

Energy from Fat Determined by Near-Infrared Reflectance Spectroscopy

SANDRA E. KAYS* AND FRANKLIN E. BARTON, II

Quality Assessment Research Unit, Richard B. Russell Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 5677, Athens, Georgia 30604-5677

The amount of energy derived from fat in foods is a requirement of U.S. nutrition labeling legislation and a significant factor in diet development by health professionals. Near-infrared (NIR) spectroscopy has been used to predict total utilizable energy in cereal foods for nutrition labeling purposes, and in the current study, was investigated as a method for evaluation of the amount of energy derived from fat. Using NIR reflectance spectra (1104–2494 nm) of ground cereal samples and reference values obtained by calorimetry and by calculation, modified PLS regression models were developed for the prediction of percent energy from fat and energy from fat/g. The models were able to predict the percent of utilizable energy derived from fat with SECV and R^2 of 1.86–1.89% of kcal ($n = 51$, range 0–43.0) and 0.98, respectively, and SEP and r^2 of 1.74% of kcal ($n = 55$, range 0–38.0) and 0.98, respectively, when used to predict independent validation samples. Results indicate that NIR spectroscopy provides useful methods for predicting the energy derived from fat in food products.

KEYWORDS: Near-infrared spectroscopy; NIR; energy; calorie; cereal; nutrition labeling

INTRODUCTION

The type of fat and the energy derived from fat are both significant considerations in diet development. Although fat is an indispensable part of the human diet, some lipids such as saturated fat, cholesterol, and trans fatty acids have adverse health effects (1, 2). Also, fat contains more energy/g than carbohydrate and protein and moderation in fat intake is recommended for weight control. The U. S. Dietary Guidelines for Americans, therefore, advises consumers to “choose a diet that is low in saturated fat and cholesterol and moderate in total fat” and “aim for a total fat intake of no more than 30% of calories” (3). Recent evidence suggests that a moderate-fat diet (25–35% of energy) may affect the lipid and lipoprotein risk profiles more favorably than a low-fat diet (<25% of energy) or a high-fat diet (>35% of energy), for the majority of individuals, and that extremes of dietary fat intake should be avoided (4). Thus, the proportion of fat energy in a food is a significant part of diet planning and a compulsory part of nutrition labeling (U. S. Nutrition Labeling and Education Act of 1990) (5).

The proportion of energy from fat in a food can be expressed in several ways. Nutrition labeling legislation requires inclusion of the energy from fat/serving of the product on the nutrition label when fat is >0.5 g/serving (5). For dietary recommendations, fat energy is expressed as percent of calories from fat, which is a different parameter from the energy from fat per serving and uses total utilizable product energy as the denominator.

Before determining total energy of a product, there are certain physiological factors to consider. Gross energy in foods is derived from carbohydrate (starch, sugar, and fiber), protein and fat. Gross energy measured by calorimetry, although a measure of the total energy in a food, does not reflect the energy physiologically available due to the incomplete utilization of protein and fiber in the human body. While protein is completely oxidized when gross energy is determined in a calorimeter, it is not in humans, as nitrogen and some carbon and hydrogen are excreted as urea. It has been estimated that, typically, 1.25 kcal/g of protein are not available (6). Fiber is completely oxidized in a calorimeter, and by definition, not digested at all by human enzymes (7). Rather, partial degradation of fiber occurs because of the action of enzymes of colonic bacteria that degrade, primarily, soluble dietary fiber to short chain fatty acids, which can be absorbed by the colon and subsequently become available to the body for energy. Thus, adjustments in gross energy for incomplete utilization of protein and undigested fiber provide a more realistic estimate of the energy utilized from a food by humans.

There are several possible variants of the percent of calories from fat, as the U. S. Code of Federal Regulations (5) allows the denominator, total utilizable energy, to be expressed in more than one way. According to the U. S. Code of Federal Regulations, the total utilizable energy value of foods can be measured by bomb calorimetry subtracting 1.25 kcal/g of protein to correct for unutilized protein. Alternatively, total utilizable energy can be calculated using the general factors of 4, 4 and 9 kcal/g for protein, total carbohydrate less the amount of insoluble dietary fiber, and total fat, respectively (6, 8). The first method takes into account the unutilized protein, and the

* To whom correspondence should be addressed. Tel.: 706-546-3338. Fax: 706-546-3607. E-mail: sekays@qaru.ars.usda.gov.

second takes into account both the unutilized protein and the non digestibility of a portion of the dietary fiber. Other methods use factors for the energy values of specific foods, ingredients, or components (5). For the purpose of this manuscript, the percent of energy from fat is calculated as (1) the energy from fat as a percent of the gross energy adjusted for unutilized protein (PEF1), and (2) the energy from fat as a percent of the gross energy adjusted for unutilized protein and undigested insoluble fiber (PEF2). Absorption of fat may be affected by interference from other food components; however, this aspect of fat energy availability is not addressed in the current work.

Although energy derived from fat is a fraction of total utilizable energy, it is not a constant fraction. The major components of cereal products are starch, fiber, protein, added fat, and added sugar (e.g., as sugar coating), which are present in a wide range of concentrations. Each contributes to the energy content of the food and the proportion of energy from fat can vary from close to zero to more than 40% in processed cereal products.

Previous work from this laboratory has described the use of NIR spectroscopy for the rapid prediction of gross and total utilizable energy in a diverse array of cereal products (9). The reference values for gross energy were derived from calorimetry, and values for utilizable energy expressed as (a) gross energy adjusted for unutilized protein and (b) gross energy adjusted for unutilized protein and undigested insoluble dietary fiber. Modified partial least squares models were developed for both (a) and (b) and tested with an independent data set of cereal products. Both models predicted utilizable energy of new samples well within the accuracy required by U. S. nutrition labeling legislation. The percent of energy derived from fat, although a component of total utilizable energy, is a different parameter and also a nutritionally important parameter. The potential of developing rapid and feasible methods for predicting fat energy in foods using NIR spectroscopy has not been reported. The current study investigates the potential of NIR reflectance spectroscopy for the evaluation of the percent of energy from fat and the energy derived from fat/g in diverse cereal products.

MATERIALS AND METHODS

Samples and Sample Preparation. The cereal food data set used in the current study is the same as that used in a prior study for development of NIR models for prediction of gross and total utilizable energy (9). The samples were obtained from commercial retailers and included breakfast cereals, crackers, flours, brans, pastas and unprocessed whole grains. The cereal grains present in products in the calibration and validation data sets are listed in **Table 1**. The proportion of samples with high-fat, high-sugar, and low-fat and low-sugar was similar for the calibration and validation data sets as shown in **Table 2**. The cereal food products used for validation were never part of the calibration data set and were different products from those used in the calibration, although in the same category. Samples were purchased, ground, and scanned over a period of 18 months.

Cereal food products were dry milled to <500 μm in a Cyclone 1093 sample mill (Perstorp Analytical, Silver Spring, MD). Samples with >20% sugar content (based on nutrition label values) were mixed with liquid nitrogen to aid grinding, and samples with >10% fat (based on nutrition label values) were ground with a coffee mill (model KSM-2, Braun Inc., Lynnfield, MA).

Spectroscopic Analysis. Ground cereal samples were scanned in duplicate with a Foss-NIRSystems 6500 spectrometer (Foss North America, Silver Spring, MD) to obtain reflectance spectra, as described previously (9).

Reference Analysis. Energy is expressed as kilocalories (kcal). A kcal is equivalent to a Calorie or one thousand "small calories," and

Table 1. Representation of Grain Types and Fat Content in the Calibration Data Set (Before and After Selection of Samples) and in the Validation Data Set of Models to Predict Energy Derived from Fat^a

product type	no. of prods	range in fat (%)	mean fat (%)	SD fat (%)
Calibration Data Set—Original				
wheat	38	0.0–20.69	4.7	5.5
oats	8	2.96–21.70	9.2	5.9
corn	8	0.00–30.00	4.4	10.4
rice	10	0.00–16.67	2.6	5.2
rye	3	0.00–1.88	1.2	1.1
barley	3	0.00–9.72	3.9	5.1
millet	1	3.19		
multiple grains ^b	54	0.00–28.57	5.8	6.6
Calibration Data Set—Selected				
wheat	14	1.67–20.69	9.1	6.7
oats	6	5.95–21.70	10.9	6.6
corn	4	0.00–30.00	7.5	15
rice	3	0.00–3.19	2.02	1.75
barley	1	2.00		
multiple grains ^c	23	0.00–25.00	9.27	7.04
Validation Data Set				
wheat	13	0.00–23.33	8.3	8.0
oats	7	7.35–17.65	14.9	3.8
corn	5	0.00–7.27	3.1	3.1
rice	4	0.00–3.45	1.5	1.8
rye	2	0.00–8.33	4.2	
millet	1	2.86		
multiple grains ^d	25	0.00–21.88	4.9	5.7

^a Values for fat content are based on nutrition label information for each product. The grains present in multiple grain products (followed by the number of products) for the ^b original calibration data set are wheat (36), oats (25), corn (19), rice (16), rye (10), barley (18), millet (3), amaranth (2), and buckwheat (5); ^c selected calibration data sets are wheat (18), oats (11), corn (8), rice (9), barley (9), and amaranth (1); ^d validation data set are wheat (17) including kamut (2), oats (9), corn (6), rice (9), rye (2), barley (5), millet (1), and amaranth (3).

Table 2. Distribution of Samples with High Fat and/or High Sugar in the Calibration and Validation Data Sets for the Prediction of Energy Derived from Fat^a

	no. of samples		
	calibration-original	calibration-selected	validation
low-fat/low-sugar	71	12	29
high-fat/low-sugar	17	17	9
low-fat/high-sugar	37	22	14
high-fat/high-sugar			5

^a High-fat = >10%; high-sugar = >20% (based on product nutrition label values for fat and sugar).

defined as the amount of heat required to raise the temperature of 1 kg water 1 °C at 15 °C (10). The energy derived from fat was calculated as (1) kcal from fat as a percent of gross energy adjusted for unutilized protein (PEF1), (2) kcal from fat as a percent of gross energy adjusted for unutilized protein and insoluble dietary fiber (PEF2), (3) kcal from fat/g.

The energy adjusted for unutilized protein and energy adjusted for unutilized protein and insoluble dietary fiber were determined in a previous study (9). Briefly, gross energy (kcal/g) was determined at The University of Georgia Poultry Nutrition Laboratory by oxygen combustion in a bomb calorimeter (Parr Instrument Company, Moline, IL), as described in Parr Manual No. 120 (10). Gross energy values were adjusted for unutilized protein and insoluble dietary fiber (a) by subtracting 1.250 kcal/g of protein (8) or (b) by subtracting 1.250 kcal/g of protein and 4.153 kcal/g of insoluble fiber (9). Fat was determined using the Soxtec 1040 Extraction System (Perstorp Analytical) with petroleum ether as the solvent (AOAC Method 945.16) (11) and dry

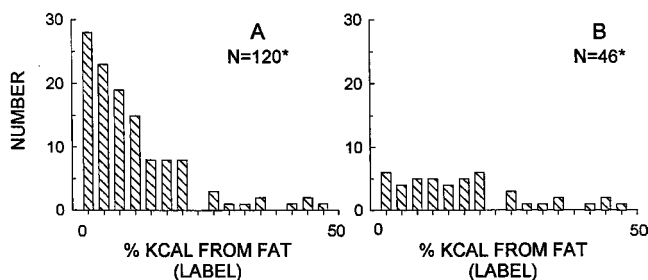


Figure 1. Distribution of number of samples in 3% intervals of values for % energy from fat in the calibration data set before (A) and after (B) application of the SELECT algorithm. * Nutrition label values were used to calculate % energy from fat but were not available for five of the calibration samples in A and B.

matter determined by the air oven method at 105 °C (AOAC Method 945.15) (12). Fat was expressed on a dry weight basis.

The parameters to be modeled (i.e., percent of energy derived from fat (PEF1 and PEF2) and kcal from fat/g sample) were calculated as follows, using a factor of 9 kcal of energy/g fat: (6, 8)

$$\% \text{ kcal from fat} = \frac{\text{g fat per g sample} \times 9 \times 100}{\text{total utilizable kcal per g sample}}$$

$$\text{kcal from fat per g sample} = \text{g fat per g sample} \times 9$$

Selection of Calibration Samples for Prediction of Energy from Fat. ISI Software NIR3 version 4.01 (ISI International Inc., Port Mathilda, PA) was used to scan and select samples and develop chemometric models. The majority of cereal products in the calibration and validation data sets were low in fat, with 70% of the samples containing <3% fat in a range of 0–25% (based on nutrition label values). This resulted in a skewed distribution of the values for the energy derived from fat as shown in **Figure 1A**, where the % energy from fat was calculated from product nutrition label values for energy. Label energy values were not available for five high fat samples that were sold from a hopper. Thus, **Figure 1A** shows 120 rather than 125 samples, and **Figure 1B** shows 46 samples rather than 51. To avoid a skewed distribution of values for the energy derived from fat, a selection algorithm (SELECT, NIR3 3.0 V. 4.01) (13, 14) was employed to select representative samples from the pool of calibration samples with <3% fat ($n = 89$). The NIR3 version 4.01 SELECT algorithm has a default neighborhood H value of 0.6, which was used to select samples. Using a wavelength range of 1104–2494 nm, spectra were transformed using a running average smooth (4 nm interval) followed by second derivative mathematical treatment (segment = 4 nm) (15) before principal component analysis (PCA). The SELECT algorithm identified samples with <3% fat that were within and outside the neighborhoods defined by the samples with >3% fat ($n = 36$). Seven PCA components were used by SELECT, and with the scores in 7-dimensional space, the neighborhood H distance was calculated between all spectral pairs in both sets of samples. Any low fat sample whose neighborhood H value was <0.6 from any sample in the set with fat content >3% was eliminated, as neighbors are considered spectrally similar and do not need to be included in the calibration. This process was continued until all of the samples with <3% fat were either selected for inclusion in the calibration or eliminated. Samples having <3% fat ($n = 15$) were selected from the pool of 89 for combination with 36 samples having >3% fat to form the calibration data set. Reference values for fat were obtained for the calibration samples ($n = 51$) using the AOAC Method 945.16 (11), and PEF1, PEF2 and kcal from fat/g were calculated.

Shenk and Westerhaus (14) used SELECT and a minimum standardized H distance value of 0.6 to select representative samples for calibrations for protein and acid detergent fiber prediction in hay, haylage, wheat, corn, and barley. The calibrations developed were found to be similar in robustness to those developed with all the samples in the data set for four out of five of the products used (hay, haylage, corn and barley). Thus, calibrations developed following sample selection can be just as robust as those developed with all the samples.

Development of Multivariate Calibrations for Prediction of Calories Derived from Fat. Using the spectra and reference data of 51 ground cereal calibration samples, models were developed to predict energy derived from fat. The wavelength range used for analysis was 1104–2494 nm. Using a commercial analysis program (NIR3, version 4.01, ISI International Inc., Port Matilda, PA), three energy prediction models were developed with reference data for (a) PEF1, (b) PEF2, and (c) energy from fat/g sample. Modified partial least squares (PLS) was the regression method used. The modification to PLS scaled the reference method data and reflectance data at each wavelength to have a standard deviation of 1.0 before each PLS term (13). Prior to PLS regression, log 1/R spectra were mean centered, transformed with multiplicative scatter correction (16) to remove interferences due to particle size, and then transformed with second derivative processing (gap = 4 nm) after a running average smoothing (interval = 4 nm). The optimum number of modified PLS factors used for fat energy prediction was determined using full cross-validation (17). During cross-validation, one calibration sample at a time was temporarily removed from the calibration set and used for prediction. Performance statistics were accumulated for each sample. The optimum number of factors for energy prediction was that which produced a minimum in overall error between modeled and reference values (standard error of cross-validation). The preprocessing used was the optimum required to improve the standard error of cross-validation (SECV) compared to modified PLS values with unprocessed data and data processed with combinations of scatter correction (none, normal MSC, SNV, detrend, SNV, and detrend) and derivatives (none, first, second).

Validation of Models. Energy prediction models developed were tested using an independent set of cereal food products ($n = 57$). Performance of the models was reported as the standard error of performance (SEP), coefficient of determination (r^2), slope, bias (18), and RPD. The RPD (19) is the ratio of the standard deviation of the reference values to the SEP and provides a standardization of the SEP. RPD values of 5.0–6.4 are considered good and applicable for quality control. Values of 6.5–8.0 are considered very applicable for process control and values of >8.1 are considered excellent and useful for any application.

RESULTS AND DISCUSSION

Spectral Characteristics of Samples and Sample Selection. Marked differences among the spectra of samples are due to the quantities of fat and sugar present. Samples with high fat content, such as many granolas and crackers, have sharper peaks at 1212, 1732, 1764, 2304, and 2346 nm, compared to low fat samples, due to absorption by C–H groups in lipids at these wavelengths. Samples with high content of crystalline sugar, such as sugar-coated cereal products, have sharper peaks at 1434 and 2076 nm, compared to low sugar samples, due to absorption by O–H groups in carbohydrates. The distribution of samples with low fat and low sugar, high fat, and high sugar, based on nutrition label values, is shown in **Table 2**.

The composition of the calibration data set after use of the SELECT algorithm was similar, in types of grains present, to the composition of the calibration data set before using the SELECT algorithm (**Table 1**). With the exception of buckwheat, all cereal grains present in products in the original calibration data set ($n = 125$) were also present in the selected calibration data set ($n = 51$). As none of the four products containing buckwheat was selected by the SELECT algorithm, it is probable that the products (all multiple-grain products) were not sufficiently different, spectrally, from products already selected. Thus, the calibration data set did not appear to be compromised by the selection process in terms of the scope of grains present (**Table 1**). Although not present in the calibration data set, quinoa was present in one multiple grain product in the validation data set. However, the sample was predicted by all the models within the acceptable error.

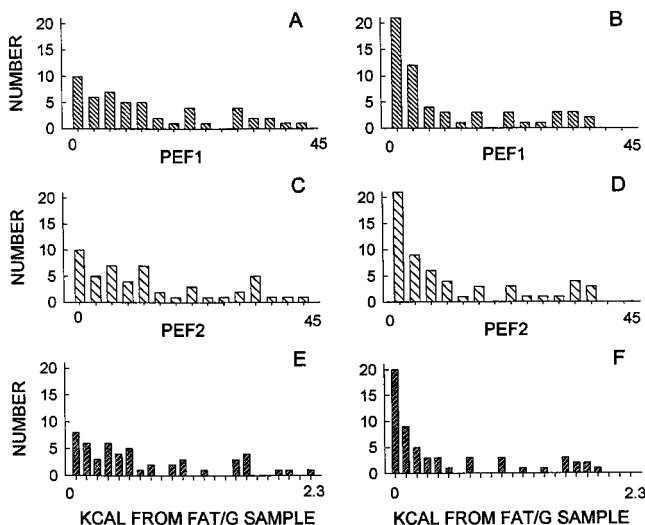


Figure 2. Distribution of number of samples in 3% intervals of reference method values for percent energy derived from fat and 0.1 kcal intervals for energy from fat/g in cereal samples used for the calibration (A, C, E) and validation (B, D, F) of models to predict PEF1, PEF2, and kcal derived from fat/g. (PEF1 is kcal from fat as a percent of energy adjusted for unutilized protein. PEF2 is kcal from fat as a percent of energy adjusted for unutilized protein and insoluble dietary fiber.)

After the selection process, the calibration data set had a more uniform distribution of nutrition label based values along the range of values for % energy from fat (**Figure 1B**), although there was still some skewing at the lower values. An even distribution of values along the range to be studied is the preferred sample distribution pattern (20). This helps to prevent bias that can be caused by weighting of samples at only one end of the range. The same calibration data set used in the current study had been used to model gross and utilizable energy in the previous study, but reference values for gross and utilizable energy had a more Gaussian-like distribution (9).

Reference Method Measurement for Energy Derived from Fat. The distribution of reference values for energy derived from fat in the calibration ($n = 51$) and validation ($n = 55$) data sets for three models are shown in **Figure 2**. For PEF1, values ranged from 0 to 42.79% for the calibration data set and 0.3–38.0% for the validation data set. For PEF2, the values ranged from 0 to 43.06% for the calibration data set and 0.3–38.1% for the validation data set. The values for kcal from fat/g ranged from 0 to 2.30 kcal for the calibration data set and 0–1.98 kcal for the validation data set. Even after the selection process, the distribution of values for energy derived from fat was somewhat skewed to the lower values. The standard error of the reference method (21) for determination of fat was 0.19% (range of values 0.2–25.6%). Standard errors for the reference methods used to determine utilizable energy were 0.035 kcal/g for gross energy, 0.21% for protein, and 0.41% for insoluble dietary fiber (9).

Calibrations for Energy Derived from Fat. NIR calibrations were developed, using modified PLS, for the determination of percent energy from fat and energy from fat/g. The number of modified PLS factors used for all the calibrations was five (**Figure 3**). This is in contrast to the models for gross and utilizable energy, which used 7–9 factors (9). Using full cross validation, the overall error between modeled and reference values (standard error of cross validation, SECV) for the PEF1 model was 1.86% (range 0–42.8%) with a multiple coefficient of determination (R^2) of 0.98 (**Table 3**). Linear regression of reference values for PEF1 against NIR predicted values gave

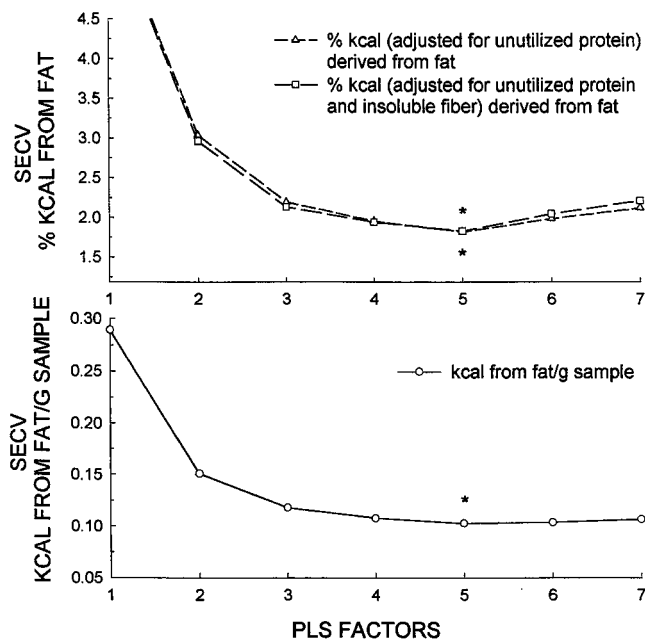


Figure 3. Number of modified PLS factors versus SECV (standard error of cross validation) for the prediction of energy derived from fat. * Number of factors used for each model.

an intercept and slope not significantly different from 0.0 and 1.0, respectively ($p > 0.05$). Independent validation samples were predicted with the model. When reference values for PEF1 ($n = 55$) were compared statistically with NIR predicted values, the SEP was 1.74% (range 0.3–38.0%) and the coefficient of determination (r^2) was 0.98. Linear regression of reference values for kcal derived from fat versus NIR predicted values for validation samples gave an intercept and slope not significantly different from 0.0 and 1.0, respectively ($p > 0.05$). Two samples in the validation data set were identified as residual outliers (having a difference between reference values and NIR-predicted values of 2.5 times or greater, the standard error of the difference between the two values) and removed. If the two samples were not removed, the SEP and r^2 were 2.05% and 0.97, respectively. The samples were not outliers in the previous study to predict gross energy and total utilizable energy (9), suggesting that the problem is in the reference analysis rather than the spectral analysis. Reference analysis (AOAC Method 945.16) was repeated for these samples with no significant change in values. The two samples were analyzed using an alternative reference method for total fat (i.e., acid hydrolysis of the sample followed by capillary gas chromatographic (GC) analysis of methyl esters of the fatty acids extracted) (AOAC Method 996.01) (22). The values for total fat measured by this method were higher than those measured by AOAC Method 945.16 (i.e., 2.67% fat compared to 1.06% fat for the first sample and 6.89% compared to 1.51% for the second sample) and gave calculated values of PEF1 that were close to the NIR predicted values, such that both samples were no longer outliers. When the two samples were included in the validation data with reference values obtained by the acid hydrolysis/GC method for total fat, the SEP and r^2 were 1.81% and 0.98, respectively. The reason for the discrepancy in reference method results is not known; however, it is possible that the outlier samples may contain significant quantities of bound lipids that are not extracted by solvent alone.

The model developed for determination of PEF2 had a SECV and R^2 of 1.89% (range 0–43.1%) and 0.98, respectively,

Table 3. Calibration and Validation Statistics for Prediction of Energy Derived from fat

	method ^c	calibration ^a					validation ^b							
		<i>n</i>	mean	SD	SECV	<i>R</i> ²	<i>n</i>	mean	SD	SEP	<i>r</i> ²	bias	slope	RPD
PEF1 ^d	REF	51	14.31	12.33			55	10.92	12.07					
	NIR	51	14.37	12.18	1.86	0.98	55	10.95	12.19	1.74	0.98	-0.03	0.98	6.94
PEF2 ^e	REF	51	14.93	12.50			55	11.44	12.31					
	NIR	51	14.99	12.37	1.89	0.98	55	11.49	12.25	1.74	0.98	-0.05	1.00	7.07
kcal from fat/g	REF	51	0.68	0.63			55	0.52	0.61					
	NIR	51	0.69	0.62	0.104	0.97	55	0.52	0.61	0.076	0.99	0.004	0.99	8.03

^a Number of samples (*n*), mean, standard deviation (SD), standard error of cross validation (SECV), and multiple coefficient of determination (*R*²) for calibration. ^b Number of samples (*n*), mean, standard deviation (SD), standard error of performance (SEP), coefficient of determination (*r*²), bias, slope and RPD (reference SD/SEP) for validation. ^c Reference method (REF); near-infrared method (NIR). ^d kcal from fat as a percent of energy adjusted for unutilized protein. ^e kcal from fat as a percent of energy adjusted for unutilized protein and insoluble fiber.

using full cross-validation and five PLS factors (Figure 3, Table 3). When the model was used to predict PEF2 in independent validation samples (*n* = 55), the SEP, *r*², bias, slope and RPD were 1.74% (range 0.3–37.0%), 0.98, -0.05%, 1.00, and 7.07, respectively. The performance statistics were very similar for both of the models to predict the kcal derived from fat as a percent of the utilizable energy.

An NIR calibration was obtained with modified PLS. For the determination of kcal from fat/g sample with modified PLS. The SECV, using full cross validation and five PLS factors, was 0.104 kcal (range 0–2.30 kcal) with a multiple coefficient of determination (*R*²) of 0.97 (Figure 3, Table 3). Linear regression of reference values for kcal from fat/g versus NIR predicted values gave an intercept and slope not significantly different from 0.0 and 1.0, respectively (*p* > 0.05). Fat energy in independent validation samples was predicted with the model. When predicted values for kcal from fat/g were compared statistically with reference values, the SEP, *r*², bias, slope, and RPD were 0.076 kcal (range 0.01–1.98 kcal), 0.99, 0.004 kcal, 0.99, and 8.03, respectively. Linear regression of reference values for kcal from fat/g against NIR predicted values gave an intercept and slope not significantly different from 0.0 and 1.0, respectively (*p* > 0.05). The RPD (19) values for the performance of the three models indicate that the models are “very good for process control” of the fat energy in diverse cereal products and should provide reliable information for development of diets.

The three formulations of energy derived from fat differ in the denominator, which is total utilizable energy for PEF1 and PEF2 and unit weight of the food or product for kcal from fat/g. The former two provide information in a form that has utility for diet planning so that overall calories from fat, as recommended for most individuals, can be adjusted for a moderate range (3, 4). The kcal from fat/unit is the formulation required for U.S.A. nutrition labeling, when expressed on a per serving basis (5).

Modified PLS Loadings. The modified PLS models for percent kcal derived from fat used five factors that accounted for 98.4–98.5% of the spectral variation. Figure 4 illustrates the loading plot for factor one of the model to predict PEF1. This is very similar to the loading plots of the model to predict PEF2 (not shown). Both plots are dominated by large variations in absorption associated with CH groups in lipids at 1212, 1728, 1764, 2304–10 and 2346–52 nm. (23, 24) Similarly, the modified PLS model for prediction of kcal from fat/g of the sample used five factors which accounted for 98.4% of the spectral variation. As for the models predicting % energy from fat, the loading plot for factor one had large variations predominantly associated with absorption by C–H in lipids at

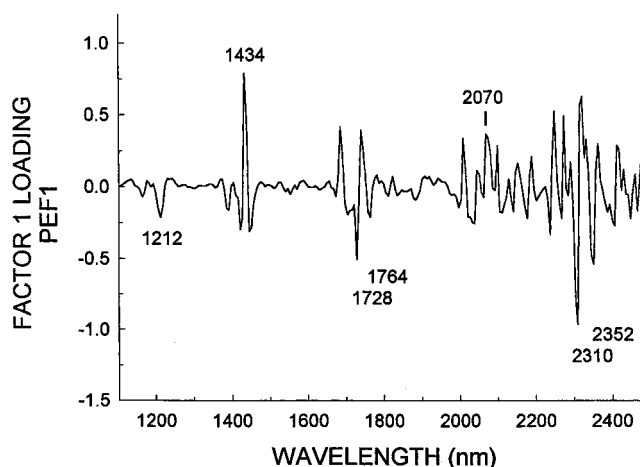


Figure 4. Loading spectrum for the first modified PLS factor for the model to predict PEF1. (PEF1 is kcal from fat as a percent of energy adjusted for unutilized protein.)

1212, 1728, 1764, 2340, and 2346 nm. However, for all models, some variation was also present due to absorption by OH groups in carbohydrates at 1434 nm. This differs from the modified PLS models for gross energy and total utilizable energy in a previous paper (9), which were dominated by effects due to absorption by CH groups in lipids with some additional influences from OH groups in carbohydrates (at 1434 and 2076 nm) and water (at 1416, 1914, and 1920) (9). Although both were dominated by fat, the models for energy derived from fat appear to have less influence from components other than fat.

In summary, near-infrared reflectance spectroscopy was found to provide a rapid and accurate method for the determination of the amount of energy that is derived from fat, in a diverse set of cereal food samples. This is the first report of the potential of NIR spectroscopy for prediction of energy derived from fat in foods. Fat energy values required for nutrition labeling (i.e., the kcal from fat/serving), can be calculated easily from kcal from fat/g using the serving size specified for each product. In addition, NIR spectroscopy can be used for the rapid and accurate determination of the percent of calories derived from fat in a product. Both parameters are important for the selection of diets by health conscious consumers and for health professionals compiling diets for those at risk for certain chronic diseases. Very little sample preparation is involved in the NIR determination, and the results are obtained rapidly without the use of chemicals and the necessity for chemical waste disposal. The modified PLS loadings for the models to predict energy derived from fat indicate that absorption by C–H groups in fat is the most important component of the models. The determi-

nation of energy derived from fat in cereal products expands the potential of NIR spectroscopy for the determination of the macro-nutrient profile in cereal products for purposes of nutrition labeling and healthy selection of foods by consumers.

ACKNOWLEDGMENT

We thank R. E. Miracle for excellent technical assistance and L. L. Vines for the GC analysis of two outlier samples.

LITERATURE CITED

- (1) Whitney, E. N.; Rolfes, S. R. *Understanding Nutrition*, 9th ed.; Wadsworth: Belmont, CA, 2002, pp 148–150.
- (2) Katan, M. B. Health effects of trans fatty acids. *Eur. J. Clin. Nutr.* **1998**, *28*, 257–258.
- (3) Dietary Guidelines for Americans; U.S. Department of Health and Human Services, Home and Garden Bulletin 232, 5th ed.; U. S. GPO: Washington, DC, 2000.
- (4) Kris-Etherton, P. M.; Binkoski, A. E.; Zhao, G.; Coval, S. M.; Clemmer, K. F.; Hecker, K. D.; Jacques, H.; Etherton, T. D. Dietary fat: assessing the evidence in support of a moderate-fat diet; the benchmark based on lipoprotein metabolism. *Proc. Nutr. Soc.* **2002**, *61*, 287–298.
- (5) Code of Federal Regulations; FDA, HHS.; 21, part 101.9; 2002.
- (6) Atwater, W. O.; Bryant, A. P. The availability and fuel value of food materials. Conn. (Storrs) Agricultural Experiment Station 12th Annual Report (1899), 1900; pp 73–110.
- (7) AACC Report. The Definition of Dietary Fiber. *Cereal Foods World*, **2001**, *46*, 112–129.
- (8) Merrill, A. L.; Watt, B. K. *Energy Value of Foods: Basis and Derivation*. United States Department of Agriculture Handbook 74 (slightly revised 1973); U. S. GPO: Washington, DC, 1955.
- (9) Kays, S. E.; Barton, F. E., II. Rapid prediction of gross energy and utilizable energy in cereal food products using near-infrared reflectance spectroscopy. *J. Agric. Food Chem.* **2002**, *50*, 1284–1289.
- (10) *Parr Manual No. 120, Oxygen Bomb Calorimetry and Oxygen Bomb Combustion Methods*; Parr Instrument Co.: Moline, IL, 1948.
- (11) *AOAC Official Methods of Analysis*, 16th ed.; Method No. 945.16, Oil in Cereal Adjuncts: Petroleum Ether Extraction Method; AOAC: Arlington, VA, 1995.
- (12) *AOAC Official Methods of Analysis*, 15th ed.; Method No. 945.15, Moisture in Cereal Adjuncts: Air Oven Method; AOAC: Arlington, VA, 1990.
- (13) Shenk, J. S.; Westerhaus, M. O. Population definition, sample selection, and calibration procedures for near-infrared reflectance spectroscopy. *Crop Sci.* **1991**, *31*, 469–474.
- (14) Shenk, J. S.; Westerhaus, M. O. Population structuring of near-infrared spectra and modified partial least squares regression. *Crop Sci.* **1991**, *31*, 1548–1555.
- (15) Barton, F. E., II. Spectra. In *Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality*; Marten, G. C., Shenk, J. S., Barton, F. E. II, Eds.; USDA Agriculture Handbook 643; U. S. GPO: Washington, DC, 1989; pp 30–31.
- (16) Martens, H.; Jensen, S. C.; Geladi, P. Multivariate linearity transformation for near-infrared reflectance spectrometry. *Proceedings of the Nordic Symposium on Applied Statistics*; Stokand Forlag: Stavanger, Norway, 1983; 205–234.
- (17) Martens, H.; Naes, T. Assessment, validation and choice of calibration method. In *Multivariate Calibration*; John Wiley and Sons: New York, 1989; pp 237–266.
- (18) Hruschka, W. R. Data analysis: wavelength selection methods. In *Near-infrared Technology in the Agricultural and Food Industries*; Williams, P. C., Norris, K. H., Eds.; Am. Assoc. Cereal Chem.: St. Paul, MN, 1987; pp 53–54.
- (19) Williams, P. C. Implementation of near-infrared technology. In *Near-infrared Technology in the Agricultural and Food Industries*, 2nd ed.; Williams, P. C., Norris, K. H., Eds.; Am. Assoc. Cereal Chem.: St. Paul, MN, 2001; pp 145–169.
- (20) Abrams, S. M. Populations. In *Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality*; Marten, G. C., Shenk, J. S., Barton, F. E. II, Eds.; USDA Agriculture Handbook 643; U. S. GPO: Washington, DC, 1989; p 37.
- (21) Windham, W. R.; Mertens, D. R.; Barton, F. E. II. Protocol for NIRS calibration: sample selection, and equation development and validation. In *Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality*; Marten, G. C., Shenk, J. S., Barton, F. E. II, Eds.; USDA Agriculture Handbook 643; U. S. GPO: Washington, DC, 1989; pp 96–103.
- (22) *AOAC Official Methods of Analysis*, 17th ed., 1998; AOAC: Arlington, VA, 1990; Method No. 996.01 (Fat (Total, Saturated, Unsaturated, and Monounsaturated) in Cereal Products).
- (23) Murray, I.; Williams, P. C. Chemical principles of near-infrared technology. In *Near-Infrared Technology in the Agricultural and Food Industries*; Williams, P. C., Norris, K. H., Eds.; Am. Assoc. Cereal Chem.: St. Paul, MN, 1987; pp 17–34.
- (24) Williams, P. C.; Norris, K. H. Qualitative applications of near-infrared reflectance spectroscopy. In *Near-infrared Technology in the Agricultural and Food Industries*; Williams, P. C., Norris, K. H., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1987; pp 241–246.

Received for review September 5, 2003. Revised manuscript received December 15, 2003. Accepted December 19, 2003.

JF030646L