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COMPOSITION AND VARIATION OF FATTY ACIDS AMONG GROUNDNUT CULTIVARS IN UGANDA

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ABSTRACT

Groundnuts (*Arachis hypogaea* L.) contain approximately 44-56% oil made up of fatty acids. Oleic and linoleic acids comprise about 80% of fatty acids in groundnuts. Groundnuts with >80% oleic are beneficial health-wise and also improve groundnut quality, flavour, and extended shelf-life, which is beneficial to traders. In Uganda, however, little is known about the fatty acids content of commercially available cultivars. This study was undertaken to determine the fatty acid composition of the most recently released commercially available groundnut cultivars in Uganda. Eleven groundnut genotypes were profiled for different fatty acids using gas chromatography (GC). Data were recorded in percentages from chromatographs and totaled up to about 100%. Oleic to linoleic ratios (O/L) and iodine values were calculated from fatty acid data. Groundnut genotypes tested differed significantly ($P < 0.05$) in oleic, linoleic, palmitic, behenic, gadoleic, arachidic and palmitoleic acids. Oleic acid was highest in Lot 197 line, and linoleic acid was lowest in the same line. Oleic acid was lowest in Serenut 7 (43.19%) and linoleic acid was highest in the same line (33.45%). The lowest iodine value was observed in 197 (84.14); and the highest in Serenut 9T (96.59). Significant correlations ($P < 0.001$) were observed between most of the fatty acids, except between arachidic versus gadoleic, stearic and gamma linoleic.

Key Words: *Arachis hypogaea*, gas chromatography, linoleic, oleic

RÉSUMÉ

L'Arachide (*Arachis hypogaea* L.) contient approximativement 44-55% d'huile faites d'acides gras. Les acides oléique et linoléique constituent environ 80% des acides gras dans l'arachide. Les arachides avec plus de 80% d'oléiques sont bénéfiques pour la santé et aussi améliorent la qualité de l'arachide, le goût, et une longue durée de conservation, avantageux pour les commerçants. En Ouganda, toutefois, très peu d'informations sont disponibles concernant la teneur en acides gras des cultivars commercialement disponibles. Cette étude a été entreprise pour déterminer la composition en acide gras de la majorité des cultivars d'arachides récemment libérés et commercialement disponibles en Ouganda. Onze génotypes d'arachide ont été profilés pour les différents acides gras en utilisant le gaz chromatographie (GC). Les données ont été collectées des chromatographes en pourcentages et additionnées

jusqu'à environ 100%. Les rapports d'oléiques au linoléiques (O/L) et les valeurs d'iode ont été calculées à partir des données des acides gras. Les génotypes d'arachide testés ont été significativement ($P < 0,05$) différents en acides oléique, linoléique, palmitique, béhénique, gadoléique, arachidique et palmitoleique. L'acide oléique était le plus élevé dans la lignée Lot 197, et l'acide linoléique était le plus faible dans la même lignée. L'acide oléique était le plus faible en Serenut 7 (43,19%) et l'acide linoléique était le plus élevé dans la même lignée (33,45%). La plus faible valeur de l'iode était observée dans 197 (84,14) ; et la valeur la plus élevée dans Serenut 9T (96,59). Des corrélations significatives ($P < 0,001$) étaient observées entre la majorité des acides gras, sauf entre l'acide arachidique contre les acides gadoléique, stéarique et gamma linoléique.

Mots Clés: *Arachis hypogaea*, gaz chromatographie, linoléique, oléique

INTRODUCTION

Groundnut (*Arachis hypogaea* L., $2n=4x=40$) is an important oilseed and cash crop for farmers of the arid and semi-arid tropics. In Uganda, groundnut is a significant source of cash income and contributes significantly to food security (Okello *et al.*, 2013). Oleic, linoleic, and palmitic fatty acids constitute ~90% of the total fatty acids (Barkley *et al.*, 2013).

Oleic and linoleic fatty acid composition of groundnuts play an important role in determining how beneficial groundnuts are for humans. For instance, many studies have reported that groundnuts with high oleic to linoleic ratio (>2) are more beneficial to human beings compared to 'normal' oleic groundnuts with a ratio of <2 . This is because a high oleic to linoleic ratio confers health benefits (Garcia *et al.*, 2006) and good seed oxidative stability, thus extended shelf life (Janila *et al.*, 2013). In addition, groundnut oil is considered one of the healthy cooking oils; since the ratio of unsaturated to saturated fatty acids is very high (Johnson and Saikia, 2009).

Fatty acids, both free and as part of complex lipids, play a number of key roles in metabolism, as major metabolic fuel (storage and transport of energy), as well as essential components of all membranes and gene regulators (Rustan and Drevon, 2005). Saturated fatty acids are 'filled' (saturated) with hydrogen and straight hydrocarbon chains, with an even number of carbon atoms; while unsaturated fatty acids generally contain double bonds in their structure (Rustan and

Drevon, 2005). Saturated fatty acids are stable and have been associated with heart problems. Saturated fat also increases high-density lipoprotein (HDL) cholesterol, the total cholesterol (TC) to HDL cholesterol ratio, a risk marker for Cardio Vascular Disease (CVD) (Barbour *et al.*, 2015).

Oleic acid, a monounsaturated fatty acid (MUFA) is, however, thought to reverse the above effects. Oleic acid (C18:1) has been associated with several human health benefits, including; decreased risk of cardiovascular disease (CVD), by reducing the levels of serum low-density lipoproteins (LDL) cholesterol; and maintaining the levels of high-density lipoproteins (HDL), without causing significant weight gain (Barbour *et al.*, 2015). MUFAs decrease plasma triglyceride levels in comparison with carbohydrates (Kris-Etherton, 1999). In addition, the MUFAs help in hindering the development of adrenoleukodystrophy (ALD) (Rizzo *et al.*, 1986) and reversing inhibitory effects of insulin production (Vassiliou *et al.*, 2009). MUFAs may also decrease platelet aggregation and increase fibrinolysis, thereby protecting against thrombogenesis (Kris-Etherton, 1999). It also has anti-inflammatory properties that activate different pathways of immune competent cells (Carrillo *et al.*, 2012).

Polyunsaturated fatty acids (PUFAs) such as linoleic (C18:2), are recognised for their susceptibility to oxidative rancidity, such that when heated at high temperatures makes it dangerous for human consumption (Isleib *et al.*, 2006). This instability leads to formation of trans-fatty acid, which has detrimental

effect on human health as it causes cardiovascular disease (CVD) (Wang *et al.*, 2015). In addition, linoleic acid is a metabolic precursor to arachidonic acid and eicosanoids, which have been associated with an increased risk of inflammation, cancers, CVD and neurological disorders (Whelan, 2008). Iodine value is directly proportional to the degree of unsaturation (number of double bonds). An increase in iodine value, therefore, indicates high susceptibility of lipid to oxidative rancidity due to high degree of unsaturation (Gupta, 2011).

Following the benefits conferred by major fatty acids in groundnuts, it is important to know the exact quantities of fatty acids present per genotype so that the genetic potential of genotypes can be exploited by breeders. This study was, therefore, carried out to profile fatty acids, especially oleic, in the commercially available Ugandan cultivars.

MATERIALS AND METHODS

Eleven groundnut cultivars (Table 1) were used for this study. Ten of these were obtained from the National Groundnut Improvement Department at National Semi-Arid Resources Research Institute (NaSARRI), Serere in Uganda. Lot 197, a high oleic line, was acquired from New Mexico State University (NMSU) Peanut Improvement Program, USA. Varieties obtained from NaSARRI were commercially available varieties and the most recently released by National Agricultural Research Organization (NARO) in Uganda.

Eleven groundnut samples were stored at room temperature at NaSARRI. Each of the samples were collected in two 250 g replicates. Fatty acid extraction procedures were carried out according to the Association of Official Agriculture Chemists (AOAC International) 19th Edition. Briefly, each of the groundnut variety sample (250 g) were thoroughly ground to a fine paste, using IKA WERKE M20 water-cooled grinding machine to obtain a homogenous mixture. Fifty micro-litres (50 µl) of the different groundnut

samples of each genotype, was extracted in 1:1 n-methyl- formamide: Fioxane solution, and dried under nitrogen. 50 µl of 0.5% (w/v) butylated hydroxytoluene was added to prevent oxidation.

Samples were then dried under nitrogen and saponified with 5% methanolic-potassium hydroxide, followed by transmethylation with 14% methanolic-boron trifluoride, to form fatty acid methyl esters (FAMES) (Shiple *et al.*, 1993). Water was removed by putting samples through anhydrous magnesium sulphate. Mini-columns and FAMES were purified on salicylic acid mini-columns. Each sample was reconstituted in 500 µl chloroform for gas chromatography analysis.

Reconstituted FAMES were analysed by gas chromatography (GC) on a HP5890 Series II Gas Chromatograph with HP7673 Auto-sampler. The above were then introduced onto a DB-225 column (30 m × 0.25 mm with 0.15 µm film thickness) (JandW Scientific), using a split injector set at 250 °C with a 1:25 split ratio. The carrier gas used was ultrapure helium at 1 ml min⁻¹. The GC procedure was carried out at 100 °C for 2 minutes, 25 °C minutes⁻¹ to 180 °C, 15 °C minutes⁻¹ to 200 °C, 4 °C minutes⁻¹ to 225 °C held at 6 minutes. A flame ionisation detector was set at 300 °C, and peak areas were recorded using HP-Chemstation software. FAME peaks were identified by using retention times compared to standard fatty acid methyl esters (FAMES, Nu Check Prep, Inc., Elysian MN).

Data analysis. Analysis of variance and mean comparison of fatty acids composition of the 11 genotypes, was done in GenStat 12th Edition, following a completely randomised design. Means were separated using Fisher's protected Least Significant Difference (LSD) scores at 0.05 probability level. Pearson correlations between the different fatty acids among genotypes were also generated. The Linear Mathematical Model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

TABLE 1. Groundnut varieties used in the fatty acids study at Chemiphar Laboratories, Kampala, Uganda

Common name	Variety name	Pedigree	Year of release	Oleic level	Other attributes
Serenut 5R	ICGV-SM 93535	ICGM 522 X RG 1	2010	Unknown	Confectionary and Butter type, attractive red market color, Leafspot resistant
Serenut 6T	ICGV-SM 99566	ICGV 93437 X ICGV-SM 93561	2010	Unknown	Butter, Confectionary type, early maturing (90 days)
Serenut 7T	SGV 99018	CG 7 X ICGV 90704	2011	Unknown	Confectionary type, good for condiments, flour
Serenut 8R	SGV 99019	CG 7 X ICGV 90704	2011	Unknown	Drought and Leafspot resistant, good for butter, attractive red market colour
Serenut 9T	SGV 99024	CG 7 X ICGV 90704	2011	Unknown	Leafspot resistant, very sweet, Confectionary type
Serenut 10R	SGV 99044	CG 7 X ICGV 90704	2011	Unknown	Leafspot and leaf miner resistant
Serenut 11T	SGV 99031	CG 7 X ICGV 90704	2011	Unknown	Leafspot tolerant, Confectionary type, ease of shelling
Serenut 12R	SGV 99048	CG 7 X ICGV 90704	2011	Unknown	Leafspot tolerant, Confectionary type, attractive red market color
Serenut 13T	SGV 99052	CG 7 X ICGV 90704	2011	Unknown	Leafspot tolerant, Confectionary type, ease of shelling
Serenut 14R	SGV 99064	CG 7 X ICGV 90704	2011	Unknown	Leafspot resistant, confectionary type, ease of harvesting
Lot 197	-	NM02322 x NuMex-01	Advanced cross	High oleic	Early maturing

Source: NaSARRI Groundnut Improvement Project, 2012; CG 7 pedigree = USA 20 X TMV 10; ICGV 90704 pedigree = RG-1 X Manipintar. All materials, except Lot 197 are known to be rosette resistant, high yielding and drought tolerant, in addition to the listed attributes

Y_{ij} is the j th observation of the i th treatment

μ is the population mean, T_i is the treatment effect of the i th treatment, e_{ij} = the experimental error

Oleic to linoleic ratio (O/L) was calculated from:

$$\frac{\% \text{ fatty acid of oleic}}{\% \text{ fatty acid of linoleic}}$$

Iodine value was calculated from:

$$(0.8601 \times \% \text{oleic acid}) + (1.7321 \times \% \text{linoleic acid}) + (0.7854 \times \% \text{Eicosapentaenic acid})$$

(Mozingo *et al.*, 1988).

RESULTS

Nine fatty acids were consistently present in groundnut samples. Oleic and linoleic acids accounted for >80% of the fatty acids; while oleic, linoleic, palmitic and stearic acids collectively accounted for ~95% (Table 2). The rest, (<5%) constituted behenic, palmitoleic, arachidic, gadoleic and gamma linoleic fatty acids. Significant differences ($P < 0.001$) were observed among oleic, linoleic, behenic and palmitoleic fatty acids. Palmitic and gadoleic fatty acids were significant at $P < 0.05$.

A moderate oleic acid composition (52.79%) and a low linoleic acid mean (26.79%) were observed in Serenut 8R (Table 2). Serenut 6T and S.7T had the highest palmitic acid means (18.63 and 18.17%, respectively). All the means for arachidic, behenic, palmitoleic, gadoleic and gamma linolenic acids ranged between 0.37-0.78%; and palmitoleic acid between 0.06 and 0.17%. Serenut 5R had the least of both behenic and palmitoleic acids (Table 2). Oleic acid mean was highest in Lot 197 line, which also had the lowest linoleic acid. Serenut 7 had the lowest oleic acid (43.19%) and highest linoleic acid mean (33.44%).

TABLE 2. Fatty acids (%) across groundnut genotypes in a study at Chemphar Laboratories in Uganda

Genotypes	Oleic acid	Linoleic acid	Palmitic acid	Stearic acid	Palmitoleic acid	Arachidic acid	Behenic acid	Gamma-linoleic acid	Gadoleic acid
Lot 197	87.25	5.25	7.51	0	0.03	0	0	0	0
S.10R	52.8	26.79	16.05	2.67	0.14	0.61	0.78	0.33	0.1
S.11T	44.07	32.29	15.2	2.35	0.13	0.69	0.63	0.41	0.11
S.12R	51.23	28.97	16.34	2.73	0.13	0.57	0.67	0.45	0.1
S.13T	49.31	29.96	14.61	2.46	0.11	0.71	0.61	0.43	0.1
S.14R	48.55	30.35	15.05	2.79	0.12	0.65	0.72	0.27	0.09
S.5R	49.11	30.4	16.27	2.61	0.06	0.59	0.37	0.27	0
S.6T	50.66	29.68	18.63	3.46	0.15	0.52	0.6	0.27	0.05
S.7T	43.19	33.44	18.17	3.3	0.14	0.57	0.72	0.34	0.05
S.8R	50.86	30.01	15.32	3.24	0.13	0.53	0.76	0.35	0.09
S.9T	48.35	31.76	15.69	2.19	0.17	0.66	0.65	0.33	0.11
LSD (5%)	9.66	6.04	3.58	1.96	0.08	0.27	0.41	0.42	0.06
CV (%)	7.84	9.08	4.1	4.8	4.5	2.9	5.2	16	20.7

Stearic, arachidic, behenic, gamma-linoleic and gadoleic acids were absent in Lot 197. The O/L increased as the amount of oleic acid increased, thus the highest ratio being in Lot 197 (16.6) and the lowest in Serenut 7T (1.30) (Table 3). However, Serenut 8R registered the highest O/L among the local cultivars. The lowest iodine value was observed in 197 (84.14) and the highest in Serenut 9T (96.59) (Table 3).

Significant correlations ($P < 0.001$) based on genotype means were observed between most of the fatty acids, except between arachidic versus gadoleic, stearic and gamma linoleic (Table 4). The highest correlation was observed between oleic and linoleic acids ($r = 0.99$); at $P < 0.001$; followed by oleic and palmitic acids ($r = 0.96$). The relationship in the two cases were, however, negative (Table 4). The lowest, but positive correlation ($r = 0.5$) was observed between palmitoleic acid, and arachidic acids and between gadoleic and stearic acids (Table 4).

A highly significant ($P < 0.001$) relationship was observed between oleic and linoleic fatty acids and iodine value (Table 5). Positive correlations were observed between linoleic, palmitic and stearic fatty acids and iodine value,

TABLE 3. Means of oleic to linoleic ratio (O/L) and Iodine values

Variety	O/L	Iodine value
Lot 197	16.6	84.14
S.8R	1.9	91.82
S.6T	1.4	93.84
S.14R	1.8	94.24
S.10R	1.7	94.31
S.12R	1.6	94.32
S.5R	1.6	94.90
S.11T	1.7	94.98
S.7T	1.3	95.08
S.13T	1.7	95.73
S.9T	1.5	96.59
LSD (5%)	0.6	5.75
CV (%)	8.36	2.6

TABLE 4. Pearson correlation between fatty acids (n=11 genotype means)

	Oleic	Linoleic	Palmitic	Palmitoleic	Arachidic	Behenic	Gadoleic	Stearic	Gamma linolenic
Oleic	1.00								
Linoleic	-0.99***	1.00							
Palmitic	-0.96***	0.91***	1.00						
Palmitoleic	-0.73***	0.69***	0.70***	1.00					
Arachidic	-0.74***	0.82***	0.53*	0.50*	1.00				
Behenic	-0.68***	0.64**	0.62**	0.90***	0.55*	1.00			
Gadoleic	-0.55**	0.53**	0.47*	0.75***	0.42ns	0.74***	1.00		
Stearic	-0.79***	0.69***	0.84***	0.67***	0.34ns	0.72***	0.50*	1.00	
Gamma linolenic	-0.60**	0.55**	0.57**	0.70***	0.32ns	0.75***	0.85***	0.66**	1.00

Values marked '***' are significantly different at probability level 0.001 '**' 0.01 '*' 0.05, ns = no significance

TABLE 5. Correlations between major fatty acids and iodine value for groundnut

	Iodine
Linoleic	0.9186***
Oleic	-0.9178***
Palmitic	0.8149**
Stearic acid	0.7129*

while a negative correlation ($r = -0.9178$) was observed between oleic acid and iodine value.

DISCUSSION

The overall number of fatty acids recorded in this study was nine (Table 2). The total number of fatty acids per genotype, however, varied slightly in Lot 197; which had oleic content of 87.25% and no stearic, arachidic, behenic, gamma-linoleic and gadoleic acids. Thus, the total number of fatty acids recorded in Lot 197 was four compared to nine consistently recorded among the Serenut 5-14 series (Table 2). This suggests that oleic acid content has an effect on the amounts; thus number of minor fatty acids since this was only observed in Lot 197 where high oleic was highest. Nonetheless, the major fatty acids consistently observed in this study were oleic, linoleic and palmitic fatty acids constituting ~90% of the fatty acids, irrespective of the genotype. This finding was also reported by Singhkam *et al.* (2012); Hassan and Ahmed (2012); Janila *et al.* (2015) and Wang *et al.* (2015), irrespective of the method of fatty acid profiling /analysis. The significant variation in both major (oleic, linoleic and palmitic acids) and minor (arachidic, behenic, palmitoleic and gadoleic) fatty acids composition among the groundnut genotypes in this study indicates genetic differences among the genotypes for the different fatty acids (Hassan and Ahmed, 2012) which is crucial for fatty acid improvement. Variation and number of fatty acids (especially minor fatty acids) among genotypes may not only be attributed to the genetic make-up of a particular cultivar, but also, conditioned by

place of origin of the genotype and the environmental conditions (such as soil type) prevailing during the crop life cycle (Hassan and Ahmed, 2012).

The significant negative correlation observed between oleic *versus* linoleic, oleic *versus* palmitic acid, oleic *versus* stearic acid, linoleic *versus* palmitic acid and linoleic *versus* stearic (Table 4) reveal clear antagonistic relationships between the fatty acids. The very high correlation coefficients observed between oleic and linoleic acids, palmitic acids suggest close linkage of genes controlling these fatty acids. It would, therefore, be beneficial for breeders to select for these fatty acids simultaneously, rather than independently. The relationships between oleic and linoleic, palmitic fatty acids also suggests that ahFAD2 genotype may have influenced the levels of oleic, linoleic and palmitic acids (Barkley *et al.*, 2013).

Generally, an increase in oleic acid, a monounsaturated fatty acid, was inversely proportional to linoleic acid a polyunsaturated acid, stearic and palmitic acids (Table 4), which are the major saturated fatty acids in groundnuts; similar to what was observed by Wang *et al.* (2015). Having identified the benefits of oleic *versus* the negative effects of linoleic, stearic and palmitic acids, this inverse relationship can be exploited to increase oleic acid with reduction in linoleic stearic and palmitic acids. The negative relationship between oleic and palmitic, stearic acids among the genotypes implies high chances of lowering health hazards by breeding for high oleic acid as the stearic and palmitic acid content is reduced. This is because saturated fatty acids such as palmitic and stearic acids are known to elevate plasma cholesterol and LDL concentrations by down-regulating the hepatic receptor for LDL (Connor, 1999). Increase in the oleic content will, therefore, lead to the reduction in the levels of the major saturated (palmitic and stearic acids) and polyunsaturated (linoleic acid) which should be the focus of breeding programmes.

All the Ugandan genotypes had low oleic acid content (<60%) and a high linoleic content (>25%), thus a low O/L (<2); while Lot 197 the line from USA had a high oleic content (87.25%) and a very low linoleic content (<5.25%) and a high O/L (16.6), therefore, qualifying it to be a 'high oleic' genotype (Singkham *et al.*, 2012) and the Ugandan cultivars; normal oleic. Lot 197 also had the lowest iodine value (oleic acid was directly proportional to O/L and inversely proportional to iodine value). The positive relationships between linoleic, stearic and palmitic fatty acids and iodine value evidently suggest that the rate of saturation of fatty acids has an effect on the iodine value which in turn increase the rancidity of oil. This finding is consisted with that of Gupta (2011), who reported that high oleic content lowers iodine value, which in turn increases oil stability. Oleic to linoleic ratio and iodine value, together are important factors in determining oil stability and shelf life, by reducing rancidity thus longer periods of storage (longer shelf life). The high oleic line Lot 197 can, therefore, be used as a donor parent to improve Ugandan cultivars for their oleic acid content during hybridisation.

The relatively high amount of oleic acid in Lot 197 line, compared to the Ugandan commercial genotypes, is attributed to the presence of a mutant allele that reverses the activity of the enzyme, *ahFAD2* that converts linoleic acid to oleic acid therefore resulting in a dysfunctional *ahFAD2* at both the A and B loci (Chu *et al.*, 2009). The activity of the mutant allele is also responsible for the reduction of linoleic acid since the relationship between the fatty acids (oleic and linoleic) is strong and inversely proportional.

CONCLUSION

All the Ugandan groundnut cultivars in this study had low to moderate oleic levels and higher linoleic, palmitic and stearic fatty acids, compared to Lot 197, which registered the highest oleic levels and the lowest linoleic,

palmitic and stearic acid levels. This variation should be exploited by groundnut breeders for the purpose of oleic content improvement of Ugandan genotypes by crossing with Lot 197 as the pollen donor with subsequent backcrossing to maintain traits such as rosette resistance in the genetic background of progeny.

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