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## Analytical Methods

# Chemometric approach to fatty acid profiles in Runner-type peanut cultivars by principal component analysis (PCA)

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### ABSTRACT

The fatty acid profiles of commercially-grown Runner-type peanut cultivars (i.e., 10 cultivars,  $n = 151$ ) collected over two production years (2005 and 2006) were determined by gas–liquid chromatography. Eight major fatty acids were identified in the sample set including palmitic (C16:0), stearic (C18:0), oleic (C18:1,  $\omega$ 9), linoleic (C18:2,  $\omega$ 6), arachidic (C20:0), gondoic (C20:1,  $\omega$ 9), behenic (C22:0), and lignoceric (C24:0) acids. Based on the oleic to linoleic acid (O/L) ratio, these cultivars were denoted as normal, mid-, and high-oleic peanut types. Correlation coefficients  $(r)$  between the eight major fatty acids identified were generated and revealed an inverse association between oleic and linoleic acids  $(r = -0.997,$  $P < 0.001$ ), while oleic acid and linoleic acid were positively correlated to gondoic acid ( $r = 0.818$ ,  $P < 0.001$ ) and palmitic acid ( $r = 0.967$ ,  $P < 0.001$ ), respectively. Principal component analysis (PCA) of the fatty acid data yielded three significant PCs (i.e., eigenvalues  $\geq 1$ ), which together account for 87.18% of the total variance in the data set; with PC1 contributing 60.45% of the total. Eigen analysis of the correlation matrix loadings of the three significant PCs revealed that PC1 was mainly contributed to by palmitic, oleic, linoleic, and gondoic acids; PC2, by behenic acid; and PC3, by lignoceric acid. The score plot generated between PC1 and PC2 successfully segregated normal, mid- and high-oleic peanut cultivars, while the PC1–PC3 plot segregated normal and the combination of mid- and high-oleic acid cultivars.

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## 1. Introduction

In the United States, production of Runner, Virginia, Spanish, and Valencia market-type peanuts (Arachis hypogaea L.) provides about 10% of the world production of peanuts. Runner peanuts, the predominant peanut type in the United States (>50%), are primarily grown in Georgia, Alabama, Florida, Texas, and Oklahoma [\(Ac](#page-7-0)[quaah, 2007; Haumann, 1998\)](#page-7-0). Peanut breeding programs throughout the world introduce many new cultivars each year with varying nutrient compositions, including differences in the fatty acid profile of the oil. Nutritional attributes and stability of peanut oil have been improved by the successful modification of Runner-type peanuts to increase oleic acid levels at the expense of linoleic acid ([Moore &](#page-8-0) [Knauft, 1989; Norden, Gorbet, Knauft, & Young, 1987\)](#page-8-0). [Ray, Holly,](#page-8-0) [Knauft, Abbott, and Powell \(1993\)](#page-8-0) reported that the high-oleate trait results from reduced activity of microsomal oleoyl–phosphatidylcholine desaturase that catalyses the chemical reaction {1-acyl-2-oleoyl-sn-glycero-3-phosphocholine +  $NAD^+ \rightleftharpoons 1$ -acyl-2-linoleoylsn-glycero-3-phosphocoline + NADH + H<sup>+</sup>} and introduces a second

double bond in oleoyl-PC giving linoleoyl-PC. The high-oleate trait relies on changes in two oleoyl–phosphatidylcholine desaturase genes, ahFAD2A and ahFAD2B. The enzyme activity of either ahFAD2A or ahFAD2B is sufficient for the normal oleate trait. The high-oleate trait is caused by reduction in the levels of ahFAD2B and a mutation in ahFAD2 ([Jung, Powell, Moore, & Abbott, 2000;](#page-8-0) [Jung, Swift, et al., 2000; Patel et al., 2004\)](#page-8-0).

Chemometrics is the combination of mathematical, statistical, and other logic-based methods to efficiently manage and interpret chemically-derived data ([Haswell, 1992](#page-8-0)). Principal component analysis (PCA) is a multivariate modelling and analysis technique commonly used in chemometric studies. PCA is a way of identifying patterns in data, and expressing the data in such a way as to emphasise their similarities and differences. It can compress the data, that is, by reducing the number of dimensions without much loss of information based on their similarities and differences, and define a limited number of ''principal components" which describe independent variation structures in the data. When more than three variables have been measured, visualisation of the data by various plotting systems is then possible ([Kamal-Eldin & Anders](#page-8-0)[son, 1997\)](#page-8-0). Therefore, PCA can indicate relationships among groups of variables in a data set and show relationships that might exist between objects.

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Because of the capability to manage and interpret large data sets, PCA has been effectively employed to define relationships that exist in fatty acid characterisation studies of food lipids ([Kad](#page-8-0)[egowda, Piperova, & Erdman, 2008; Matos et al., 2007\)](#page-8-0). For example, almond cultivars have been classified in several investigations using chemometric techniques. [García-López, Grané-Teruel, Beren](#page-8-0)[guer-Navarro, García-García, and Martín-Carratalá \(1996\)](#page-8-0) used cluster analysis of the major fatty acids in almond lipids to classify the cultivars into three groups. In a subsequent study, these three groups were further subdivided by including minor fatty acids in the multivariate analysis [\(Martín-Carratalá, García-López,](#page-8-0) [Berenguer-Navarro, & Grané-Teruel, 1998](#page-8-0)). Recently, [Sathe, Seeram,](#page-8-0) [Kshirsagar, Heber, and Lapsley \(2008\)](#page-8-0) applied cluster analysis to show that fatty acid composition of California grown almonds is influenced by cultivar, location of growth, and production year. With regard to peanuts, [Sato \(1994\)](#page-8-0) successfully clustered nine vegetable oils (including peanut oil) from their PCA score plots (PC1–PC2 and PC2–PC3) generated from near-infrared (NIR) spectral data. Brodnjak-Vončina, Kodba, and Novič (2005) applied PCA for the discrimination of various vegetable oils, and observed a peanut lipid cluster from the PCA score plot PC1–PC2, as well as a nearly perfect negative correlation between oleic and linoleic acids (-0.97). In early work, [Brown, Cater, Mattil, and Darroch](#page-7-0) [\(1975\)](#page-7-0) were successful in segregating Spanish peanuts from other peanut varieties via PCA, and grouped cultivars from different locations based on their fatty acid composition.

At the request of the peanut industry, we conducted a study which re-examined and updated compositional information of peanuts, including the levels of key bioactives, grown in the United States. To ensure the most accurate data, an intensive sampling program was designed and implemented by the industry to provide cultivars in current production by accepted agricultural practices employed in the United States over two production years. In our opinion, the strength of this research initiative lies in the care and detail which went into the sampling effort of peanut types and cultivars. Data resulting from the analyses of 151 Runner peanut samples distinguishes this work from all other peanut fatty acid investigations. Findings of the research will be shared with the USDA for consideration of inclusion in their Nutrient Database for Standard Reference, as there is currently no data for Runner peanuts (i.e., both normal and high-oleate genotypes). This paper – only a portion of the complete study – reports on the employment of PCA, a multivariate statistical method, to discriminate and classify US field-grown normal, mid-oleic, and high-oleic Runner-type peanut cultivars based upon their fatty acid profiles. Although peanut lipids have been included in some chemometric studies comparing vegetable oil fatty acid compositions, this paper provides more and advanced information concerning the application of PCA to the fatty acid profiles of a distinct sample set of only the most predominant commercial Runner peanuts. Utilizing PCA will effectively reduce the number of variables (e.g., type, crop year, geographic location, fatty acid profile) needed to classify peanut cultivars, and in this manner will permit peanut researchers (e.g., breeders, geneticists) to more easily develop significant relationships between important peanut characteristics.

## 2. Materials and methods

## 2.1. Collection of samples

Field-grown peanut samples  $(n = 151)$  comprising six normal and four mid/high-oleic Runner-type cultivars from the 2005 and 2006 production years were provided for this study by The Peanut Institute, the USDA-ARS National Peanut Laboratory, and personnel from the peanut industry. In brief, the sampling effort involved the development of a uniform sampling plan that accurately defined major Runner-type cultivars grown by US peanut farmers. Based on sheller input and seed sales to reflect present-day percentages of peanuts in the US market, cultivars were chosen. After selection of these cultivars, seed growers were identified in each of the three peanut-growing regions (i.e., Virginia/Carolina, Southeast, and Southwest), and samples were taken from the seed wagons after initial drying to a moisture content between 8% and 10%. An official sample was pulled from each wagon and graded. The sheller obtained the back half of the official-grade sample and subdivided the sample down to three pounds. This sample was then cleaned using a grade-room Farmerstock cleaner and forwarded to the USDA-ARS National Peanut Research Laboratory in Dawson, GA, for further processing. The samples were shelled and then sent to the Department of Food Science and Technology, University of Georgia (UGA) in Athens. Upon arrival at UGA, the peanuts were packaged in labelled vacuum pouches (Prime Source, Kansas City, MO) with a vacuum system (Henkelman 600, Henkelman, Hertogenbosch, The Netherlands) to prevent their degradation. The vacuum-packaged peanuts were stored at  $-40\,^{\circ}\textrm{C}$  until analysed.

## 2.2. Chemicals

Methanol, chloroform, hexanes, carbon disulphide, anhydrous sodium sulphate, sulphuric acid, and sodium chloride were ACSgrade and purchased from Fisher Scientific Company LLC (Suwanee, GA). Heptadecanoic acid (98% purity) and hydroquinone were acquired from the Sigma Chemical Company (St. Louis, MO). A variety of lipid standards were ordered from Nu-Chek Prep, Inc. (Elysian, MN).

## 2.3. Lipid extraction

Raw peanuts were removed from the freezer and tempered until reaching room temperature. Total lipids were extracted according to the classical [Bligh-Dyer method \(1959\)](#page-7-0) with slight modifications. For each sample, 20 g of shelled peanuts were ground in a commercial coffee mill (Tipo 203, Krups, New York, NY). Five grams of the ground sample were accurately weighed and transferred to a 250-ml Erlenmeyer flask. Twenty millilitres of deionised H<sub>2</sub>O (*i.e.*, to adjust the moisture content to  $\sim$ 80%), 50 ml of CH<sub>3</sub>OH, 25 ml of CHCl<sub>3</sub>, and  $\sim$ 10 mg of hydroquinone (as antioxidant) were added, and the contents were blended at 5400 rpm for 2 min using a Polytron<sup>®</sup>-type homogenizer (Pro Scientific Inc., Monroe, CT). After the initial blending, an additional 25 ml of  $CHCl<sub>3</sub>$  were added, and homogenisation was repeated for 1 min. Next, 25 ml of deionised  $H_2O$  and 35 ml of CHCl<sub>3</sub> were added, the mixture was blended again for 1 min. The slurry was filtered through Whatman No. 1 filter paper using a Büchner funnel under slight vacuum. Approximately 1 g of NaCl crystals was added to the filtrate to facilitate phase separation. The filtrate was quantitatively transferred to a 250-ml separatory funnel and allowed to stand overnight to permit complete separation of the layers. The CHCl<sub>3</sub> phase was passed through Whatman No. 1 filter paper containing anhydrous  $Na<sub>2</sub>SO<sub>4</sub>$  to eliminate moisture and collected in a 100-ml round-bottom flask. The CHCl<sub>3</sub> was removed under vacuum at <40  $\degree$ C using a Rotavapor/Heating Bath (model R-210 and B-491, respectively, Büchi Corporation, New Castle, DE). The resultant lipid was transferred via a Pasteur pipette to an amber-coloured vial. The round-bottom flask was rinsed with a small portion of  $CHCl<sub>3</sub>$  to ensure a quantitative transfer of the extracted lipids. Residual CHCl $_3$  was removed from the vial using a nitrogen evaporator (N-EVAP<sup> $M$ </sup> 111, Organomation Associates, Inc., Berlin, MA). Samples were stored under a  $N_2$ -headspace at  $-80$  °C until further analysed.

#### 2.4. Fatty acid methylation

The extracted peanut lipids were used for fatty acid analysis. Fatty acid methyl esters (FAMEs) were prepared according to [Dhanda, Pegg, and Shand \(2003\)](#page-7-0) with slight modifications. Briefly, extracted lipids ( $\sim$ 70 mg) were transferred to a Reacti-vial<sup>M</sup> small reaction vial (5 ml, Thermo Fisher Scientific, Rockford, IL), and the mass was accurately weighed. Heptadecanoic acid was employed as the internal standard (IS) for this work. One hundred microlitres of IS (i.e., 2.5 mg heptadecanoic acid/ml in hexane) were added to each Reacti-vial<sup>M</sup> via a 100-µl gastight syringe (Hamilton Co., Reno, NV). Peanut lipids were hydrolysed with a transmethylation reagent consisting of 6% (v/v) concentrated  $H<sub>2</sub>SO<sub>4</sub>$  in anhydrous CH<sub>3</sub>OH containing ca. 10 mg of hydroquinone. Two millilitres of the transmethylation reagent and a Reacti-vial<sup> $M$ </sup> magnetic stirrer were added to each reaction vial, which was tightly capped, vortexed for 1 min, and placed in Reacti-Block<sup> $M$ </sup> B-1 aluminum block within a Reacti-Therm III<sup> $M$ </sup> Heating/Stirring Module (Thermo Fisher Scientific, Rockford, IL) at 65  $\degree$ C for 16 h. After derivatisation, samples were removed and allowed to cool to room temperature. Next, 1 ml of deionised  $H_2O$  was added to each reaction vial, the solution was vortexed for 30 s and FAMEs were extracted  $3 \times$  with 1.5 ml of hexanes. The hexane layers were combined in a test tube (13  $\times$  100 mm, Fisher Scientific) and then washed  $2 \times$  with 1.5 ml of deionised H<sub>2</sub>O. After the second wash, the hexane layer was transferred to a new test tube via a Pasteur pipette. Hexane was removed using the N-EVAP. The FAMEs were redissolved in 1.5 ml of  $CS_2$  and transferred to 2-ml wide-opening crimp top vials (Agilent Technologies, Wilmington, DE). Vials were capped with 11-mm silver aluminum caps, clear PTFE/red rubber septa, and then crimped with an electronic crimper (Agilent Technologies).

## 2.5. Gas–liquid chromatography

An Agilent Technologies 6890N Network gas chromatograph system (configuration: capillary split/splitless inlet with electronic pneumatic control [EPC] and a flame ionisation detector [FID] with EPC, for packed & capillary column) equipped with a 7683 autosampler tray module, 7683B autoinjector module, and GC Chem-Station software (Rev. A0803 (847), Agilent) was employed for fatty acid analysis profiling. Operating conditions were as follows: the column was a (50%-cyanopropyl)methylpolysiloxane J&W fused-silica DB-23 capillary column (60 m  $\times$  0.25 mm *i.d.*, 0.25lm film thickness, Agilent Technologies); ultra-high purity helium was the carrier gas at a flow rate of 2.7 ml/min and analyses were performed in constant flow mode; a split liner with glass wool was installed in the injector; the injector temperature was set at  $250 °C$ for split injection at a split ratio of 50:1, the FID temperature was set at  $250$  °C; ultra-high purity hydrogen and scientific-grade air were the fuel gases for the FID and set at 40 and 450 ml/min, respectively, with 25 ml/min of nitrogen as the makeup gas; the initial oven temperature was set at 130  $\degree$ C and held for 5 min before ramping up at 4 °C/min to 240 °C, this temperature was maintained for an additional 15 min. Analyses were performed in triplicate.

## 2.6. Identification of the fatty acids

A Nu-Chek Prep GLC-463 FAME reference standard (i.e., 52 components from C4:0 to C24:1) was used to identify and quantity individual FAMEs from peanut samples. A relative response factor was calculated for each FAME using methyl heptadecanoate as an internal standard according to [Ngeh-Ngwainbi, Lin, and Chandler](#page-8-0) [\(1997\).](#page-8-0) Each FAME has a different response to the FID depending on chain length, saturation, and cis/trans configuration:

$$
R_i = (Ps_i \times Ws_{c17:0})/(Ps_{c17:0} \times Ws_{is})
$$

where  $R_i$  = relative response factor for fatty acid *i*; Ps<sub>i</sub> = peak area of individual FAME *i* in FAMEs standard solution;  $Ws_{c17:0}$  = mg of C17:0 FAME in injected FAMEs standard solution;  $Ps<sub>c17:0</sub> = peak$ area of C17:0 FAME in FAMEs standard solution; and  $Ws_{is}$  = mg of individual FAMEs i in injected FAMEs standard solution.

## 2.7. Statistical analysis

All samples ( $n = 151$ ) were analysed in at least triplicate. Correlations between fatty acids were determined by the Pearson correlation coefficient, which describes the strength of the linear relationship between two quantitative variables, at  $P < 0.05$ , 0.01, and 0.001, respectively. Pearson correlation coefficients were calculated using the Statistical Analysis System (SAS, ver. 9.0, SAS Institute Inc., Cary, NC) software [\(O'Rourke, Hatcher, & Stepanski,](#page-8-0) [2005\)](#page-8-0).

The classification and discrimination of Runner-type peanut cultivars using fatty acid profiles were achieved by PCA using XLSTAT Software (XLSTAT, 2008, Addinsoft, New York, NY). PCA is a multivariate statistical method that entails data reconstruction and reduction. PCA generates a set of new orthogonal axes or variables known as principal components (PCs) from the original variables. The data sets presented on the orthogonal axes are uncorrelated with one another, and express much of the total variability in the data set through comparison of only a few PCs ([Sola-Larrañaga & Navarro-Blasco, 2009](#page-8-0)). The maximal amount of variance in the data set and its direction are often explained by the first PC (*i.e.*, PC1). Each PC is defined by a vector known as the eigenvector of the variance–covariance matrix. The variance along the vector is known as the eigenvalue. Each eigenvalue, the amount of variance that is explained by a given component, was used for the determination of variances of the major PCs. The loadings (or scores) corresponding to the PCs were calculated from the correlation matrix ([Massart, Vandeginste, Deming, Michotte, &](#page-8-0) [Kaufman, 1988](#page-8-0)). The arithmetic value of each PC is determined by the equation:

$$
(PC)=a_1\frac{x_1-\bar{x}_1}{SD_1}+\cdots
$$

where  $x_1$  are measurements of the original variables,  $\bar{x}_1$  are mean values for the corresponding variables,  $SD<sub>1</sub>$  are standard deviations for the corresponding variables, and  $a_1$  are loadings of the linear transformation [\(Tsimidou, Macrae, & Wilson, 1987\)](#page-8-0). Each loading of variables was used for the contribution of the original variable to the PC. Variance reduction was achieved by neglecting the unimportant directions in which samples' variance are insignificant. The important variables are along several significant directions, and the number of these directions approximates the dimensionality of the sample set. For a visualisation of the data discrimination, PCA plots mapped variables (eight fatty acids) and samples ( $n = 151$ ) through loadings and scores in dimensional spaces determined by PCs with eigenvalues >1.0 based on a Kaiser's rule ([Kaiser, 1960; O'Rourke](#page-8-0) [et al., 2005; Sola-Larrañaga & Navarro-Blasco, 2009\)](#page-8-0). The loading plot depicts the identification of important variables, and the score plot indicates if samples are similar or dissimilar, typical, or represent an outlier.

## 3. Results and discussion

## 3.1. Fatty acid profiles and correlations

A representative GC chromatogram of the FAMEs from peanut lipid extracts is depicted in [Fig. 1.](#page-3-0) Eight major fatty acids were identified in this study by retention time mapping with external

<span id="page-3-0"></span>

Fig. 1. Representative GC chromatogram of fatty acid methyl esters (FAMEs) from the lipid extract of normal Runner-type peanuts (peak 1, palmitic acid (C16:0); 2, internal standard, heptadecanoic acid (C17:0); 3, stearic acid (C18:0); 4, oleic acid (C18:1,  $\omega$ 9); 5, linoleic acid (C18:2,  $\omega$ 6); 6, arachidic acid (C20:0); 7, gondoic acid (C20:1,  $\omega$ 9); 8, behenic acid (C22:0); and 9, lignoceric acid (C24:0)).

standards and quantified relative to an internal standard (i.e., methyl heptadecanoate, the FAME resulting from the derivatization of C17:0). [Table 1](#page-4-0) reports the fatty acid compositions of lipid extracts from 151 Runner-type peanut cultivars collected in 2005 and 2006. Palmitic acid (C16:0) ranged from 5.31% to 11.49%; stearic acid (C18:0), 1.46% to 4.76%; oleic acid (C18:1,  $\omega$ 9), 44.78% to 82.17%; linoleic acid (C18:2,  $\omega$ 6), 2.85% to 33.92%; arachidic acid (C20:0), 0.87% to 2.18%; gondoic acid (C20:1,  $\omega$ 9), 1.09% to 3.13%; behenic acid (C22:0), 0.73% to 4.37%; and lignoceric acid (C24:0), 0.41% to 2.12%. These results correspond with those from other studies and show that the sum of oleic and linoleic acids accounts for almost 80% of the total fatty acids detected in peanut samples ([Andersen & Gorbet, 2002; Davis, Dean, Faircloth, & Sand](#page-7-0)[ers, 2008\)](#page-7-0). The oleic to linoleic acid (O/L) ratio is a quality index employed for the determination of genetic peanut characteristics classified as normal, mid-, and high-oleic types. In this work, cultivars assayed included the following: a normal group comprising Georgia Green, Tamrun 96, C99-R, Georgia-01R, Georgia-03L, and AP-3; a mid-oleic group of Tamrun OL01; and a high-oleic group comprising Tamrun OL02, Flavorunner 458, and Georgia-02C. Pearson correlation coefficients between fatty acid variables are given in [Table 2.](#page-6-0) The correlation between oleic acid and linoleic acid was strong but negative ( $r = -0.997$ ,  $P < 0.001$ ); that is, an increase in one fatty acid leads to a corresponding decrease in the other. In previous studies involving multiple peanut cultivars, a strong negative correlation (i.e., an inverse association) between oleic acid and linoleic acid in peanut lipids was also observed [\(Andersen &](#page-7-0) [Gorbet, 2002; Brown et al., 1975\)](#page-7-0). This negative relationship originates from the biochemical pathways of peanut development: in the peanut germplasm, palmitoyl CoA is elongated to stearoyl CoA followed by desaturation forming oleic acid. Then, the action of oleoyl-phosphatidylcholine desaturase (a  $\Delta^{12}$ -fatty acid desaturase) synthesizes linoleic acid from oleic acid ([Jung, Powell,](#page-8-0) [et al., 2000; Jung, Swift, et al., 2000; Patel et al., 2004](#page-8-0)). In addition, palmitic acid is positively correlated to linoleic acid ( $r = 0.967$ ,  $P < 0.001$ ), while negatively correlated to oleic  $(r = -0.971,$  $P < 0.001$  ) and gondoic acids ( $r = -0.860$ ,  $P < 0.001$ ). [Hammond,](#page-8-0) [Duvick, Wang, Dodo, and Pittman \(1997\)](#page-8-0) also reported these negative correlations between palmitic acid with oleic and gondoic acid contents, but [Andersen and Gorbet \(2002\)](#page-7-0) only described the negative relationship between palmitic and oleic acids. Positive correlations between stearic and arachidic acids  $(r = 0.862,$  $P < 0.001$ ) as well as oleic and gondoic acids ( $r = 0.818$ ,  $P < 0.001$ ) were observed in our study. [Andersen and Gorbet \(2002\)](#page-7-0) suggested that arachidic and gondoic acids can be synthesized from stearic and oleic acid by the incorporation of an acyl group, respectively. Evidently, as the level of stearic and oleic acids increases, the chance for elongation to the minor fatty acids arachidic and gondoic acids also goes up.

## 3.2. Principal component analysis (PCA)

The data matrix of variables analysed *(i.e.*, the eight predominant fatty acids in Runner-type peanut cultivars) was subjected to PCA in order to decrease the number of descriptors associated with the data set while still explaining the maximum amount of variability present in the data. In early PCA applications of peanut research, [Brown et al. \(1975\)](#page-7-0) reported the effect of variety and growing location on the fatty acid composition of Runner-, Virginia-, and Spanish-type peanuts by PCA. The authors not only obtained the unique and independent information characterising the varieties and locations, but also a smaller number of recombined variable sets (i.e., the principal components, PCs). [Table 3](#page-6-0) shows the most significant PCs generated from the peanut fatty acid data and their statistical loadings in the current study. A new set of eight orthogonal variables (PCs) was generated by PCA. The first principal component (*i.e.*, PC1) had the highest eigenvalue of 4.84, and accounted for 60.45% of the variability in the data set. The second and third PCs (i.e., PC2 and PC3) had eigenvalues of 1.14 and 1.00, and accounted for 14.21% and 12.53% of the variance in the data, respectively (NB, only eigenvalues of  $\geq 1.0$  are considered significant descriptors of data variance: Kaiser's rule). The remaining five generated PCs (i.e., PC4 to PC8) yielded progressively smaller eigenvalues (i.e., <1; 0.75, 0.16, 0.09, 0.03, and 0.00, respectively) and did not explain significant variability in the data (<13% total). Therefore, according to Kaiser's rule, only the first three PCs were used for further study. Loading values >0.90 in PC1 and values >0.50 in PC2 and PC3 are marked throughout [Table 3](#page-6-0) in boldface type. These numbers represent significant contributions of individual fatty acid variables to the total variability explained by the generated PCs; whereas, [Brown et al. \(1975\)](#page-7-0) did not report on this. PC1 describes 60.45% of the variance in the data set, and its loadings indicate that it has high contributions from palmitic  $(-0.93)$ , oleic  $(0.94)$ , linoleic  $(-0.92)$ , and gondoic (0.90) acid variables. Whereas palmitic and linoleic acids exhibited negative loadings, oleic and gondoic acids had positive loadings denoting the sign relationship of their contributions to the data variability. PC2 showed a high positive loading for behenic acid (0.59) and PC3 was most described by lignoceric acid content (0.84). Together PC2 and PC3 comprised 26.74% of the remaining variance in the data set. In the data set of fatty acids from peanut cultivars, [Brown et al. \(1975\)](#page-7-0) observed 86% of variance as PC1 and 9% of variance as PC2 for 10 peanut varieties, and 60% of variance as PC1 and 27% of variance as PC2 from seven growing locations.

Specific patterns of correlation between the variables tested can be visualised when one compares loading plots between the PCs; see PC1–PC2 and PC1–PC3 plots [\(Fig. 2](#page-6-0)). The objective of a loading projection is to visualise the position of the variables with respect to one another in two-dimensional space and their corresponding correlations. Variables closest to one another and far from the plot origin are positively correlated (or directly proportional; e.g., see C18:1 and C20:1 in the PC1–PC2 plot), while variables opposite one another on the plot are negatively correlated (or inversely proportional; e.g., see C18:1 and C18:2 in the PC1–PC2 plot). The two loading plots generated from the data of [Table 3](#page-6-0) can explain the relationships between two variables by their angle from the centre. The correlation coefficient between two variables is defined as the cosine of the angle between their respective vectors on the plot.

<span id="page-4-0"></span>**T[a](#page-6-0)ble 1**<br>Fatty acid compositions of lipid extracts from Runner-type peanuts (*n* = 151).ª



## Table 1 (continued)



#### <span id="page-6-0"></span>Table 1 (continued)



<sup>a</sup> Data represent the mean of triplicate analyses for each sample.

**b** Cultivars were used by ten Runner-type cultivars.

<sup>c</sup> Year represents the production year ( $n = 2$ , 2005 and 2006).

 $d$  O/L represents the oleic acid to linoleic acid ratio. Normal ranges from 1 to 1.5; mid, 1.5 to 9.0, and high, above 9.0, respectively.

## Table 2

Pearson correlation coefficients between percentage levels of eight fatty acids from lipid extracts of Runner-type peanuts ( $n = 151$ ).



 $a^*$ ,  $^{**}$ , and  $^{***}$ correspond to significance at  $P < 0.05$ , 0.01, and 0.001, respectively.

#### Table 3

Eigen analysis of the correlation matrix loadings of the significant principal components (PCs).



<sup>a</sup> The most significant loadings are highlighted in boldface.

The cosine of  $180^\circ$  (*i.e.*, the angle between C18:1 and C20:1 on the PC1–PC2 plot) is -1; therefore, they are negatively correlated. Based on this mathematical rule, uncorrelated variables occur at right angles to one another because the cosine of the angle between them is cosine  $90^\circ = 0$ , or not correlated. Similarly, the cosine of  $0^\circ$  is 1, which denotes a positive correlation between the variables [\(Kaiser, 1970; López, Montaño, García, & Garrido, 2006\)](#page-8-0). In Fig. 2A, the PC1–PC2 plot is explanatory to 74.66% of the total variation in the data set, and is composed of positive loadings of oleic and gondoic acids as well as negative loadings of the other six fatty acids (as seen by their spatial relationship to one another and the x-axis). PC1 revealed negative correlations between a cluster of oleic and gondoic acids and a cluster of palmitic and linoleic acids (NB, the correlation was close to –1 based on their cosine angle,  $180^\circ$ ). Moreover, lignoceric acid was less correlated with palmitic, oleic, linoleic, and gondoic acids (their correlation was approaching 0 based on their cosine angles,  $\sim$ 90°). PC2 (i.e., the y-axis) distinguished two clusters of fatty acid variables: one cluster containing palmitic and linoleic acids in negative loadings and the other cluster containing all other fatty acids in positive loadings. Behenic acid (0.59) had the highest loading at the PC2 axis thereby denoting its significant contribution to the variance ex-



Fig. 2. Loading plot of PC1-PC2 and PC1-PC3 for Runner-type peanut cultivars. (A) PC1–PC2 and (B) PC1–PC3.

plained in PC2. In the PC1–PC2 axes, all saturated fatty acids, except for palmitic acid, showed a similar position; that is, <span id="page-7-0"></span>negatively loaded to the PC1 axis and positively loaded to the PC2 axis. In [Fig. 2,](#page-6-0) the PC3 axis distinguished lignoceric acid (0.84) from all other fatty acids and revealed that it had the highest contribution to the variance expressed in PC3. What is more, lignoceric acid was not correlated to all other fatty acids based on their cosine angles (all  $\sim$ 90°). These results correlate well with the eigenvalues discussed in [Table 3,](#page-6-0) which singles out lignoceric acid as being explicative to the variability in the generated PC3.

The score plots of fatty acids from peanut lipid extracts  $(n = 151)$  generated from comparisons of the first three PCs (*i.e.*, PC1–PC2 and PC1–PC3) are depicted in Fig. 3. The score plot of the PC1–PC2 comparison revealed three distinct groups of samples. Group A was related to normal peanut cultivars positively correlated to palmitic, stearic, linoleic, and arachidic acids, respectively, based on the loading plot for PC1 in [Fig. 2](#page-6-0)A. Group B indicated a weak positive correlation to oleic and gondoic acids, which is thought to be typical for mid-oleic cultivars (e.g., Tamrun OL01). Lastly, group C consisted predominantly of high-oleic cultivars due to a strong positive correlation to oleic and gondoic acids. In explanation, cultivars which have higher contents of oleic and gondoic acids were oriented in the positive PC1 axis (i.e., mid- & higholeic cultivars), whereas normal cultivars possessing higher contents of palmitic and linoleic acids were centred more towards the negative PC1 axis. There was one outlier contained in the PC1–PC2 plot (i.e., Georgia-01R), due to a higher contribution of



Fig. 3. Score plots for Runner-type peanut cultivars. (A) PC1–PC2 and (B) PC1–PC3. Cultivar symbols: ( $\bullet$ ) Georgia Green; ( $\circ$ ) Tamrun 96; ( $\nabla$ ) C99-R; ( $\triangledown$ ) Georgia-01R;  $(\blacksquare)$  Georgia-03L; ( $\square$ ) AP-3; ( $\blacklozenge$ ) Tamrun OL01; ( $\diamond$ ) Tamrun OL02; ( $\blacktriangle$ ) Flavorunner 458; and  $(\triangle)$  Georgia-02C.

behenic acid in that cultivar (NB, behenic acid is of high contribution to PC2). The score plot for the PC1–PC3 grouping is illustrated in Fig. 3B. Given that this grouping accounts for less of the total variability of the dataset (*i.e.*, when compared to that of PC1-PC2), it is not surprising there is less distinction between groups. In fact in the PC1–PC3 grouping, there are only two distinct groups of peanut cultivars. Group A accounts for normal cultivars, while group B contains both the mid- and high-oleic cultivars. This observation is likely due to the fact that PC3 was less capable of explaining the variance between peanut cultivars than that of PC2. There were two noticeable outliers in the PC1–PC3 grouping (i.e., Georgia-01R and Tamrun-OL02), most notably due to their low content of lignoceric acid, which is of a highly negative contribution to PC3.

## 4. Conclusions

This research specifically focused on US field-grown Runner peanut cultivars and showed how the contributions of individual fatty acids related to the generated PCs; individual fatty acid contributions to the total variability of a PC are often omitted from other investigations, but are invaluable. Eigen analysis of the correlation matrix loadings of the three significant PCs revealed that PC1 was mainly contributed to by palmitic, oleic, linoleic, and gondoic acids; PC2, by behenic acid; and PC3, by lignoceric acid. When the loading and score plots for Runner-type peanuts were projected as PC1–PC2 and PC1–PC3 groupings, there was an evident reduction in the number of variables necessary for the discrimination of peanut cultivars. PCs 1 through 3 together were found to be explanatory of more than 87% of the total variability in the data set. Statistical examinations need to be conducted routinely with data generated by the most up-to-date scientific methods/technologies in order to maintain the highest degree of validity. Furthermore, this study clearly indicates that the combination of experimental GC fatty acid data along with a chemometric approach (PCA, in this case) can be successfully employed by peanut researchers in collaboration with the peanut industry to give more information on variation in peanut cultivars than is capable with the experimental data alone.

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