Nondestructive NIR Reflectance Spectroscopic Method for Rapid Fatty Acid Analysis of Peanut Seeds

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ABSTRACT

Near Infrared Reflectance Spectroscopy (NIRS) was used to rapidly and nondestructively analyze the fatty acid concentration present in peanut seeds samples. Absorbance spectra were collected in the wavelength range from 400 nm to 2500 nm using NIRS. The oleic, linoleic and palmitic fatty acids were converted to their corresponding methyl esters and their concentrations were measured using a gas chromatograph (GC). Partial least square (PLS) analysis was performed on a calibration set, and models were developed for prediction of fatty acid concentrations. The best model was selected based on the coefficient of determination (R^2), Root Mean Square Error of Prediction, residual percent deviation (RPD) and correlation coefficient percentage between the gas chromatography measured values and the NIR predicted values. The NIR reflectance model developed yielded RPD values of three and above for prediction of the three fatty acids, indicating that this nondestructive method would be suitable for fatty acid predictions in peanut seeds.

Key Words: Nondestructive, NIR reflectance spectroscopy, Partial Least Squares, Peanut kernels, Seeds, Fatty acids.

Near infrared spectroscopy (NIRS) techniques have become more common in food quality analysis. NIRS has been used to detect the harmful pathogens in milk, dairy products, meat and eggs (Pérez-Vich *et al.,* 1998; Velasco and Becker, 1998; Daun *et al.,* 1994). NIRS works on the principle of interaction of electromagnetic radiation with matter and the energy transfer. The spectral response for larger samples, such as whole grain, in the NIR region was found to be more pronounced than in the mid IR region. Thus, NIR spectra can be obtained on whole samples without the additional work of sample preparation. Electromagnetic response in the NIR region is primarily from the vibration response of O-H, C-H, C-O and N-H molecular bonds. Hydrogen bonds have more spectral stability in the NIR region, which helps greatly in food quality analysis, and may yield possible structural information of the chemical composition of the food material. Food components such as water, ethanol, sugars (fructose and glucose), organic acids, fatty acids, phenolic compounds, and food oxidative products contain many chemical bonds that absorb energy in the NIR spectrum, facilitating the application of NIRS in the measurement of food quality (Sundaram *et al.*, 2010a,b).

NIRS is useful for the quantitative measurement of composition and constituents such as, protein, carbohydrates, moisture content, oil content, fatty acid, acidity, total soluble solids, as well as physical properties such as freshness, color and maturity measurements of food materials (Sundaram *et al.*, 2010a,b, Windham *et al.*, 2010). Combining NIR measurements with chromatographic techniques would be helpful for the identification and quantification of chemical constituents present in the food and agricultural products. The NIR technique was first used in 1964 for the measurement of moisture in agricultural products (Norris, 1964). After that, it was applied to moisture measurement of grain and oil seeds in 1965 by Norris and Hart (Norris and Hart, 1965). Since then, it has been used in the grain industries for the rapid measurements of oil, protein and moisture (Rosenthal, 1973).

In the peanut industry, peanuts are shelled and graded by Federal-State Inspection Service to determine the price paid to the grower. The overall grade of the peanuts is determined by considering different quality factors such as moisture content, meat content, and size of pods, kernel size, damaged kernels and foreign material present in the peanuts. Peanut samples of a recommended weight are cleaned, shelled and subjected to different tests. Similarly, while the peanuts are processed for making them into different commercial products it is important to know the moisture content, total oil content, fatty acid profile, protein and starch present.

Holman and Edmondson (1956) and Murray (1987) studied the near-infrared spectra of homologous series of fatty acids. Based on these studies, NIRS has been used to differentiate various types of oils, and to estimate the fatty acid composition of the oil in intact-seeds of rapeseed, mustard, and husked sunflower seeds (Panforda and DeManb, 1990; Bhatty, 1991; Sato, 2002). NIRS was first

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Table 1. The pedigree information and high oleic donor parents of thirty genotypes used in the test.

Code	Genotype	Pedigree	Oleic	Linoleic	Palmitic	Location of Seed Production
10	07TX3_3,4	GK-7 X F435*	67.52	10.44	9.28	Brownfield, TX
17	07TX3_23,24	AT108 X Sunoleic 95R*	72.21	6.63	7.85	Brownfield, TX
18	07TX3_25,26	AT108 X Sunoleic 95R*	75.86	3.71	7.18	Brownfield, TX
19	07TX4_21,22	GA Brown X AT108-HO*	70.40	7.85	8.51	Brownfield, TX
29	07TX7_9,10	ViruGard X NC-7	43.14	31.76	12.85	Brownfield, TX
36	07TX7_27,28	ViruGard X NC-7	42.40	33.23	12.81	Brownfield, TX
37	07TX8_1,2	ViruGard X NC-7	47.31	28.51	11.99	Brownfield, TX
40	07TX8_11,12	ViruGard X NC-7	50.49	26.30	11.32	Brownfield, TX
42	07TX8_19,20	ViruGard X NC-7	43.87	32.06	12.53	Brownfield, TX
45	07TX9_3,4	ViruGard X NC-7	45.40	30.42	12.27	Brownfield, TX
48	07AL1_61,62	AT-50-1114* X AT 97-688	75.60	2.59	8.43	Headland, AL
57	07AL2_57,58	AT-50-1114* X AT 97-688	74.04	4.31	8.95	Headland, AL
73	07AL4_43,44	GK7-HO* X H95	74.53	4.83	9.10	Headland, AL
74	07AL4_49,50	GK7-HO* X H95	75.48	4.12	8.96	Headland, AL
75	07AL4_51,52	GK7-HO* X H95	76.39	3.47	8.43	Headland, AL
77	07AL4_55,56	GK7-HO* X H95	73.12	4.76	9.21	Headland, AL
58	07AL3_3,4	ViruGard X (Southern Runner X GK-7)	54.63	15.82	10.27	Headland, AL
59	07AL3_7,8	ViruGard X (Southern Runner X GK-7)	55.40	15.91	10.53	Headland, AL
60	07AL3_11,12	ViruGard X (Southern Runner X GK-7)	60.11	17.44	10.46	Headland, AL
62	07AL3_25,26	ViruGard X (Southern Runner X GK-7)	58.89	17.72	10.73	Headland, AL
63	07AL3_27,28	ViruGard X (Southern Runner X GK-7)	60.99	16.19	10.96	Headland, AL
67	07AL3_37,38	ViruGard X (Southern Runner X GK-7)	60.16	16.50	11.22	Headland, AL
82	07AL5_47,48	ViruGard X (Southern Runner X GK-7)	55.37	21.07	11.70	Headland, AL
83	07AL5_49,50	ViruGard X (Southern Runner X GK-7)	58.53	18.09	11.50	Headland, AL
52	07AL2_31,32	ViruGard X AT108-HO*	52.19	22.79	12.34	Headland, AL
53	07AL2_33,34	ViruGard X AT108-HO*	51.34	23.55	12.40	Headland, AL
80	07AL5_35,36	ViruGard X AT108-HO*	50.29	25.31	12.64	Headland, AL
69	07AL4_15,16	ViruGard X GA Green	53.02	23.39	11.79	Headland, AL
78	07AL5_7,8	ViruGard X GA Green	46.45	24.05	11.73	Headland, AL
96	07AL6_49,50	ViruGard X GA Green	48.17	27.23	13.08	Headland, AL

applied to determine oil, protein, water, and fiber contents in sunflower meal samples (Robertson and Barton, 1984 and Kaffka *et al.,*1982), and later for fatty acid composition (Sato *et al.,*1995). Oil, protein, chlorophyll and glucosinolate content of whole rapeseed kernels were also analyzed using NIRS by Tkachuk *et al.,* (1988). Nimaiyar *et al.,* (2004) used Fourier-Transform (FT) NIRS to develop calibrations equations to measure palmitic, stearic, oleic, linoleic and linolenic acids in soybeans. FT-NIR calibration models predicted the fatty acids with correlation coefficients ranging from 0.49 to 0.87, Root Mean Square Error of Prediction values from 0.39% to 3.46% of total oil and RPD values from 1.0 to 1.9 for the five fatty acids.

It is well documented that NIRS could be used successfully for the measurement of protein, moisture, oil content and fatty acid composition of peanuts. Misra *et al.*, (2000) used NIRS for measuring the oil content in different cultivars of peanuts from different harvests. Tillman *et al.,* (2006) showed that NIRS could be used for the nondestructive determination of oleic and linoleic acid concentrations in single peanut seeds. During the breeding programs, breeders usually acquire small quantities of samples for testing, and the ability to analyze the samples nondestructively would be valuable to them. NIRS analysis could be performed rapidly compared to other conventional methods such as gas chromatography and it does not need any sample preparation. Working with larger sample groups instead of single seeds during NIRS analysis would be useful in analyzing bulk samples.

Materials and Methods

Peanut Samples

Thirty genotypes of runner-type peanut seeds were used in this study (Table 1). These genotypes were derived from nine crosses with considerable variations of oleic acid content. Seeds were provided by the peanut breeding program at USDA-ARS National Peanut Research Laboratory and were harvested in fall 2007 either in Headland, AL or Brownfield, TX. High oleic content lines among 30

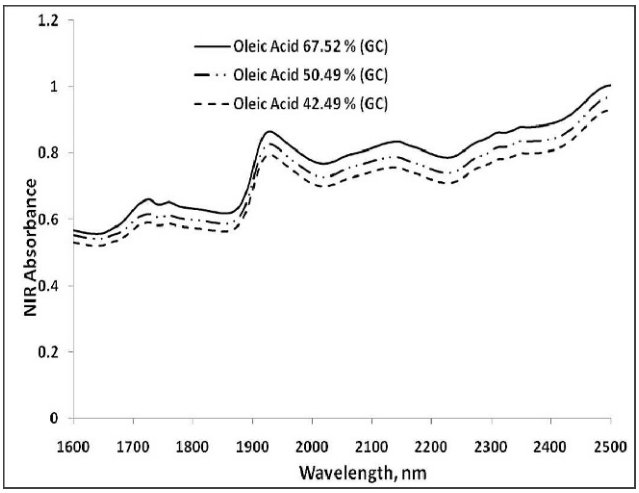

Fig. 1. Primary absorption spectrum of peanut kernels varying with oleic acid percentage.

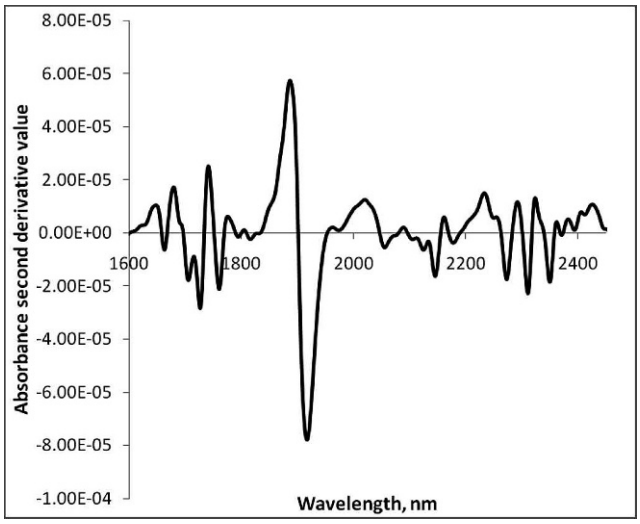

Fig. 2. Absorption derivative spectra of peanut kernel.

genotypes were contributed by five high oleic donor parents: 'F435', 'Sunoleic 95R', 'AT108-HO', 'AT-50-1114', and 'GK-7-HO' (Table 1). All seeds were dried to a safe moisture level of ≤ 10.5%, wet basis (USDA, 2000). Petroleum ether (Fisher Chemicals, USA) was used for oil extraction. Cyclohexane, acetone and sodium methoxide (Sigma-Aldrich, St. Louis, MO, USA) were used to prepare oil samples for fatty acid analysis using gas chromatography, to develop fatty acid reference values (Sundaram *et al.*, 2010a).

Oil extraction

A Soxtec[3] semi automatic method was used to extract the total oil from peanut samples with petroleum ether. Well-developed and intact kernels were selected from the different cultivars of the peanuts for the measurements. Kernel samples were frozen before grinding to avoid loss of any oil while grinding. About one gram of homogeneously ground fine peanut meal was weighed and placed in a cellulose thimble. The thimble was covered using a thin layer of defatted cotton, to avoid any spilling of petroleum ether. The thimble was fixed on to a metal ring through which the thimble could be attached to the Soxtec extractor. Metal cups were placed below each of the thimble to collect the extracted oil from a sample. Weights of the empty cups were recorded and the cups were placed on the metal plate. About 90 ml of petroleum ether was added to each thimble from the top. Thimbles were heated to boil the petroleum ether. While boiling, the petroleum ether extracted oil contained in the peanut meal. Temperature was maintained at 135°C ± 2°C during boiling, for 45 minutes. By end of the boiling time, petroleum ether and oil mixture was collected in the metal cups placed below the thimbles. Heating the collection cups was continued for another 45 minutes at 135°C ± 2°C to evaporate the petroleum ether from the mixture. The cups were cooled for 15 minutes. During the cooling process, evaporated petroleum ether that had condensed was collected separately. The

[3]Mention of company or trade names is for the purpose of description only and does not imply endorsement by the U.S. Department of Agriculture.

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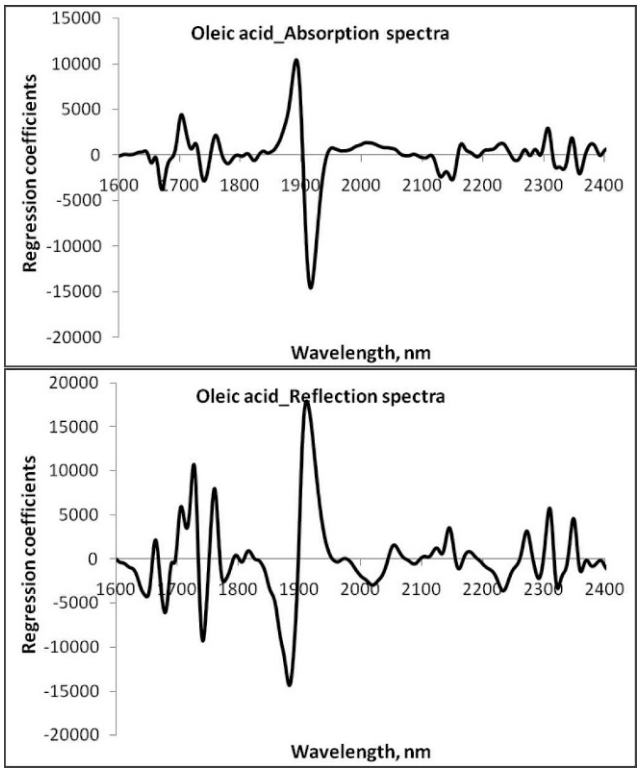

Fig. 3a. Absorption and reflection derivative regression coefficients of oleic acid.

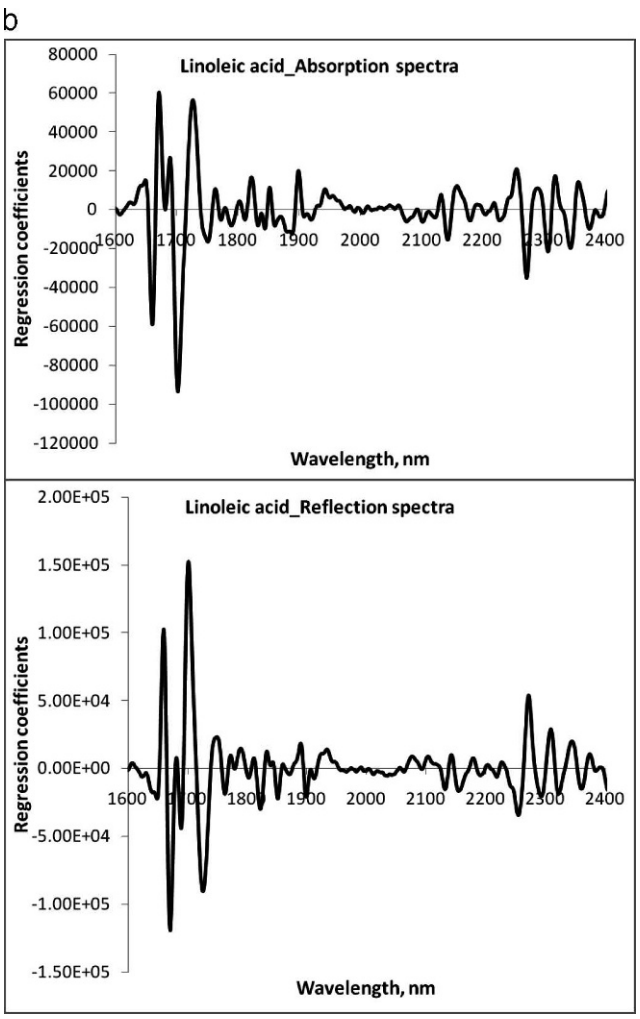

Fig. 3b. Absorption and reflection derivative regression coefficients of linoleic acid.

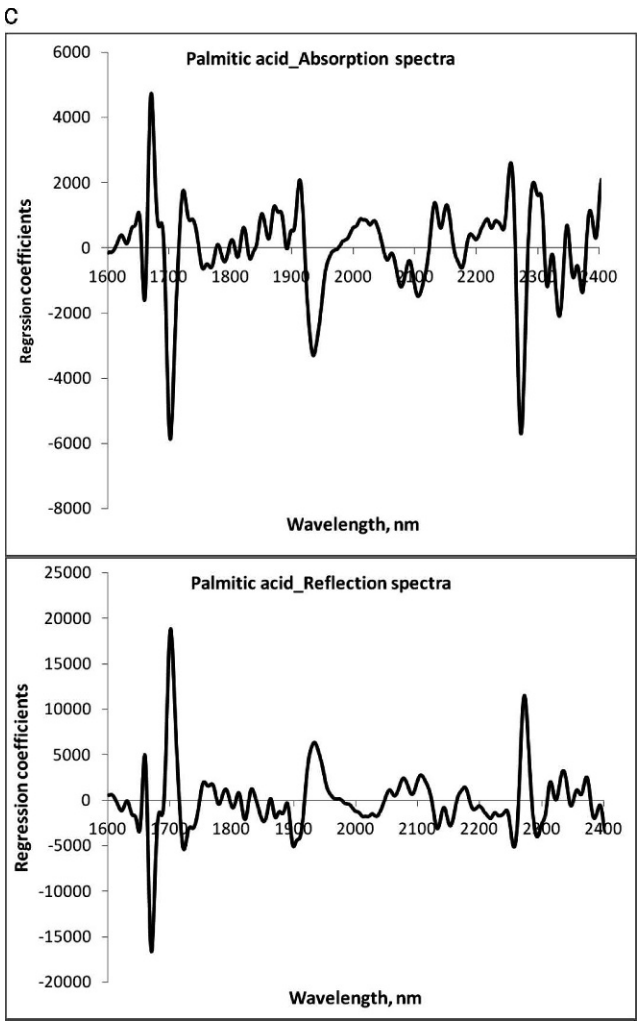

Fig. 3c. Absorption and reflection derivative regression coefficients of palmitic acid.

collecting cups were removed and kept in a hot-air oven at 40°C for 30 minutes to drive out any traces of petroleum ether. From the weights of, empty collecting cups, cups with oil, and initial ground peanut kernel meal, the total oil percentage was calculated. The measurements were done in triplicate for each cultivar (Sundaram *et al.*, 2010a).

Fatty acid analysis

Fatty acids present in the peanut samples were analyzed using Gas Chromatography (GC) (Agilent Technologies, 6890N Network GC). Oils extracted from each peanut group, using the method described above, were used for fatty acid analysis. About 10–12 mg of the extracted peanut oil was reacted with 0.2 ml of sodium methoxide to convert the fatty acids into corresponding esters. To complete this process, the oil and sodium methoxide mixture was heated to 50°C, and thoroughly mixed using a sonicator until it was derivatized completely. About 0.5 ml of cyclohexane was then added to the mixture to recover the derivatized esters and the whole mixture was sonicated for about 10–15 minutes and allowed to undergo phase separation. From the upper phase, 10–20 µl was withdrawn and diluted using one ml of acetone. This mixture was taken in a glass vial and analyzed using a GC fitted with a SP 2380 fatty acid column. The oven temperature in GC was maintained at 185°C. Hydrogen, air, and helium gas flow rates were 40, 450 and 0.6 ml/minute respectively. One µl of the sample was injected for each measurement. Total run time was 20 minutes. Within 20 minutes, esters of all major fatty acids namely oleic, linoleic and palmitic acids were separated and well-identified. Before running the oil samples, a fatty acid methyl ester (FAME) standards were run by diluting FAME in acetone. Based on the FAME peak references peanut fatty acid peaks were identified.

NIRS measurements

The peanut seed samples were separated into calibration and validation groups. NIRS measure-

Table 2. Fatty acid composition in different peanut cultivars determined using gas chromatography method and near infrared spectroscopy predicted values in calibration model.

Oleic GC area %			Linoleic GC area %			Palmitic GC area %		
GC**	NIR-absorption	NIR-reflection	GC**	NIR-Absorption	NIR-Reflection	GC**	NIR-Absorption	NIR-Reflection
67.52	69.94	67.32	10.44	10.44	10.49	9.28	9.28	9.24
72.21	73.31	72.46	6.63	6.61	6.72	7.85	8.16	7.87
75.86	70.23	75.92	3.71	3.72	3.71	7.18	7.08	7.20
70.40	68.64	70.09	7.85	7.83	7.73	8.51	8.53	8.52
42.40	43.59	42.20	33.23	33.23	33.24	12.81	12.84	12.87
47.31	47.21	47.31	28.51	28.51	28.67	11.99	11.86	11.93
50.49	50.37	49.83	26.30	26.29	26.54	11.32	11.96	11.36
43.87	44.92	44.30	32.06	32.07	31.94	12.53	12.07	12.55
45.40	47.94	45.73	30.42	30.41	30.23	12.27	12.19	12.26
75.60	72.88	75.63	2.59	2.58	2.34	8.43	8.26	8.41
52.19	50.95	52.58	22.79	22.79	22.52	12.34	12.21	12.22
51.34	48.94	51.64	23.55	23.54	23.64	12.40	12.35	12.41
74.04	75.21	73.72	4.31	4.314	4.60	8.95	9.21	8.89
54.63	56.20	54.55	15.82	15.83	16.04	10.27	10.48	10.35
55.40	57.32	55.61	15.91	15.90	15.85	10.53	10.09	10.58
60.11	57.13	59.63	17.44	17.43	17.31	10.46	10.79	10.49
58.89	60.90	59.12	17.72	17.73	17.67	10.73	10.68	10.67
60.99	56.67	60.58	16.19	16.18	16.21	10.96	10.79	11.04
60.16	58.20	60.32	16.50	16.49	16.57	11.22	11.21	11.23
53.02	52.19	52.78	23.39	23.38	23.54	11.79	11.80	11.79
74.53	79.67	74.76	4.83	4.82	4.89	9.10	8.718	9.07
75.48	77.65	75.87	4.12	4.13	3.90	8.96	9.34	9.05
76.39	71.56	76.53	3.47	3.46	3.44	8.43	8.47	8.33
73.12	73.53	72.74	4.76	4.76	4.83	9.21	8.99	9.23
46.45	42.44	46.25	24.05	24.04	24.03	11.73	11.86	11.68
50.29	51.52	50.11	25.31	25.30	25.25	12.64	12.41	12.59
55.37	57.36	55.36	21.07	21.05	20.93	11.70	11.71	11.57
58.53	60.97	58.92	18.09	18.10	18.14	11.50	11.67	11.52
48.17	50.64	48.01	27.23	27.23	27.20	13.08	13.12	13.15
43.14	44.21	43.29	31.76	31.75	31.75	12.85	12.76	12.81

ments were made using a scanning monochromator (Model 6500, FOSS NIRSystems, Silver Springs, MD, USA). Spectral data were collected using Vision software (Version 1.0, FOSS NIRSystems, Silver Springs, MD, USA). Each replicate sample consisted of 100–200 g of peanut seeds depending on the amount required to adequately fill the sample holder. The room temperature during the measurements varied from 21°C to 23°C. NIR light passed through the bottom of the sample holder and was incident on the in-shell peanuts. The transmitted and reflected energy spectrum over the wavelengths of 400 nm to 2500 nm that carried absorption information of the samples was collected. This spectral data was collected for 30 replicates of each peanut cultivar.

Data analysis

NIRS data were analyzed using multivariate data analysis software (Unscrambler Version 9.7 CAMO ASA, USA). Reflectance spectra between 400–2500 nm with 0.5 nm intervals were taken as independent variables. Fatty acid concentration was the dependent variable in the analysis. Using the spectral data from the calibration dataset, partial least square (PLS) regression analysis was conducted to develop an empirical equation to estimate the concentrations of oleic, linoleic and palmitic acids. Before analysis, raw data often needs mathematical pretreatments to remove any defects in the spectra. Before applying the PLS analysis, absorption spectral data were converted in to reflectance spectral data. The derivatives of the absorption and reflection spectral data with respect to wavelength were computed. The derivative computation was used to remove any baseline shifting that may have occurred and to avoid overlapping of peaks. The fatty acids constitute a small fraction (less than 5%) of the mass index of total oil and moisture contents in the samples, and their spectral characteristics could be easily overshadowed by the total oil and moisture contents. By applying the derivative treatment, the modified

Table 3. Fatty acid composition in different peanut cultivars determined using gas chromatography method and near infrared spectroscopy predicted values in cross validation.

Oleic GC area %			Linoleic GC area %			Palmitic GC area %		
GC**	NIR-absorption	NIR-reflection	GC**	NIR-Absorption	NIR-Reflection	GC**	NIR-Absorption	NIR-Reflection
67.52	68.65	69.86	10.44	6.38	7.47	9.28	8.24	8.49
72.21	73.38	72.90	6.63	8.32	5.38	7.85	9.14	8.71
75.86	68.68	73.51	3.71	6.11	6.28	7.18	7.58	8.00
70.40	68.31	70.78	7.85	8.66	7.68	8.51	8.30	8.67
42.40	46.24	48.19	33.23	24.05	30.80	12.81	10.96	12.53
47.31	47.53	49.57	28.51	28.97	28.90	11.99	11.53	11.66
50.49	50.40	48.58	26.30	28.71	28.66	11.32	12.60	12.14
43.87	45.60	44.41	32.06	27.75	29.83	12.53	11.33	11.24
45.40	48.84	48.71	30.42	27.60	26.95	12.27	11.85	11.45
75.60	70.60	76.19	2.59	0.13	1.97	8.43	8.07	8.00
52.19	50.82	54.51	22.79	23.78	20.21	12.34	12.17	12.12
51.34	48.23	51.65	23.55	24.30	23.34	12.40	11.91	12.08
74.04	74.65	72.43	4.31	5.40	4.56	8.95	9.07	8.17
54.63	57.77	54.47	15.82	17.98	18.16	10.27	10.98	11.89
55.40	57.72	56.40	15.91	13.14	15.93	10.53	9.87	9.96
60.11	57.07	56.41	17.44	19.42	18.24	10.46	11.45	11.21
58.89	61.09	60.50	17.72	15.00	15.75	10.73	10.73	10.34
60.99	56.16	56.05	16.19	20.26	20.65	10.96	11.13	11.18
60.16	58.02	57.96	16.50	19.18	18.20	11.22	10.97	11.37
53.02	52.59	49.97	23.39	24.39	24.62	11.79	11.80	12.91
74.53	80.75	76.12	4.83	1.24	3.94	9.10	8.37	8.50
75.48	77.63	72.78	4.12	5.66	6.05	8.96	9.64	9.41
76.39	68.25	74.31	3.47	8.76	5.22	8.43	8.50	8.39
73.12	72.36	73.94	4.76	2.03	1.85	9.21	8.35	8.83
46.45	38.26	43.41	24.05	29.91	28.44	11.73	13.30	12.38
50.29	53.13	56.045	25.31	21.58	22.52	12.64	12.06	12.81
55.37	57.71	55.73	21.07	16.51	18.37	11.70	11.54	11.26
58.53	62.09	60.19	18.09	20.57	18.58	11.50	11.75	11.17
48.17	50.49	47.36	27.23	25.34	25.21	13.08	12.18	12.45
43.14	46.85	42.50	31.76	33.27	36.92	12.85	12.84	12.86

wavelength spectrum showed more details by diminishing the influence of the lower frequencies, which hides the clarity of the spectral signatures and enhances the higher frequencies to show the spectral signature of fatty acids clearly. However, the derivative treatment was limited to second order because at higher orders excessive smoothing may take place that would diminish the contributions of the characteristic peaks.

Table 4. Correlation coefficient obtained for gas chromatography measured and near infrared spectroscopy predicted fatty acid concentrations.

Composition	Absorbance derivative spectra Correlation coefficient	Reflectance derivative spectra Correlation coefficient
Oleic	0.95	0.98
Linoleic	0.94	0.97
Palmitic	0.89	0.92

Results and Discussion

The NIR region selected for fatty acid analysis was 1600–2400 nm, because the absorption bands of *cis*-unsaturation and length of carbon chain in fatty acids appear around this region of wavelengths (Panforda and DeManb, 1990). The primary NIR absorption spectra of peanut kernels, at three different percentage contents of oleic acid are shown in Fig 1. The peaks and valleys in the NIR region represent absorption of electromagnetic energy. Strong NIR absorption bands near 1400–1440 nm and 1900–1950 nm are often used for quantitative analysis of water content in foods. The NIR spectra consisted of peaks at 1722, 1735, 1757, 1768, 1787, 2127 and 2144 nm for saturated and *trans-unsaturated* fatty acids (Fig. 2). The peaks around 1660 nm and 2145 nm are due to *cis –unsaturation.* All these peaks appeared more clearly than in primary spectra given in Fig. 1.

Table 5. Fitness measures of calibration model for peanut fatty acids.

	Absorbance derivative spectra			Reflectance derivative spectra		
Composition	R^2	RMSEC*	Bias	R^2	RMSEC	Bias
Oleic	0.99	2.71	0.02	0.99	−0.02	2×10^{-4}
Linoleic	0.99	0.01	1×45^{-5}	0.99	−0.01	2.5×10^{-6}
Palmitic	0.99	0.24	0.000	0.99	0.00	-8×10^{-5}

*Root mean square error of calibration.

The regression coefficients of the calibration model were developed for the fatty acids, oleic acid (Fig.3a), linoleic acid (Fig.3b) and palmitic acid (Fig. 3c) using absorbance and reflectance derivative data and PLS regression. Wavelengths corresponding to many of the peaks appearing in this figure are related to fatty acid composition of peanuts. The peaks ranging between 1600–1800 nm and 2100–2400 nm contributed significantly to the regression coefficients of the calibration equation developed using the reference values of fatty acids obtained from GC methods. Table 2 shows the fatty acid concentration determined using GC and also the values predicted using NIR through the PLS calibration equation. Table 3 shows the GC measures as well as the NIR predicted values through the cross validation of the model. The correlation coefficient obtained between the GC- measured and NIR- predicted fatty acid concentrations in cross validation are given in Table 4. Both NIR absorption and reflection models gave a correlation coefficient of 0.94 (94%) or better for oleic and linoleic acids. Palmitic acid gave a correlation coefficient of 0.89 (89%) with the absorption model and 0.92 (92%) with the reflection model. Fitness measures of PLS calibration regression curves are given in Table 5. R^2 obtained in this table is 0.99 for all the models and fatty acid concentrations. The PLS calibration curve fitted well with the fatty acid concentration percentages and the NIR wavelengths selected for the prediction model development. Also, their root mean square error of calibration (RMSEC) values, were low. Based on guidelines for interpretation of R^2, outlined by Williams and Norris (2001) and Williams (2001), NIR calibration equations for fatty acids were found suitable for quality assurance applications. A previous study by Pazdernik *et al.,* (1997), on the applicability of NIRS for fatty acid composition determination in soybeans, resulted in R^2 values (for validation samples) of 0.18 (palmitic), 0.38 (oleic), and 0.52 (linoleic) for whole-seeds.

Table 6 shows the fitness measures of the cross validation of the fatty acid predictions using the PLS calibration model. Relative percent deviation (RPD) values (standard deviation divided by Standard Error of Prediction) obtained for the fatty acid prediction through the absorption and reflection models are greater than 2. The reflection model gave the RPD values for linoleic and palmitic acids greater than 3 and thus they would be useful for estimating the respective fatty acid concentrations in peanut kernels. Because higher values indicate a stronger calibration model to predict the composition accurately in the unknown samples (Sundaram *et al.*, 2010a; Fearn, 2002; Williams, 2001). The same model gave an RPD value close to 3 for oleic acid, making it suitable for predicting oleic acid in peanuts. However, the absorption models did not perform as well as the reflectance model. The performance may improved by increasing the sample size and introducing more samples with very high and low values of fatty acids into corresponding calibration data sets, predictive ability of NIRS for these constituents may be improved. Therefore, for this analysis, it could be concluded that reflectance derivative model is the best model for fatty acid concentration prediction.

Table 6. Fitness measures of cross validation of peanut fatty acids.

	Absorbance derivative spectra			Reflectance derivative spectra		
Composition	RPD*	RMSEP**	R^2	RPD	RMSEP	R^2
Oleic	2.52	4.45	0.99	2.94	3.82	0.99
Linoleic	2.84	3.40	0.99	3.69	2.70	0.99
Palmitic	2.17	0.77	0.99	6.80	0.66	0.99

*Relative percent deviation (Standard deviation ÷ Standard error of prediction).
**Root mean square error of prediction.

Summary and Conclusion

NIRS could be a useful tool for the nondestructive analysis of fatty acid concentration of peanut seeds (kernels). An NIR reflectance derivative model was found to be the best model for the prediction of all three fatty acids viz., oleic, linoleic and palmitic based on the RPD values and correlation coefficients. NIR measurements are procedurally very simple, considerably reducing the time required for measurements to approximately 5 to 10 minutes, compared to standard analytical procedures such as gas chromatography which typically requires 2 to 4 hours. The use of NIR spectroscopy as described in this paper would result in significant savings in time and labor and is suitable for use in peanut breeding program to select and classify the seeds based on their fatty acid concentrations. Further improvement in the analysis of NIR data may make it possible to estimate the fatty acid profile with sufficient accuracy to improve quality control of peanut samples for peanut breeders.

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