatty acids was in the overtone and combination regions for lipid absorption.

KEYWORDS: NIR; near infrared; *trans*-fatty acid; *cis*-fatty acid; cereal products; foods; total fat

INTRODUCTION

Intake of *trans*-fatty acids in the diet has been associated with increased risk for cardiovascular disease (*1*, *2*); thus, *trans*-fat content is now mandated for nutrition labeling by the U.S. Food and Drug Administration (*3*). *trans*-Fats occur in foods primarily as hydrogenated or partially hydrogenated vegetable oils added during processing and also as a result of ruminant biohydrogenation. Current official methods for the determination of the amount of *trans*-fat include the gas chromatographic (GC) methods, which are used for analysis of foods, including oils, and infrared spectroscopic methods for use on oils (*4*−*9*). The most sensitive spectroscopic methods for the quantification of *trans*-fats in oils involve Fourier transform infrared (FTIR) spectroscopy in conjunction with an attenuated total reflection (ATR) accessory [American Oil Chemists' Society (AOCS) method Cd 14d-99] (*4*, *5*). The FTIR methods are dependent upon the C−H out-of-plane deformation band observed at 966 cm^{-1}, which is uniquely characteristic of isolated double bonds with *trans* configuration. Although the FTIR methods are usually applied to fats or oils, Kim et al. (*10*) reported prediction of *trans*-fatty acids in ground cereal products without prior oil extraction. The fingerprint region around 966 cm^{-1} (1500−900 cm^{-1}) was as effective in modeling for *trans*-fat as was the entire IR region (4000−650 cm^{-1}), emphasizing the importance of the unique C−H deformation band in modeling for *trans*-fat.

Although FTIR has been used traditionally for spectroscopic determination of the quantity of *trans*-fat, several studies have reported the successful use of Fourier transform near-infrared (FT-NIR) spectroscopy for quantification of *trans*-fats in oils (*11*−*13*). There is not a strong, unique band associated with *trans* absorption in the NIR spectrum. Broad overlapping bands, due to overtones and combinations of fundamental vibrations, occur in the NIR region in contrast to sharper peaks in the IR region. However, the use of advanced chemometric techniques, such as partial least-squares (PLS) regression, enables correlations to be made between information in the NIR spectra and parameters measured. Li et al. (*13*), when developing NIR calibrations for *cis*- and *trans*-fat using oil samples from a variety of sources, compared the correlation spectra of separate *cis* and *trans* calibrations using the 1000−2222 nm region. Significant absorptions in the correlation spectra were observed in three main areas, i.e., the CH stretch first- and second-overtone regions at 1640−1870 and 1150−1250 nm, respectively, and the combination region of CH stretch plus other vibrational modes at 2040−2222 nm (*13*). In addition, minor variation was observed at the area of the first overtone of the combination region at around 1389 nm. Although the correlation spectra of *cis* and *trans* calibrations both showed variations in the same overtone and combination regions, there were quantitative differences in absorption (*13*). When calibrations were developed for *cis*- and *trans*-fatty acids using process samples taken from a soybean oil hydrogenation process where there was inherent correlation between the two parameters, the *cis* and *trans* correlation spectra closely resembled each other quantitatively as well as in absorption regions.

Previous methods to measure total *trans*- and *cis*-fat content by NIR spectroscopy have been limited to use on fats and

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oils (*11*−*13*). The current study was conducted to investigate the potential of NIR spectroscopy for the quantitative determination of *trans*-fatty acid content in ground cereal products directly without the need for oil extraction. In addition, *cis*-fatty acid content was also predicted by NIR spectroscopy, so that regression coefficients for PLS models for both *trans*- and *cis*-fatty acids could be examined and compared.

MATERIALS AND METHODS

Samples and Sample Preparation. Commercial cereal products with a wide range of total fat (4.81−36.46%) were purchased from local retailers. Samples were the same as those investigated in a previous study using ATR−FTIR spectroscopy ($n = 110$) (*10*), with the addition of five new samples (*trans*-fat range and mean 7.2−10.7 and 9.13%, respectively) added to improve the distribution of *trans*-fatty acid values in the high end of the range. The sample set was selected to represent the diversity of processed cereal food products commercially available and included crackers, cookies, chips, meal kits, cakes, snacks, and breakfast cereal. Grain types present in the data set were wheat, oats, corn, rice, millet, and multiple grain mixtures, including combinations of rye, barley, triticale, amaranth, buckwheat, and quinoa. Products also contained a wide range of concentrations of sugar, fat, dietary fiber, and protein and a variety of food additives, such as cocoa, honey, salt, herbs, dried vegetables, soy, dried fruits, nuts, and spices. Processing methods used in the formation of the products included baking, extrusion, milling, frying, and air puffing. Several types of oil were present as ingredients in the products, i.e., soybean, cottonseed, corn, canola, palm, and sunflower oils, as well as butter and lard. A total of 83% of products contained partially hydrogenated vegetable shortening or oil, as indicated in the ingredients list on the nutrition label of the product. Cereal products were ground as described by Kim et al. (*10*) and stored at −20 °C until use. All samples were analyzed by spectral and reference methods within 5 days of grinding. It was established in duplicate low and high *trans*-fatty acid samples measured by the reference method that total and *trans*-fatty acid content were stable for at least 7 days at −20 °C.

Reference Methods. Total fat and *trans*- and *cis*-fatty acid content were measured in ground cereal product samples using AOAC Method 996.01 with modifications (*6*, *10*). The modifications were to improve the separation of *trans* and *cis* isomers and consisted of a longer GC column (100 versus 30 m) and changes in GC temperature programming (described later). Briefly, duplicate aliquots of sample were weighed into Mojonnier tubes, and 1 mL of 10 mg/mL tritridecanoin (C13:0, T-3882, Sigma, St. Louis, MO) in chloroform was added as an internal standard. The sample size was between 0.75 and 2 g and adjusted depending upon the amount of total fat (on the basis of the nutrition label value) in the sample. The sample was digested with hot (80 ± 2 °C) 8 N HCl in a water bath for 40 min, and the hydrolyzed fat components were subsequently extracted with diethyl and petroleum ethers. The diethyl and petroleum ethers were evaporated on a steam bath, and the extracts were saponified and esterified. Fatty acid methyl esters (FAMEs) formed were analyzed in parallel with FAME standards (Supelco 37 Component FAME mix, Supelco, Bellefonte, PA) using an Agilent 6890N gas chromatogram (Agilent Technologies, Inc., Palo Alto, CA) with an SP-2560 flexible fused silica capillary column (100 m × 0.25 mm internal diameter × 0.2 μm film thickness, Supelco, Bellafonte, PA). Helium was the carrier gas with a linear velocity of 18 cm/s. The split ratio was 50:1. A single injection of 1 μL was made per sample replicate. The injection port and detector (flame ionization detector) were kept at 200 and 250 °C, respectively, with gas flows of 40 mL/min for hydrogen and 450 mL/min for air. The modified oven temperature programming consisted of an initial temperature of 120 °C held for 5 min, an increase in temperature of 3 °C/min to 240 °C, and a hold time of 20 min at 240 °C. Sample FAMEs were measured against the C13:0 internal standard; Supelco 37 Component FAME mix was used in the identification and quantification of individual fatty acids according to their percentage areas. The *trans*-fatty acids were assigned according to AOCS method Ce 1 h-05 (*8*) and Ratnayake et al. (*14*) as well as identified by a comparison of retention times with standard FAME (from the Supelco 37 FAME standard mix) including elaidic acid methyl ester (*t*-9-octadecanoic acid), oleic acid methyl ester (*c*-9-octadecanoic acid), linoelaidic acid methyl ester (*t*-9,*t*-12-octadecadienoic acid), and linoleic acid methyl ester (*c*-9,*c*-12-octadecadienoic acid). The *cis*/*trans*- and *trans*/*cis*-18:2 isomers identified were included as *trans* isomers for the quantification of *trans*-fatty acids and as *cis* isomers for the quantification of *cis*-fatty acids. All FAMEs were converted to corresponding fatty acids or triglycerides by the appropriate conversion factors (*6*), and triglycerides were summed to obtain the total fat measurement. The total fat was expressed as a percentage weight of the sample. *trans*- and *cis*-fatty acids were individually summed to obtain the totals and also expressed as a percentage weight of the sample. Infant formula (SRM 1846, National Institute of Standards and Technology; 27.1 ± 0.59% total fat) was used as a standard reference material for total fat, on a daily basis.

NIR Spectroscopy. NIR spectra of individual samples were obtained by packing samples into triplicate cylindrical sample cells (internal diameter = 38 mm, depth = 9 mm) with quartz windows. Samples were scanned over muliple days with a NIRSystems 6500 monochromator (Foss North America, Inc., Eden Prairie, MN) using ISI software (NIRS3 version 4.01, Foss North America, Inc., Eden Prairie, MN). Temperature and relative humidity conditions during scanning ranged from 20 to 22 °C and from 50 to 55%, respectively. Diffusely reflected radiation was detected from 400 to 2498 nm at 10 nm resolution and a data interval of 2 nm. Triplicate spectra were averaged. The 700−2498 nm region was used for analysis because of interference occurring in the 400−698 nm region.

Data Processing. Samples were scanned over a period of several months; therefore, all of the spectral data from different dates were standardized to spectra from the first month of this study using the WinISI monochromator instrument standardization software (Foss North America, Inc., Eden Prairie, MN). Spectra were then converted into JCAMP format and imported into the Unscrambler version 9.0 (CAMO, Trondheim, Norway) multivariate analysis software. Unscrambler version 9.0 was used to develop NIR models. Principal component analysis (PCA) was applied to define sample outliers based on Hotelling T2 ellipse, and four outliers were removed from the sample set. After sorting by ascending order for each parameter, samples were divided into calibration and validation sets, such that the first three samples were assigned to the calibration set ($n = 84$), the fourth was assigned to the validation set ($n = 27$), etc. PLS1 models for *cis*- and *trans*-fatty acids were developed using the 700−2498, 1100−2498, and 1600−2498 nm regions. The preprocessing methods used to develop each model were selected on the basis of the preprocessing options that gave optimum performance, i.e., minimum error with full (leave one out) cross-validation (*15*). PLS regression (*15*) was the regression method used, and it was found that combining PLS with jack knifing or the Marten's uncertainty test (*16*, *17*) was an advantage in modeling the broad wavelength regions, i.e., 700−2498, 1100−2498, and 1600−2498 nm, for this data set. Spectra for models for *trans*- and *cis*-fatty acids prediction were pretreated with multiplicative scatter correction (MSC) followed by second-derivative (Savitzky-Golay, seven-point quadratic fit) processing. A multiplicative scatter correction removes effects that result from variation in sample particle size and gave the best results, with this data set, when applied before second-derivative processing.

Calibration Performance. Calibration performance was calculated as the standard error of cross-validation (SECV) and multiple coefficient of determination (R^2) (*15*). The best PLS1 regression models were tested with the validation samples, and performance was calculated as the standard error of performance (SEP), the coefficient of determination (r^2) (*15*, *18*), and the RPD (*19*). The RPD is the ratio of the standard deviation of the reference values of the validation samples to the standard error of performance (SEP) and provides a standardization of the SEP. In general, RPD values of 2.4−3.0 indicate that the model is suitable for very rough screening; RPD values of 3.1−4.9 indicate that the model is suitable for screening; RPD values of 5.0−6.4 indicate that the model is suitable for quality control; RPD values of 6.5−8.0 indicate that the model is suitable for process control; and RPD values of above 8.0 indicate that the model is suitable for any application (*19*).

RESULTS AND DISCUSSION

Reference Method Measurement of *trans*-Fatty Acids, *cis*-Fatty Acids, and Total Fat. The ranges, means, and standard deviations for total *trans*- and *cis*-fatty acid contents are given in **Table 1** for

Table 1. Range, Mean and Standard Deviation of Reference Method Measured *trans*- and *cis*-Fatty Acid Content for Cereal Products in the PLS Calibration and Validation Data Sets[a]

	calibration				validation			
	n	AOAC range	mean	SD	*n*	AOAC range	mean	SD
trans-fatty acids	84	0.01−11.81	5.11	3.27	27	0.05−11.74	5.37	3.37
cis-fatty acids	84	2.00−21.75	9.10	3.96	27	2.74−16.64	8.92	3.55

[a] *n*, number of samples; SD, standard deviation.

the calibration and validation data sets. The standard error of the laboratory (*20*) for total *trans*-fatty acids using the modified AOAC 996.01 method was 0.13% and for total *cis*-fatty acids was 0.20%. *trans*-Fatty acids included in the calculation were the 18:1 *trans*, 18:2 *cis*−*trans*, and 18:2 *trans*−*cis* isomers (**Figure 1**). Isomers of 18:2 *trans*−*trans*, in general, occur between the C18:1 *c* isomers and the C18:2 *ct*/*tc* isomers (*8*, *14*) and were not included in the calculation because peaks in this region (1) could not be identified as specific isomers by gas chromatography

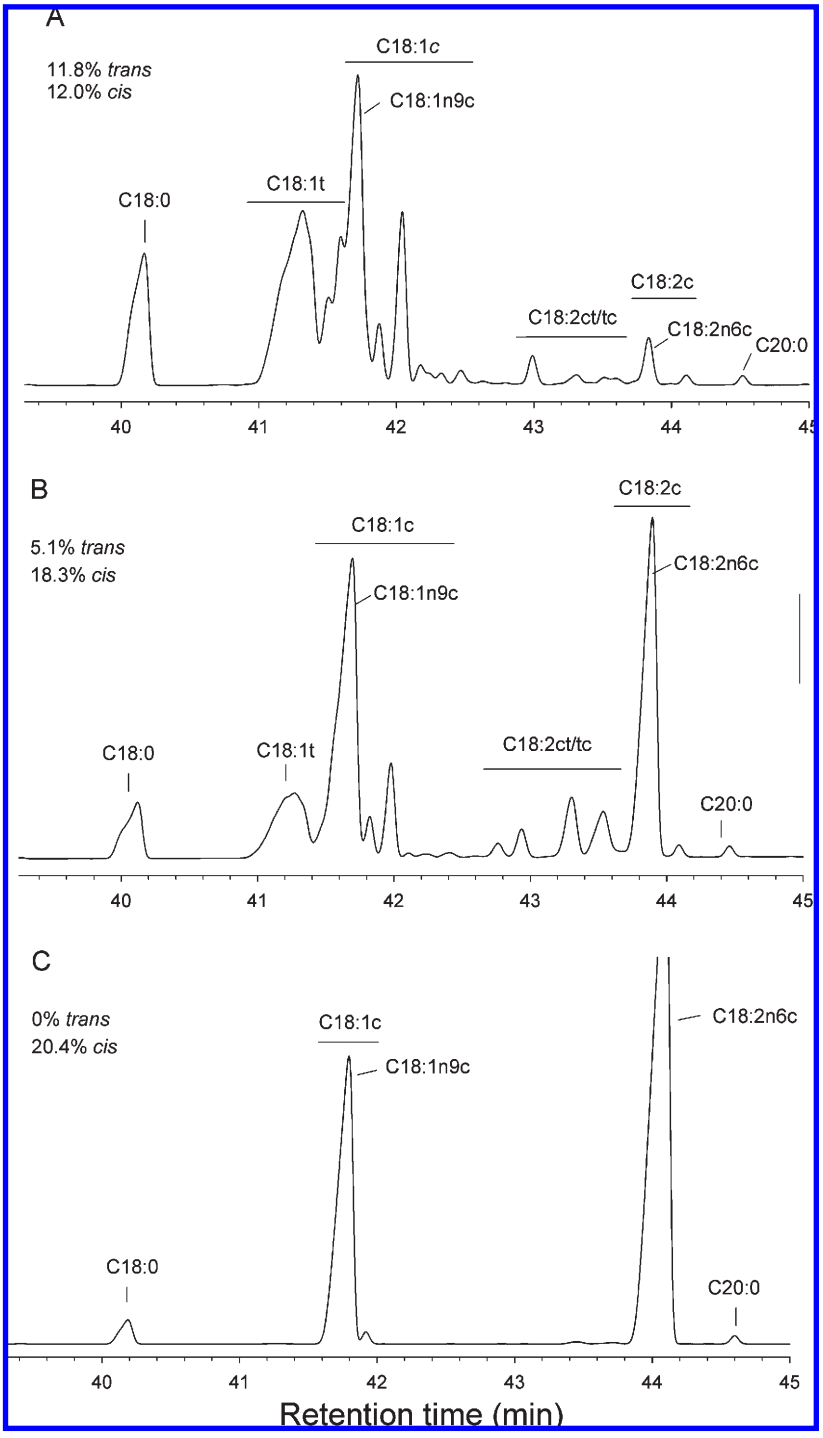

Figure 1. C18:0−C20:0 region of gas chromatograms of the FAME of three ground cereal products with (A) high, (B) medium, and (C) no *trans*-fatty acid. Sample analysis involved acid hydrolysis of ground cereal samples, extraction of lipids, and saponification and esterification of fatty acids followed by gas chromatography using an SP-2560 flexible fused silica column (100 m × 0.25 mm internal diameter × 0.2 μm film thickness) and temperature programming as described in the text.

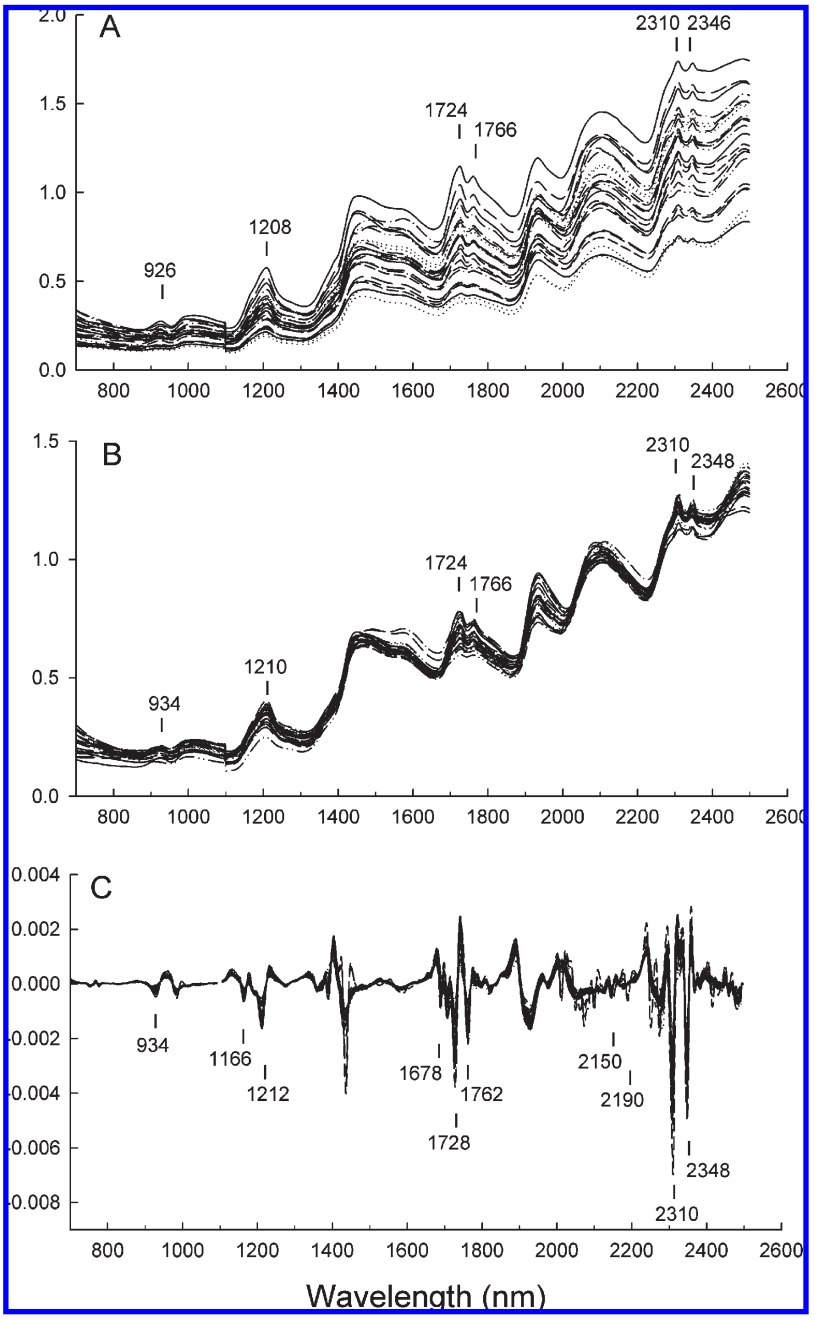

Figure 2. NIR spectra of 24 representative ground cereal product samples (*trans*-fatty acid content = 0.49−11.81%, *cis*-fatty acids = 3.13−20.89%, and total fat = 5.75−36.46%) showing (A) log 1/*R* spectra, (B) spectra after multiplicative scatter correction (MSC), and (C) spectra after MSC followed by second-derivative processing.

alone and (2) consistently occurred in trace amounts in the current study (see **Figure 1**) and in previous studies using partially hydrogenated vegetable oils (*8*, *14*).

Spectral Characteristics. Representative NIR spectra of ground cereal products are shown in **Figure 2** as log 1/*R* spectra (**Figure 2A**), spectra treated with multiplicative scatter correction (MSC, **Figure 2B**), and spectra treated with MSC followed by second-derivative processing (**Figure 2C**). For log(1/*R*) spectra and spectra treated with MSC, bands related to lipids were observed at 926−934 nm (third overtone of CH stretch), 1208−1210 nm (second overtone of CH stretch), 1724−1766 nm (first overtone of CH stretch), and 2310−2346 nm (CH stretch in combination with other vibrational modes) (*21*−*23*). After second-derivative processing, combination band peaks for *cis* isomers were also evident at 2150 and 2190 nm (*21*) and for the first overtone of *cis*- or *trans*-dialkyl CH stretch (−CH═CH−) at 1678 nm (*24*).

NIR Models for *trans*-Fatty Acids. The model developed with the wavelength range of 700−2498 nm had the best performance in cross-validation ($n = 84$) and validation ($n = 27$) with SECV of 1.05% (range of 0.01−11.81%) *trans*-fatty acids, R^2 of 0.89, SEP of 0.70% (range of 0.05−11.74%) *trans*-fatty acid, r^2 of 0.97, and RPD of 4.8 (**Table 2** and **Figure 3**). The bias was low, and the RPD indicates that the model is well-suited for screening samples for *trans*-fat content. The models developed with the 1100−2498 and 1600−2498 nm ranges were also deemed suitable for screening, although the SEP was higher and RPD was lower, showing a decreased utility with decreasing wavelength range. When the 700−2498 nm model was developed with the combination regions removed (1350−1450 and 2100−2450 nm removed), the

performance was reduced (RPD of 3.5 for the 700−2498 nm model; data not shown). A reduced performance of models developed with the 1200−2498 and 1600−2498 nm ranges and models developed without the combination regions suggest that all three overtone regions and the combination region are required for the most accurate prediction of *trans*-fatty acid (**Table 2**). Only two factors were selected for the 1600−2498 nm range model because there was little reduction in SECV with models having more than two factors. A total of six factors slightly improved the error from a SECV of 1.51 to 1.42%; however, more than six factors resulted in increased SECV.

Table 2. Cross-Validation and Validation Statistics for Prediction of *trans*- and *cis*-Fatty Acids by Second Derivative NIR Models Developed with PLS and Marten's Uncertainty Regression Using Broad Wavelength Regions Based on Lipid Absorption[a]

	calibration				validation				
model	*n*	factors	SECV	R^2	*n*	SEP	r^2	bias	RPD
				trans-Fatty Acids					
700−2498	84	6	1.05	0.89	27	0.70	0.97	−0.07	4.8
1100−2498	84	8	1.11	0.88	27	0.81	0.97	0.05	4.2
1600−2498	84	2	1.51	0.79	27	1.01	0.91	−0.36	3.3
				cis-Fatty Acids					
700−2498	84	4	1.05	0.93	27	0.90	0.94	−0.02	4.0
1100−2498	84	4	1.06	0.93	27	0.88	0.94	−0.02	4.0
1600−2498	84	4	1.12	0.92	27	1.01	0.92	0.05	3.5

[a] *n*, number of samples; SECV, standard error of cross-validation; R^2, multiple coefficient of determination; SEP, standard error of performance; r^2, coefficient of determination; RPD, ratio of the standard deviation of the AOAC values to the SEP.

The regression coefficients for the 700−2498 nm model (**Figure 4A**) confirm the importance of the overtone regions (926, 1172, and 1700−1780 nm), the combination region (2000−2450 nm), and the first overtone of the combination region (1380−1400 nm) in the prediction of total *trans*-fatty acid by NIR. Variation in the combination region at 2124−2128 nm in the regression coefficients of the model developed with the 700−2498 nm region (**Figure 4A**) and that developed with the 1100−2498 nm region (regression coefficients not shown) is surprising because this is an area uniquely related to the absorption of vibrations in fatty acids with *cis* double bonds (*21*, *23*).

NIR Models for *cis*-Fatty Acids. Performance results for second-derivative models ($n = 84$) to predict *cis*-fatty acid content using the wavelength regions of 700−2498, 1100−2498, and 1600−2498 nm are given in **Table 2** and scatter plots for the 700−2498 nm model are given in **Figure 3**. Models developed with the 700−2498 and 1100−2498 nm ranges had the lowest SECVs with similar cross-validation performance. When the models were used to predict *cis*-fatty acids in the validation data set ($n = 27$), both models again had similar performance, with SEPs of 0.90 and 0.88%, and each had a RPD of 4.0, indicating suitability for screening purposes. The bias was low for all three *cis*-fatty acid prediction models. The model developed with the 1600−2498 nm region had similar cross-validation statistics to the prior two models and lower RPD of 3.5 and was also deemed suitable for screening samples. Modeling of the 700−2498 nm model without the combination regions (1350−1450 and 2000−2450 nm removed) resulted in reduced performance (RPD of 3.4). Overall, the prediction of total *cis*-fatty acid content was not improved in this study by including the third-overtone region of the CH stretch but was somewhat improved by including the second

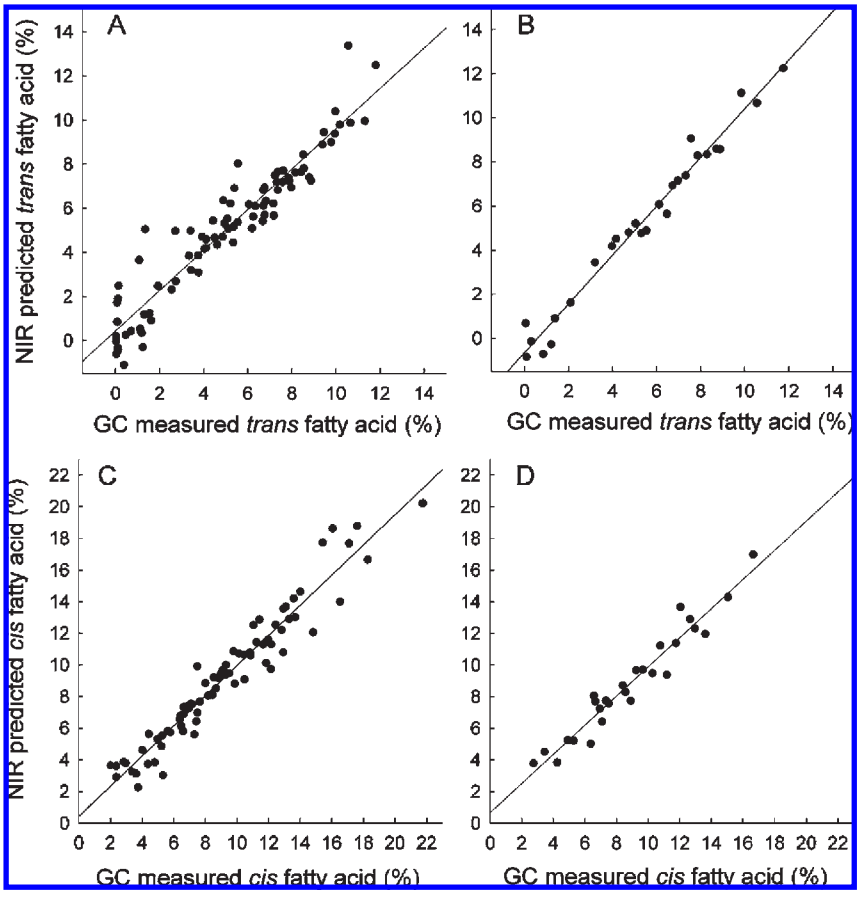

Figure 3. (A and C) Calibration and (B and D) validation plots of NIR-predicted versus GC-measured (A and B) *trans*-fatty acids and (C and D) *cis*-fatty acids in ground cereal products. The models for *trans*- and *cis*-fatty acids were developed with the 700−2498 nm region of the NIR spectrum.

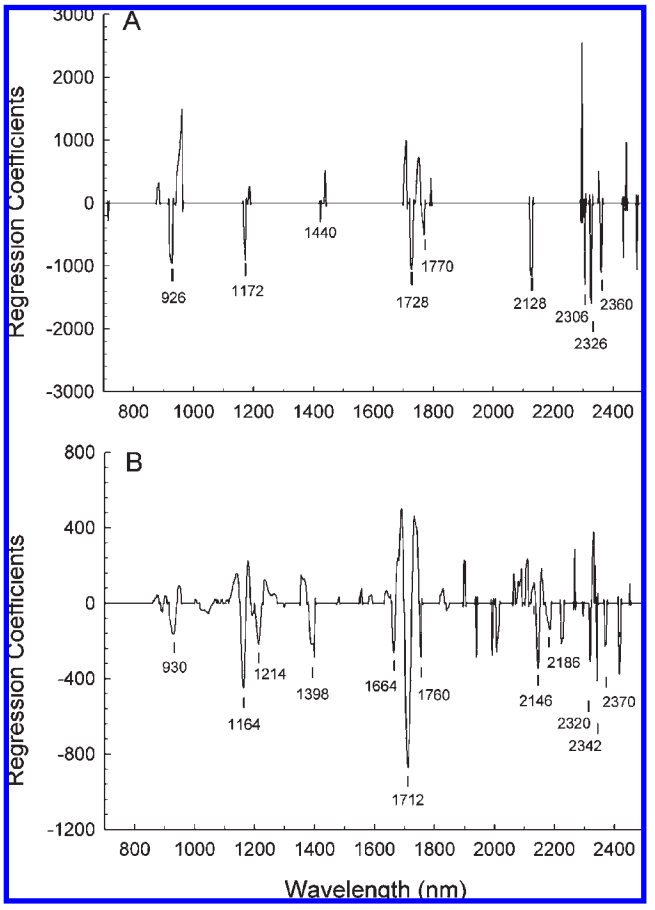

Figure 4. Regression coefficients for NIR PLS1 second derivative models to predict (A) *trans*-fatty acid and (B) *cis*-fatty acid content of ground cereal products using the wavelength range of 700−2498 nm.

overtone of the CH stretch and the first overtone of the combination region.

Regression coefficients for the model to predict total *cis*-fatty acid content using the 700−2498 nm region show that, as for total *trans*-fatty acids, analytically useful absorbance was found in the first-, second-, and third-overtone regions of the CH stretch and in the region of combination of the CH stretch with other vibrational modes in addition to the first overtone of the combination region (**Figure 4B**). The regression coefficients of all *cis* models had variation at 1664 nm, which is consistent with vibrations due to the *cis* and/or *trans* dialkyl CH stretch (−CH═CH−) (*24*), and at 2146 nm, which is characteristic of absorption of vibrations in fatty acids with *cis* double bonds (*21*, *23*). Similar basic regions of the NIR spectrum appear to hold useful absorbance for the prediction of both *trans*- and *cis*-fatty acids in ground cereal products (panels **A** and **B** of **Figure 4**). These regions are the first-, second-, and third-overtone regions of the CH stretch (1660−1780, 1160−1220, and 920−940 nm, repectively), the overtone region of the CH stretch with other vibrational modes (2100−2450 nm), and the first overtone of the combination region (1350−1460 nm). These regions are consistent with the regions observed by Li et al. (*13*) in the correlation spectra of calibrations for the prediction of *cis*- and *trans*-fats, the major absorption bands observed in edible oils by Hourant et al. (*23*), and the absorbance bands in fatty acids observed by Holman and Edmondson (*21*). For prediction of total *cis*-fatty acids, equivalent prediction accuracy of models was achieved whether or not the third overtone of the CH stretch was included.

A previous study from this laboratory (*10*) showed the prediction of total *trans*-fatty acids directly in ground cereal products by ATR−FTIR spectroscopy with a RPD of 3.4 and SEP and r^2 of 0.96% (range of 0.05−12.15%) and 0.92, also indicating suitability of the model for screening samples as is the case for NIR spectroscopy. However, a direct comparison between NIR and IR cannot be made because the IR model data set did not include the extra samples added in the current NIR study to improve the distribution of values in the higher end of the *trans*-fat range.

Effectiveness of NIR modeling and accuracy of prediction of parameters in unknown samples can be affected by the matrix variations of the samples and the conditions during scanning, such as temperature and relative humidity. For this reason, a wide range of processed cereal products was selected for use in this study to represent the range of products of this type that are commercially available. Additionally, the temperature and humidity conditions during scanning have been specified.

In summary, NIR spectroscopy, using a dispersive NIR instrument, can be used to predict total *trans*- and *cis*-fatty acid content of ground cereal products with the accuracy required for screening samples. The models developed employed PLS1 with Marten's uncertainty regression and second-derivative processing. Regression coefficients for the PLS1 models consistently showed that optimum wavelengths to predict both *trans*- and *cis*-fatty acids are in the overtone regions of CH stretch vibrations and in the regions of combination of the CH stretch with other vibrational modes.

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