RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
46 (2) 305-312, 2014

OLEIC ACID DETERMINED BY GAS LIQUID CHROMATOGRAPHY AND NEAR-INFRARED REFLECTANCE SPECTROSCOPY IN SEGREGATING POPULATIONS OF PEANUT

N. SINGKHAM[1], S. JOGLOY[1*], T. KESMALA[1], P. SWATSITANG[2], P. JAISIL[1], N. PUPPALA[3] and A. PATANOTHAI[1]

[1]Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand
[2]Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand
[3]Agricultural Science Center at Clovis, New Mexico State University, Clovis, New Mexico, 88101, USA
*Corresponding author's email: sanun@kku.ac.th

SUMMARY

A rapid and cost-effective method is needed to evaluate parental lines and breeding populations for seed chemistry for use in peanut breeding and genetics. This study was initiated to compare oleic acid in segregating populations of peanut using gas liquid chromatography (GLC) and near–infrared reflectance spectroscopy (NIR). Twenty F_2 crosses originating from 5 parent diallel crosses, and the generations of F_2, F_3, $BC_{11}S$ and $BC_{12}S$ originating from 3 parental lines were evaluated in 2 separate experiments during the 2008 rainy season. The bulk seeds were analyzed for oleic acid using GLC method, and a single seed was predicted for NIR method. The ranges for oleic acid were 47.5-79.6 for GLC and 59.6-85.2for NIR estimates in both experiments. Oleic acid contents determined by NIR were relatively higher than those determined by GLC. However, estimated from both the methods showed high correlation coefficients 0.83 ($P \leq 0.01$) in the 20 F_2's of 5 parent diallel cross and 0.86 ($P \leq 0.01$) in F_2, F_3, $BC_{11}S$ and $BC_{12}S$ of the 3 crosses. NIR method could be used to discard breeding lines with lower range in oleic acid and those retained should be further evaluated for accurate analysis of oleic content by GLC.

Keywords: Groundnut, O/L ratio,rapid method, segregating population, breeding, seed quality

Manuscript received: July 17, 2013; Decision on manuscript: August 25, 2014; Manuscript accepted: September 16, 2014.

Communicating Editor: Bertrand Collard

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an important oilseed crop worldwide. The seeds contain 42–49% oil (Jonnala *et al*., 2005) and oleic acid constitutes about 40–80% of total fatty acids (Andersen and Gorbet, 2002; Singkham *et al*., 2010). Peanut seeds with high oleic acid have greater flavor stability and longer shelf-life than low oleic peanuts (O'Keefe *et al*., 1993; Braddock *et al*., 1995; Mugendi *et al*., 1998). Regular consumption of high oleic peanut reduces blood serum cholesterol and low density lipoproteins (LDL) and thus minimizes the risk of cardiovascular diseases in humans (Alper and Mattes, 2003; O'Byrne *et al*., 1997).

Gas liquid chromatography (GLC) is the most common and accurate method of determining fatty acids in oil crops. However, it is destructive, expensive, labor-intensive and time-consuming (Kim *et al*., 2009). Pérez–Vich *et al*. (1998) successfully used near-infrared reflectance spectroscopy (NIR) as a rapid, simple and cost-effective alternative method for

large-scale screening of intact sunflower seeds for oil content and fatty acid composition. Tillman *et al*. (2006) developed an equation for calibrating oleic acids in peanut by NIR, compared it with GLC using the same single seeds of pure lines, and found NIR gave reasonable precision of predicted values of oleic acid. Moreover, NIR method can predict oleic, linoleic and palmitic acid contents as well as GLC method in large seed samples of peanuts (Sundaram *et al*., 2011). However, the use of NIR method in screening of segregating populations of peanut has not been reported. The objective of this study was to compare oleic acid contents as determined by GLC with those predicted by NIR in 20 F_2 populations originating from 5 parental full diallel crosses and of segregating populations (F_2, F_3, BC_1P_1 and BC_1P_2) originating from 3 crosses of peanut.

MATERIALS AND METHODS

Experiment 1

Five peanut genotypes with large variation in seed oil chemistry [high oleic acid peanut lines– SunOleic 97R (Gorbet and Knauft, 2000) and Georgia–02C (Branch, 2003); peanut with intermediate oleic acid – [(NC17090 × B1)-9-1/KK60-3]$_{F6-8-3}$, designated as F_{6-8-3} (Singkham *et al*., 2010); and low oleic acid peanut lines – KKU 1 and KKU 5 (Singkham *et al*., 2010)] were crossed in a full diallel mating design to generate 20 F_1 hybrids. SunOleic 97R and Georgia–02C are released peanut cultivars in USA, while KKU 1, KKU 5 and F_{6-8-3} are advanced breeding lines developed at Khon Kaen University, Thailand.

The 20 F_1 hybrids were grown at Khon Kaen (16°26′N, 102°50′E, 190 masl), in Northeast Thailand during the 2008 rainy season. The experiment was conducted in a randomized complete block design with 4 replications. The plot size was 2-row plot with 1 m long and spacing of 50 cm between rows and 20 cm between plants within row. Locally adopted standard agronomic practices were followed, including application of lime at the rate of 625 kg/ha, to prepare the soil for conducting the experiment. The seeds were treated with ethrel (2-chloroethylphosphonic acid) 48% at the rate of 2 mL/L water to break seed dormancy and also treated with captan (3a, 4, 7, 7a-tetrahydro-2-[(trichloromethyl)thio]-1*H*-isoindole-1, 3(2*H*)-dione) at the rate of 5 g/kg of seeds before planting to control soil-born fungal diseases. Pre-emergence herbicide, Alachlor (2-cholro-2′, 6′-diethyl-*N*-(methoxymethyl) acetanilide 48%, w/v, emulsifiable concentrate) at the rate of 3.75 L/ha, was applied immediately after sowing. N-P-K at the rate of 23.4, 10.2, and 19.4 K kg/ha, respectively,were applied at 14 days after emergence (DAE), while gypsum ($CaSO_4$) at the rate of 312 kg/haat 45 DAE. Carbofuran (2, 3-dihydro-2, 2-dimethylbenzofuran-7-ylmethylcabamate 3% granular) was applied at early pod development stage to control subterranean ants (*Dorylusorientalis*). Pests and diseases were controlled by regular spray of carbosulfan [2-3-dihydro-2, 2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20% w/v, water soluble concentrate] at 2.5 L/ha, methomyl [*S*-methyl-*N*-((methylcarbamoyl)oxy) thioacetimidate 40% soluble powder] at 1.0 kg/ha. Supplementary irrigation was given during the dry periods in the rainy season with an overhead sprinkler system. Ten plants per plot were harvested manually at maturity and the F_2 seeds from each plot were bulked and divided into 2 sets for oleic acid determination by GLC and NIR methods.

Experiment 2

The F_1, F_2, backcross to female parent (BC_{11}) and backcross to male parent (BC_{12}) involving SunOleic 97R and Georgia–02C crossed with KKU 1 were grown in a randomized complete block design with 4 replications in the 2008 rainy season at Khon Kaen, Thailand. Each plot was comprised of 3 rows of 1 m length, spaced at 50 cm between rows and 20 cm between plants within a row. Standard agronomic practices were followed peanut crop as was the case with experiment 1. Fifteen plants in each plot were harvested at maturity. Seeds from each plot were bulked and divided into 2 sets. The first set was analyzed for oleic acid using GLC, while the second set was used for analysis of oleic acid using NIR.

NIR predicted oleic acid

Oleic acid content was predicted for each plot using a sample of 10 kernels following NIR method (Tillman *et al*., 2006). Each individual seed was scanned by a ThermoNicolet Industrial Solutions (Fitchburg, WI) Nexus 670 FT-IR scanning monochronometer equipped with a NearIRUpDrift Smart Accessory and the data on 10 kernels for each plot were averaged.

GLC measured oleic acid

A sample of 20 kernels for each plot was ground and oven-dried at 70°C for 15 to 20 h. Moisture content was determined by the difference of weights prior to drying and after drying. Two grams of oven–dried sample was used for oil extraction by the Soxtec extractor in 50 mL of petroleum ether.

The extracted oil was determined for fatty acid content by gas liquid chromatography (GLC). The protocol of fatty acid analysis was modified from Bannon (Bannon *et al*., 1982). Fatty acid methyl esters (FAME) were prepared by adding 1 ml of 2.5% H_2SO_4/MeOH in 10 mg of oil sample and 100 µl of 0.01 g/ml C17:0 an internal standard. The mixture was incubated at 80°C for 2 h. After incubation, 200 µl of 0.9% (w/v) NaCl and 200 µl of heptane were added to the mixture and mixed well. The FAME was extracted into heptane. The concentration of oil sample was 33 µg, which was dissolved in a 1 µl of FAME. The FAME sample (2 µl) was injected to GLC (with Flame Ionization Detector: FID) for fatty acid analysis. Fatty acid analysis was conducted on Shimadzu Gas Chromatograph GC-14B-CR7A and SGE fort GC capillary column (30 m×0.25 mm ID BPX70 0.25 µm) was used. Helium was the carrier gas at a flow rate of 30 ml/min. Hydrogen and air were used at the rate of 30 and 300 ml/min, respectively for the ignition of the FID. Oven temperature was maintained at 130 °C for 2 min. Then it was programed at 5 °C/min to 220 °C and held at this temperature for 8 min. The injector temperature and detector temperature were 250 and 300 °C, respectively. The standard fatty acids that were used to identify the fatty acid content in peanut genotypes consisted of myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, eicosenoic, behedic, erucic and lignoceric acids.

The significant differences in the mean of oleic acid between GLC and NIR methods were analyzed byt-test. Simple correlation was used to determine the relationship between oleic acid content between GLC and NIR methods. All calculations were done using MSTAT–C package (Bricker, 1989).

RESULTS

The oleic acid content in the experiment 1 ranged from 47.5 to 79.6 for GLC method and 59.6 to 85.2 for NIR method (Table 1). Mean differences in the experiment 1 between oleic acid determined by GLC and NIR method were not significant for SunOleic 97R/Georgia–02C, SunOleic 97R/$F_{6\text{-}8\text{-}3}$, Georgia–02C/SunOleic 97R, and $F_{6\text{-}8\text{-}3}$/Georgia–02C.

For the experiment 2, oleic acid content ranged from 49.4 to 79.0 for GLC method and 59.8 to 83.9 for NIR method (Table 2). All crosses in different generations exhibited significant differences for oleic acid contents determined between GLC and NIR methods except (Georgia–02C/KKU1)$BC_{11}S$, (SunOleic 97R/KKU1)F_3, (SunOleic 97R/GA02C)F_2, (SunOleic 97R/GA02C)F_3 and (SunOleic 97R/GA02C)$BC_{11}S$.

The correlation coefficients between oleic acid contents determined by the 2 methods were high for both experiments with the values of 0.83 ($P \leq 0.01$) in the F_2 generation of the 20 crosses and 0.86 ($P \leq 0.01$) in 3 crosses involving F_2, F_3, $BC_{11}S$ and $BC_{12}S$ (Figure 1 and 2), with slope of 0.72 in 20 F_2's (Figure 1) and 0.82 in 4 segregating generation in 3 peanut crosses (Figure 2).

DISCUSSION

The GLC method is commonly used in determining fatty acids in oil crops. Although this method is accurate and reliable, it is destructive, time-consuming, laborious and expensive. There is a need for a non-destructive and inexpensive method for screening segregating populations in peanut breeding

programs. The NIR method is non-destructive, rapid and inexpensive.

The oleic acid contents predicted by NIR were significantly higher than those determined by GLC in all the segregating populations (Table 1 and 2) and should be calibrated for more accurate estimation of the values. Tillman *et al*. (2006) found in peanut that the range of oleic acid measured by GLC at the extremes was smaller than those predicted by NIR, similar to those reported in sunflower (Velasco *et al*., 1999).In this study, the rather high predicted oleic acids at the extreme were not observed.

Table 1. Mean of oleic acid contents and standard error (SE) as measured by GLC and predicted by NIR methods in 20 F_2 populations from 5 parent diallel crosses.

Crosses	GLC	NIR	T-test
SunOleic 97R/Georgia–02C	79.6	83.5	Ns
SE	0.7	2.0	
SunOleic 97R/$F_{6\text{-}8\text{-}3}$	72.0	79.0	Ns
SE	0.8	3.7	
SunOleic 97R/KK5	61.8	73.9	**
SE	0.7	2.0	
SunOleic 97R/KKU1	64.6	72.5	*
SE	2.2	1.6	
Georgia–02C/SunOleic 97R	79.3	85.2	Ns
SE	0.7	2.4	
Georgia–02C/$F_{6\text{-}8\text{-}3}$	72.5	79.8	*
SE	1.2	2.0	
Georgia–02C/KK5	64.1	71.7	**
SE	0.6	0.8	
Georgia–02C/KKU1	59.5	74.6	**
SE	0.9	2.4	
$F_{6\text{-}8\text{-}3}$/SunOleic 97R	71.2	75.6	*
SE	0.8	1.1	
$F_{6\text{-}8\text{-}3}$/Georgia–02C	72.2	78.6	Ns
SE	0.6	2.6	
$F_{6\text{-}8\text{-}3}$/KK5	59.6	70.8	**
SE	0.9	0.9	
F6-8-3/KKU1	60.1	73.8	**
SE	0.9	2.6	
KK5/SunOleic 97R	55.5	66.5	**
SE	0.9	1.3	
KK5/Georgia–02C	58.3	67.5	*
SE	0.8	2.9	
KK5/$F_{6\text{-}8\text{-}3}$	59.0	67.2	**
SE	1.2	1.5	
KK5/KKU1	49.9	61.2	**
SE	1.2	1.3	
KKU1/SunOleic 97R	60.6	67.0	**
SE	0.6	1.5	
KKU1/Georgia–02C	57.0	67.3	**
SE	1.3	2.2	
KKU1/$F_{6\text{-}8\text{-}3}$	56.7	67.1	*
SE	0.5	3.5	
KKU1/KK5	47.5	59.6	**
SE	0.8	1.3	

*, ** and ns significant at 0.05, 0.01 and non-significant probability levels, respectively.

Correlation between the 2 methods (GLC and NIR) provided an indication about the reliability of oleic acid predicted by NIR in comparison to GLC method. Such high correlations between the 2 methods were also reported in previous studies, 0.89 to 0.91 in sunflower segregating populations (Velasco *et al*., 1999) or 0.99 amongst the pure lines of peanut (Tillman *et al*., 2006). In addition, the correlation coefficient between GLC method and NIR absorption and reflection models in bulk peanut seed were 0.95 and 0.98, respectively (Sundaram *et al*., 2011). There is greater emphasis in plant breeding programs worldwide to select and or develop cultivars that are high yielding with nutritious grains. However, timely and cost-effective determination of quality traits of a large number of samples is needed to make progress in breeding for quality traits. Obviously, there is a need to develop analytical assays that are non-destructive, high throughput, cost-effective and accurate, as well as requiring relatively small samples for simultaneous profiling of multi-nutrient elements. Assays based on wet chemistry are the most accepted to measure the levels of seed components.

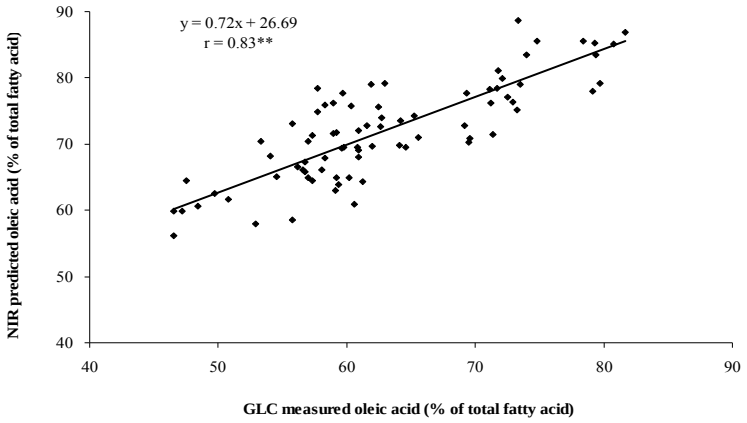

Figure 1. Correlation of oleic acid between GLC and NIR methods involving 20 F_2 populations of peanut.

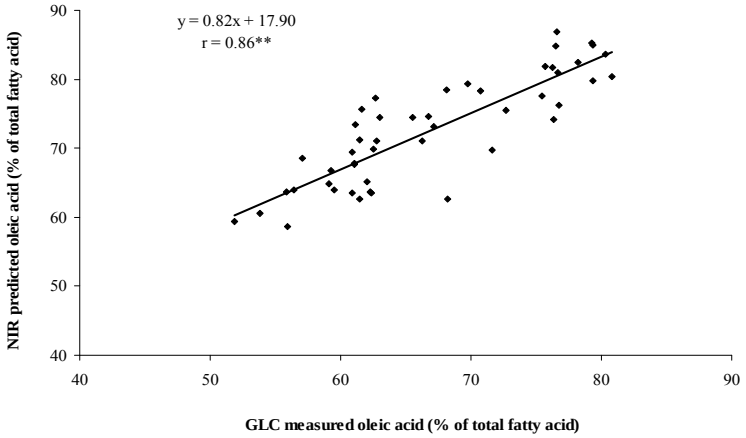

Figure 2. Correlation of oleic acid between GLC and NIR methods involving the parents, F_2, F_3, and $BC_{11}S$ and $BC_{12}S$ of 3 crosses in peanut.

Table 2. Mean of oleic acid contents and standard error (SE) as measured by GLC and predicted by NIR methods in the parents, F_2, F_3, and $BC_{11}S$ and $BC_{12}S$ populations of 3 crosses in peanut.

Crosses	GLC	NIR	T-test
(Georgia–02C/KKU1)P_1	78.0	83.8	*
SE	0.7	1.8	
(Georgia–02C/KKU1)P_2	49.9	60.7	**
SE	0.8	1.2	
(Georgia–02C/KKU1)F_2	63.0	72.1	**
SE	1.0	1.4	
(Georgia–02C/KKU1)F_3	62.1	66.8	*
SE	0.4	1.7	
(Georgia–02C/KKU1)$BC_{11}S$	68.2	72.2	Ns
SE	0.9	3.3	
(Georgia–02C/KKU1)$BC_{12}S$	54.7	63.1	*
SE	1.1	2.0	
(SunOleic 97R/KKU1)P_1	79.0	83.7	*
SE	0.9	1.6	
(SunOleic 97R/KKU1)P_2	49.4	59.8	**
SE	1.0	1.5	
(SunOleic 97R/KKU1)F_2	64.5	75.0	**
SE	1.6	1.7	
(SunOleic 97R/KKU1)F_3	63.0	65.8	Ns
SE	2.9	1.6	
(SunOleic 97R/KKU1)$BC_{11}S$	61.6	69.6	*
SE	0.2	2.5	
(SunOleic 97R/KKU1)$BC_{12}S$	58.1	62.7	*
SE	1.2	1.4	
(SunOleic 97R/GA02C)P_1	79.4	83.9	*
SE	0.9	1.4	
(SunOleic 97R/GA02C)P_2	77.6	82.3	*
SE	0.8	1.6	
(SunOleic 97R/GA02C)F_2	78.4	80.8	Ns
SE	0.6	1.9	
(SunOleic 97R/GA02C)F_3	73.6	76.7	Ns
SE	1.5	1.1	
(SunOleic 97R/GA02C)$BC_{11}S$	79.3	82.6	Ns
SE	0.9	1.1	
(SunOleic 97R/GA02C)$BC_{12}S$	76.3	83.9	**
SE	0.2	1.2	

*, ** and ns significant at 0.05, 0.01 and non-significant probability levels, respectively
P_1 = Female, P_2 =Male, F_2 = Second filial generation, F_3 = Third filial generation, $BC_{11}S$ = First backcross generation with female self, $BC_{12}S$ = First backcross generation with male self.

However, these require a relatively large sample size, and are destructive, time consuming, and relatively slow when a large numbers of samples need to be screened in a short period to allow breeders to make decisions about the breeding lines for generation advance in the following crop season. NIR technology has found wide application in estimating nutrient profiles of the seeds of staple crops (Covalence *et al*., 2006; Kim *et al*., 2007; Tallada *et al*., 2009; Hacisalihoglu *et al*., 2010; Patil *et al*., 2010; Baianu *et al*., 2012).

In the present study, we have found that NIR could be used to predict oleic acid in segregating populations of peanut with relatively high precision, which could be used to discard the breeding lines in the lower range of oleic acids and those with high oleic acid (as determined by NIR) should be evaluated by GLC to get accurate estimates of oleic acid.

ACKNOWLEDGEMENTS

This study was funded by the Thailand Research Funded by the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0035/2548) and the Thailand Research Fund, the Commission of Higher Education and Khon Kaen University for providing financial support through the Distinguished Research Professor Grant of Professor Dr. Aran Patanothai. Assistance was also received from the Peanut and Jerusalem Artichoke Improvement for Functional Food Research Group and the Plant Breeding Research Center for Sustainable Agriculture. Thanks are extended to Dr. Barry L. Tillman, University of Florida, North Florida Research and Education Center, Florida, 32446, USA, for his assistance in NIR analysis and the Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand for providing laboratory facilities.

REFERENCES

Alper CM, Mattes RD (2003). Peanut consumption improves indices of cardiovascular disease risk in healthy adults. *J. Am. Coll. Nutr.* 22: 133–141.

Andersen PC, Gorbet DW (2002). Influence of year and planting date on fatty acid chemistry of high oleic acid and normal peanut genotypes. *J. Agric. Food Chem.* 50: 1298–1305.

Baianu IC, You T, Costescu DM, Lozano PR, Presecaru V, Nelson RL. (2012). Determination of soybean oil, protein and amino acid residues in soybean seeds by high resolution nuclear magnetic resonance (NMRS) and near infrared (NIRS). *Nat. Proc.* doi:10.1038/npre.2012.7053.1

Bannon CD, Craske JD, Hai N, Harper NL, O'Rourke KL. (1982). Analysis of fatty acid methyl esters with high accuracy and reliability. III. Literature review of an investigation into the development of rapid procedures for the methoxide-catalysedmethanolysis of fats and oils. *J. Chromatogr.* 247: 71–89.

Braddock JC, Sims CA, O'Keefe SF (1995). Flavor and oxidative stability of roasted high oleic acid peanut. *J. Food Sci.* 60: 489–493.

Branch WD (2003). Registration of 'Georgia–02C' Peanut. *Crop Sci.* 43: 1883–1884.

Bricker AA (1989). MSTAT–C user's guide. Michigan State University.

Gorbet DW, Knauft DA (2000). Registration of 'SunOleic 97R' peanut. *Crop Sci*. 40: 1190–1191.

Hacisalihoglu G, Larib B, Settles AM (2010). Near-infrared reflectance spectroscopy predicts protein, starch and seed weight in intact seeds of common bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* 58: 702–706.

Jonnala RS, Dunford NT, Dashiell KE (2005). New high–oleic peanut cultivars grown in the Southwestern United States. *J. Am. Oil Chem. Soc.* 82: 125–128.

Kim KS, Park SH, Choung MG, Jang YS (2007). Use of near-infrared spectroscopy for estimating fatty acid composition in intact seeds of rapeseed. *J. Crop Sci. Biotechnol.* 10: 15–20.

Kim KS, Park SH, Choung MG, Jang YS (2009) Used of near–infrared spectroscopy for estimating fatty acid composition in intact seeds of rapeseed. *J. Crop Sci. Biotech.* 10: 15–20.

Kovalenko L, Rippke G, Hurburgh C (2006). Determination of amino acid composition of soybeans (*Glycine max*) by near-infrared spectroscopy. *J. Agric. Food Chem.* 54: 3485–3491.

Mugendi JB, Sims CA, Gorbet DW, O'Keefe SF (1998). Flavor stability of high–oleic peanut stored at low humidity. *J. Am. Oil Chem. Soc.* 75: 21–25.

O'Byrne DJ, Knauft DA, Shireman RB (1997). Low fat–monounsaturated rich diets containing high–oleic peanuts improve serum lipoprotein profiles. *Lipids.* 32: 687–695.

O'Keefe SF, Wiley VA, Knauft DA (1993). Comparison of oxidative stability of high – and oleic peanut oils. *J. Am. Oil Chem. Soc.* 70: 489–492.

Patil AG, Oak MD, Taware SP, Tamhankar SA, Rao VS (2010). Non-destructive estimation of fatty acid composition in soybean [*Glycine max* (L.) Merrill] seeds using near-infrared transmittance spectroscopy. *Food Chem.* 120: 1210–1217.

Pérez–Vich B, Velasco L, Fernández–Martínez JM (1998). Determination of seed oil content and fatty acid composition I sunflower through the analysis of intact seeds, husked seeds, meal and oil by near–infrared reflectance spectroscopy. *J. Am. Oil Chem. Soc.* 75: 547–555.

Singkham N, Jogloy S, Kesmala T, Swatsitang P, JaisilP,Puppala N (2010). Genotypic variability and genotype by environment interactions in oil and fatty acids in high, intermediate and low oleic acid peanut genotypes. *J. Agric. Food Chem.* 58: 6257–6263.

Sundaram J, Kandala CV, Butt CL, Chen CY, Sobolev V (2011). Non-destructive NIR reflectance spectroscopic method for rapid fatty acid analysis of peanut seeds. *Peanut Sci*. 38: 85–92.

Tallada J, Palacios-Rojas N, Armstrong P (2009). Prediction of maize seed attributes using a rapid single kernel near infrared instrument. *J. Cereal Sci.* 50: 381–387.

Tillman BL, Gorbet DW, Person G (2006). Predicting oleic and linoleic acid content of single peanut seeds using near–infrared reflectance spectroscopy. *Crop Sci.* 46: 2121–2126.

Velasco L, Pérez–Vich B, Fernández–Martínez JM (1999). Nondestructive screening for oleic and linoleic acid in single sunflower achenes by near–infrared reflectance spectroscopy. *Crop Sci.* 39: 219–222.