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Fingerprinting of Volatile Organic Compounds as an Advance Technology to Assess the Seed Quality of Groundnut Through Correlation and Principal Component Analysis Method

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ABSTRACT

Background: Groundnut seeds are highly sensitive to deterioration mainly due to their chemical composition and moisture content. The high oil content and fatty acids of groundnut seeds reduces the seed viability well in advance due to lipid peroxidation process, which is the most frequent cause of seed ageing. As a result, not only lipid peroxidation, but also a series of reactions that produce toxic products occur. Stored seeds produce increased level of volatile organic compounds that leads to seed deterioration.

Methods: The experiment was conducted to profile volatile organic compounds emitted from groundnut seeds during storage and also to analysis the volatile organic compounds through correlation and principal component analysis method. Volatile organic compounds profiling of stored groundnut seeds was done through Gas Chromatography Mass Spectrometry (GC-MS). The air sample was taken at monthly intervals from the glass bottle using solid-phase micro extraction (SPME) method.

Result: The results of the study clearly demonstrated that, totally 52 volatile compounds comprising of 10 alcohols, 5 aldehydes, 15 acids, 9 esters, 7 alkanes, 3 alkenes, 2 ketones and 1 ether were trapped in stored groundnut seeds. Finally the study concluded that ethanol, 1-butanol, 1-hexanol, acetaldehyde, hexanal, Nonenal, 9,12,15-octadecatrionic acid, acetic acid and 3-methyl acetate were found to be highly negatively correlated with seed germination.

Key words: Groundnut, Seed ageing, Seed quality, Volatile organic compounds.

INTRODUCTION

Groundnut seeds are highly sensitive to deterioration mainly due to their chemical composition and moisture content. Seeds deteriorate with time and a number of factors are associated with ageing. Among them, strongly accountable factors are temperature and relative humidity which influence the seed moisture, leading to physiological, biochemical and molecular changes. In addition, poor storability of seeds results in less availability of quality seeds for sowing (Navtiyal *et al.,* 1997).

Groundnut seeds contain oleic (18:1), linoleic (18:2) and linolenic (18:3) fatty acid chain. These fatty acids are highly sensitive to peroxidative degradation. As a result, not only lipid degradation, but also a series of reactions that produce toxic products occur. Degree of unsaturation has a significant influence on degree of lipid degradation. Lipid peroxidation reactions also yield unsaturated aldehydes such as hexenal and hydroxyalkenals (4-hydroxynonenal), which are common biomarkers of lipid peroxidation and oxidative stress and the final product of lipid peroxidation is lipid hydroperoxid (ROOH) from which various aldehydes are formed, including malonyl-dialdehyde (MDA) (Zhang *et al*., 1993). When these types of damages occur in seed, they may cause decrease in seed germination and vigour. Hence, an attempt was made in this study to assess the seed quality of groundnut through volatile organic compounds using correlation and principal component analysis method.

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MATERIALS AND METHODS

Genetically pure seed lots of Groundnut var. VRI 8 obtained from the Regional Research Station, Vridhachalam were used as the base seed materials for this study. The storage experiment was carried out at the Department of Seed Science and Technology and Gas Chromatography Mass Spectrometry (GC-MS) analysis of volatile organic compounds

were carried out at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2020-2022.

Groundnut seeds were stored in air tight containers (screw capped glass bottles) of 500 g capacity. The cap was provided with septum to facilitate the air sampling from the glass bottle. The glass bottles were filled 300 g of seeds. The glass bottles were kept under ambient conditions for a storage period of 10 months.

Sampling and extraction of volatile organic compounds through GC-MS

The air sample was taken at monthly intervals from the glass bottle using solid-phase micro extraction (SPME) fiber. While taking the sample the SPME fiber was inserted just nearing the surface of the seed layer. Adsorption time for collection of air sample using SPME fiber was 30 min. Then the collected air sample of SPME fiber was directly injected to GC-MS (Thermo Scientific Trace GC Ultra chromatograph system, coupled to thermo scientific DSQ II quadruple mass spectrometer). The Helium (99.9%) gas was used as a carrier gas with flow rate of 1.0 ml/min and pressure of (60-100 Psi, 400-700 Kpa). Volatile compounds from air sample were separated by phenyl methyl silicon fused-silica capillary column (TG-5 MS, 30 m in length, 0.25 µm and 0.25 µm film thicknesses).

Volatiles were extracted and concentrated using SPME manual holder assembly equipped with SPME fiber conditioned at 250°C for 30 min. The fiber was desorbed at 250°C injector temperature in splitless mode. The GC oven was programmed as 1 min hold at 50°C, ramped to 100°C at the rate of 4°C/min and was further ramped to 240°C at the rate of 50°C /min with final hold of 2 min. Injection volume of 1 µl was taken in split less mode. The injector and detector were constantly maintained at temperature of 250°C and 260°C respectively with a total run time of 1hr for good separation of the diverse compounds. Volatile organic compounds were identified by the fragmentation pattern of individual compound and confirmed with the NIST (National Institute of Standards and Technology) Library database (Mathure *et al.,* 2011).

Standard germination test

Germination test was conducted by following the procedure outlined by ISTA (2019) with sand method with four replications of 100 seeds in a germination room maintained at 25±2°C temperature and 95±2% RH. The groundnut seedlings were evaluated at the end of final count period of 10 days. The number of normal seedlings was counted and the mean values were expressed in per cent.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

Statistical analysis

Correlation analysis was carried out to assess the relationship between volatile organic compounds and germination percentage. Volatile organic compounds were taken as dependent variables and correlated with germination per cent as independent variable. Significance of correlation coefficients was tested by Pearson correlation method by using SPSS 16.0 software. The principal component analysis (PCA) of volatile organic compounds released by the stored groundnut seeds was done by analyzing the area percentage contribution/strength of the most abundant volatiles from each group. The analysis was done employing the XLSTAT 2019 software.

RESULTS AND DISCUSSION

Profiling of volatile organic compounds in groundnut seeds during storage

Volatile organic compounds profiling of stored groundnut seeds was done through GC-MS. The volatiles were analysed at monthly interval up to 10 months of storage and the results showed that the several volatile components were trapped in groundnut seeds and all these components are falling into eight major groups such as alcohols, aldehydes, acids, esters, alkanes, alkenes, ketones and ether. Totally 52 volatile compounds comprising of 10 alcohols, 5 aldehydes, 15 acids, 9 esters, 7 alkanes, 3 alkenes, 2 ketones and 1 ether were trapped in stored groundnut seeds (Table 1).

Correlation analysis between volatile organic compounds and germination per cent

Correlation analysis was carried out to assess the relationship between volatile organic compounds and germination per cent. In alcohol group, ethanol (-0.927**), 1-hexanol (-0.785**) and 1-butanol (-0.776**) had highly significant negative correlation with the germination per cent followed by 2-nonen-1-ol (-0.747*) and methanol (-0.622*). In aldehyde group, acetaldehyde (-0.898**), nonenal (-0.878**) and hexanal (-0.789**) had highly significant negative correlation with the germination per cent followed by 2,4-nonadienal (-0.672*) and benzaldehyde (-0.621*). In acid group, 9,12,15-octadecatrionic acid (-0.953**), acetic acid (-0.935**), benzoic acid (-0.924**) and 9,12-octadecatrionic acid (-0.798**) had highly significant negative correlation with the germination per cent followed by docosanoic acid (-0.711*) and decanoic acid (-0.634*). In ester group, trimethylsilyl ester (-0.839**), 3-methyl acetate (-0.774**) and methyl ester (-0.767**) had highly significant negative correlation with the germination per cent followed by propyl ester (-0.728*), ethyl ester (-0.726*), dimethyl ester (-0.714*), 1,2-ethanediyl ester (-0.653*) and 1,2,3-propanetriyl ester (-0.634*). Similarly, other minor volatile groups, 3,3,3-hexafluropropane (-0.860**), trimethylsilyl ether (-0.846**) and butane (-0.793**) had highly significant negative correlation with the germination per cent followed by tetra tetracontane (-0.732*), octadecane (-0.713*), 17-pentatriacontane (-0.712*) and 2,6-dihydroxy acetophenone (-0.668*). However, the 1-propanol, 1-pentanol, 1,4-benzenediol, phenol, 1-monolinoleoyl glycerol, butonoic

Table 1: Volatile profiles of groundnut seeds during ambient storage.

Compounds	Storage period (Months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Alcohols (10)										
Methanol	+	+	+	+	-	-	-	-	-	-
Ethanol	-	-	-	-	+	+	+	+	+	+
1-Butanol	+	+	+	+	+	+	-	-	-	-
1-Propanol	+	+	+	+	+	-	-	-	-	-
1-Pentanol	+	+	+	+	+	-	-	-	-	-
1-Hexanol	+	+	+	+	+	+	+	+	-	-
1,4-Benzenediol	-	-	-	+	+	+	+	+	-	-
Phenol	-	-	-	-	+	+	-	-	-	-
2-Nonen-1-ol	-	-	-	-	-	-	+	+	+	+
1-Monolinoleoyl glycerol	-	-	-	-	-	-	-	+	+	-
Aldehydes (5)										
Hexanal	+	+	+	+	+	+	+	+	-	-
Acetaldehyde	+	+	+	+	+	+	-	-	-	-
Benzaldehyde	+	+	+	+	+	+	-	-	-	-
Nonenal	-	-	-	-	-	-	+	+	+	+
2,4-Nonadienal	-	-	-	-	-	-	-	+	+	+
Acids (15)										
Acetic acid	+	+	+	+	+	+	+	+	+	+
Butonoic acid	-	-	-	-	+	+	+	-	-	-
Proponoic acid	-	-	-	-	+	+	+	-	-	-
Hexadecanoic acid	+	+	+	+	+	+	+	+	+	+
Octadecanoic acid	+	+	+	+	+	+	+	+	+	+
9,12-Octadecatrionic acid	-	-	+	+	+	+	+	+	+	+
9,12,15-Octadecatrionic acid	-	-	-	-	+	+	+	+	+	+
Benzoic acid	-	-	-	-	+	+	+	+	+	+
Docosanoic acid	-	-	-	-	-	-	+	+	+	+
Decanoic acid	-	-	-	-	-	-	+	+	+	+
Tetra decanoic acid	-	-	-	-	-	-	+	+	+	+
2,4,6-Decatrienoic acid	-	-	-	-	-	-	+	+	+	+
Arsenous acid	-	-	-	-	-	-	-	+	+	+
Glutaranoic acid	-	-	-	-	-	-	-	+	+	+
Tetracontanedioic acid	-	-	-	-	-	-	-	-	+	+
Esters (9)										
Methyl ester	+	+	+	+	+	+	+	+	+	+
Ethyl ester	+	+	+	+	+	+	+	+	-	-
Butyl ester	-	-	-	-	+	+	+	+	+	+
Propyl ester	-	-	-	-	-	-	-	+	+	+
Dimethyl ester	-	-	-	-	-	-	-	-	+	+
Trimethylsilyl ester	-	-	-	-	-	+	+	+	+	+
1,2-Ethanediyl ester	-	-	-	-	-	-	-	-	+	+
1,2,3-Propanetriyl ester	-	-	-	-	-	-	-	-	+	+
3-Methyl acetate	+	+	+	+	+	+	+	-	-	-
Alkanes (7)										
Butane	+	+	+	+	+	+	+	-	-	-
Hexane	-	-	-	-	+	+	+	-	-	-
Octadecane	-	-	-	-	+	+	+	+	+	+
9-Octadecane	-	-	-	-	+	+	+	+	+	+

Table 1: Continue.....

Table 1: Continue.....

3,3,3-Hexafluropropane	-	-	-	-	-	+	+	+	+	+
Tetratetracontane	-	-	-	-	-	-	-	-	+	+
17-Pentatriacontane	-	-	-	-	-	-	-	-	+	+
Alkenes (3)										
1-Pentene	+	+	+	+	+	+	-	-	-	-
Benzene	-	-	-	+	+	+	+	+	+	+
à-Pinene	-	-	-	+	+	+	+	+	+	+
Ketones (2)										
2,6-Dihydroxy acetophenone	-	-	-	-	-	+	+	+	+	+
Ethanone	-	-	-	-	-	-	+	+	-	-
Ether (1)										
Trimethylsilyl ether	-	-	-	-	-	-	-	+	+	+

MAS: Month after storage; (-) Absent, (+) Present.

Table 2: Correlation between volatile alcohol compounds and germination percentage of groundnut seeds.

Variables	Methanol	Ethanol	1-Butanol	1-Propanol	1-Pentanol	1-Hexanol	1,4-Benzenediol	Phenol	2-Nonen-1-ol	1-Monolinoleoyl glycerol	Germination
Methanol	1										
Ethanol	-0.555	1									
1-Butanol	0.219	-0.612	1								
1-Propanol	0.506	-0.540	0.793**	1							
1-Pentanol	0.354	-0.611*	0.963**	0.893**	1						
1-Hexanol	-0.014	-0.628*	0.718*	0.482	0.566	1					
1,4-Benzenediol	-0.335	0.404	-0.313	-0.306	-0.330	-0.225	1				
Phenol	-0.262	-0.222	-0.135	-0.215	-0.233	0.339	0.055	1			
2-Nonen-1-ol	-0.430	0.895**	-0.509	-0.429	-0.488	-0.622*	0.528	-0.317	1		
1-Monolinoleoyl glycerol	-0.288	0.641*	-0.340	-0.287	-0.326	-0.466	0.344	-0.212	0.889**	1	
Germination	-0.622*	-0.927**	-0.776**	0.471	0.510	-0.785**	-0.306	0.044	-0.747*	-0.572	1

**Significant at 0.01 level; *Significant at 0.05 level.

Table 3: Correlation between volatile aldehyde compounds and germination percentage of groundnut seeds.

Variables	Hexanal	Acetaldehyde	Benzaldehyde	Nonenal	2,4-Nonadienal	Germination
Hexanal	1					
Acetaldehyde	-0.263	1				
Benzaldehyde	0.404	0.515	1			
Nonenal	-0.553	-0.484	-0.587	1		
2,4-Nonadienal	-0.581	-0.428	-0.519	0.956**	1	
Germination	-0.789**	-0.898**	-0.621*	-0.878**	-0.672*	1

**Significant at 0.01 level; *Significant at 0.05 level.

acid, hexadecanoic acid, octadecanoic acid, butyl ester, hexane, 9-octadecane, 1-pentene, benzene, à-pinene and ethanone had no correlation with the germination per cent (Table 2-7).

The results of present study are in accordance with Mathure *et al.* (2011), who reported that hexanal, pentanal, heptanal, octanal, nonanal, *trans*-2-octenal, decanal, *trans*-2-nonenal, (*E,E*)-2,4-nonadienal, 1-octen-3-ol and 1-hexanol are the products derived from either oxidation or degradation of lipids. Hence, they are related with each other resulting in significant correlation with seed quality in groundnut. The significant positive correlation between 2-methyl pyrazine and 2,5-dimethyl pyrazine, 2-ethyl-6-methyl pyrazine, 2-acetyl pyrazine, 3-ethyl-2,5-dimethyl-pyrazine and 2,3,5-trimethyl-6- ethyl pyrazine were 0.903**, 0.963**, 0.987**, 0.862** and 0.909**, respectively (Lin *et al.,* 2016). Ethanol was the predominant volatile component and it tended to increase as flavour quality decreased. The correlation coefficients, significant at the 1% level, between the flavour scores and the ratios of ethanol-to-methanol and ethanol-to-total volatiles were -0.87** and -0.88**, respectively. Meanwhile, the volatile compound of ethanol was found to be the most and highly negatively correlated with seed quality in groundnut as already suggested by Greene *et al.* (2008).

Zhang *et al.* (1994) observed that acetaldehyde, acetic acid, ethanol, ethyl acetate, acetone and isopropanol evolved from seeds of rice and lettuce found to decline the germination and vigour during storage and all these volatile compounds were negatively correlated with seed germination. According to the report of Min *et al.* (2017), Oenal *et al.* (2017) and Bhattacharjee (2019), the release of alcohols, aldehydes, ketones, alkanes, carboxylic acids and other volatile compounds produced due to lipid peroxidation process have found to diffuse and easily penetrate in to the biological membrane in seed and also affect other cellular and extracellular matrix components of the cell that leads to reduce the biochemical and molecular properties of seeds.

Principal component analysis of volatile organic compounds

The principal component analysis was carried out for identification of closely associated volatile organic compounds released during storage of groundnut seeds. The key component with more than one Eigen value and variables with more loading value factors were considered as a true representation of the variance in the data set and accordingly the components were grouped as PC-1, PC-2 and PC-3 which covered 91.63 per cent total variability. Hence, The PC-1, PC-2 and PC-3 exhibited 56.75%, 24.21% and 10.66% variability respectively for those particular volatile components among the different components (Table 8).

The PCA-Correlation circle showed that the distribution of variables with respect to the PCs designated as X and Y axis. The data visualization using the correlation circle revealed that all the volatile components emanated from groundnut seeds were distributed in all quadrants and out of 16 components ethanol, 1-butanol, 1-hexanol, nonenal, acetic acid, 9,12-octadecatrionic acid, 9,12,15-octadecatrionic acid, 3-methyl acetate, exhibited higher variability, so these are concluded as closely associated volatile components for seed deterioration (Fig 1). The results of present study are in accordance with Meenakshi (2020), who reported that the principal component analysis done with 15 most abundant volatile compounds emitted under seed ageing of sunflower seeds. In this analysis, the PCA-Correlation circle interpretation clearly indicated that out of 15 components, most of the components *viz.,* 1,2-ethanediol, ethanol, acetaldehyde, hexanal, acetic acid, hexanoic acid and ethyl ester had higher variability except verbinone which had lesser variability so that they

Table 4: Correlation between volatile acid compounds and germination percentage of groundnut seeds.

Variables	Acetic acid	Butonoic acid	Hexade--canoic acid	Octade--canoic acid	9,12-Octadec--atrionic acid	9,12,15-Octadec--atrionic acid	Benzoic acid	Docosanoic acid	Decanoic acid	Germi-nation
Acetic acid	1									
Butonoic acid	0.283	1								
Hexadecanoic acid	0.419	0.821**	1							
Octadecanoic acid	0.264	0.650*	0.936**	1						
9,12-Octadecatrionic acid	0.829**	0.137	0.177	-0.003	1					
9,12,15-Octadecatrionic acid	0.732*	-0.092	-0.211	-0.405	0.745*	1				
Benzoic acid	0.762**	-0.124	-0.169	-0.341	0.810**	0.978**	1			
Docosanoic acid	0.567	-0.265	-0.355	-0.494	0.548	0.928**	0.880**	1		
Decanoic acid	0.638*	-0.322	-0.360	-0.500	0.719*	0.957**	0.955**	0.955**	1	
Germination	-0.935**	0.020	0.017	0.187	-0.798**	-0.953**	-0.924**	-0.711*	-0.634*	1

**Significant at 0.01 level; *Significant at 0.05 level.

Table 5: Correlation between volatile ester compounds and germination percentage of groundnut seeds.

Variables	Methyl ester	Ethyl ester	Butyl ester	Propyl ester	Dimethyl ester	Trimethylsilyl ester	1,2-Ethanediyl ester	1,2,3-Propanetriyl ester	3-Methyl acetate	Germination
Methyl ester	1									
Ethyl ester	0.602*	1								
Butyl ester	0.833**	0.167	1							
Propyl ester	-0.286	-0.597	0.122	1						
Dimethyl ester	-0.384	-0.529	-0.040	0.720*	1					
Trimethylsilyl ester	-0.210	-0.580	0.181	0.806**	0.939**	1				
1,2-Ethanediyl ester	-0.304	-0.394	-0.130	0.292	0.815**	0.768**	1			
1,2,3-Propanetriyl ester	-0.384	-0.528	-0.042	0.713	1.000**	0.939**	0.823**	1		
3-Methyl acetate	0.539	0.951**	0.107	-0.539	-0.456	-0.540	-0.339	-0.455	1	
Germination	-0.767**	-0.726*	-0.396	-0.728*	-0.714*	-0.839**	-0.653*	-0.634*	-0.774**	1

**Significant at 0.01 level; *Significant at 0.05 level.

Table 6: Correlation between volatile alkane compounds and germination percentage of groundnut seeds.

Variables	Butane	Hexane	Octadecane	9-Octadecane	3,3,3-Hexafluropropane	Tetratetracontane	17-Pentatriacontane	Germi--nation
Butane	1							
Hexane	0.702*	1						
Octadecane	0.113	0.593	1					
9-Octadecane	0.478	0.894**	0.851**	1				
3,3,3-Hexafluropropane	-0.446	-0.180	0.611	0.178	1			
Tetratetracontane	-0.495	-0.308	0.500	0.002	0.955**	1		
17-Pentatriacontane	-0.481	-0.300	0.495	0.012	0.972**	0.994**	1	
Germination	-0.793**	0.021	-0.713*	-0.398	-0.860**	-0.732*	-0.712*	1

**Significant at 0.01 level; *Significant at 0.05 level.

Table 7: Correlation between volatile alkene, ketone, ether compounds and germination percentage of groundnut seeds.

Variables	1-Pentene	Benzene	à-Pinene	2,6-Dihydroxy acetophenone	Ethanone	Trimethylsilyl ether	Germination
1-Pentene	1						
Benzene	0.131	1					
à-Pinene	0.079	0.982**	1				
2,6-Dihydroxy acetophenone	-0.623	-0.062	-0.017	1			
Ethanone	-0.393	-0.093	-0.058	0.876**	1		
Trimethylsilyl ether	-0.509	-0.295	-0.248	0.157	-0.084	1	
Germination	0.610	-0.002	-0.052	-0.668*	-0.320	-0.846**	1

**Significant at 0.01 level; *Significant at 0.05 level.

Table 8: Principal component analysis (PCA) - Eigen value of volatile organic compounds in groundnut seeds.

Factors	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Eigen value	9.080	3.875	1.706	0.741	0.220	0.178	0.117	0.065	0.017
Variability (%)	56.751	24.217	10.664	4.631	1.376	1.114	0.733	0.406	0.107
Cumulative (%)	56.751	80.968	91.632	96.263	97.639	98.754	99.486	99.893	100.000

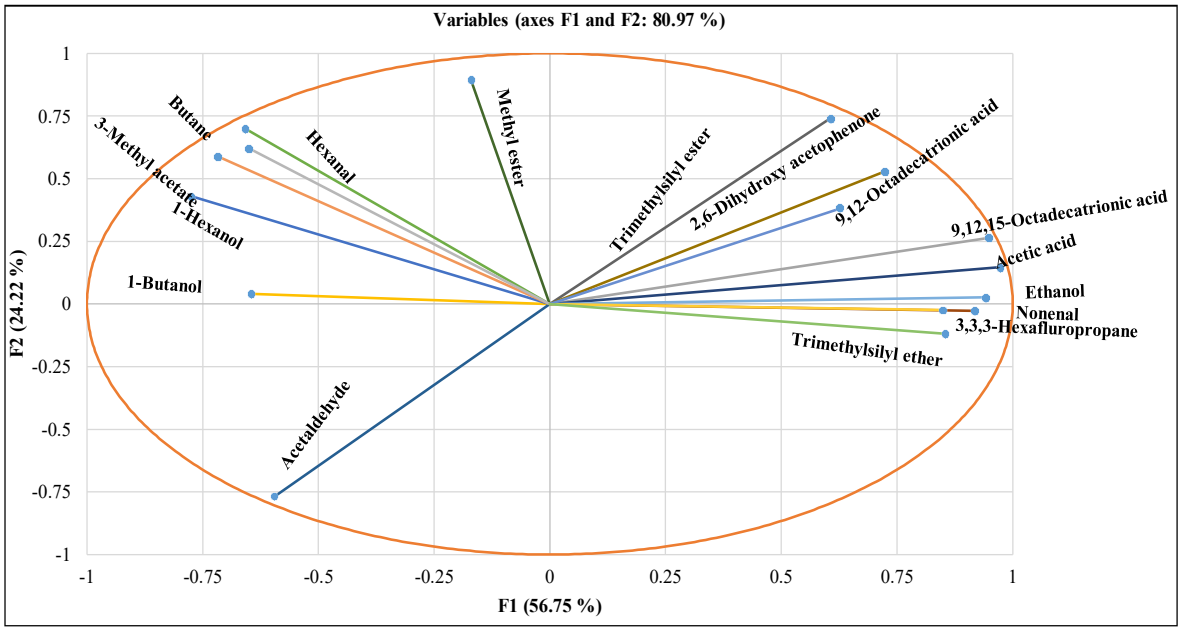

Fig 1: Principal component analysis (PCA) of volatile organic compounds in groundnut seeds.

are concluded as closely associated components with seed deterioration under ageing of sunflower.

CONCLUSION

The study concluded that there were eight different categories of 52 volatile organic compounds profiled in groundnut seeds during storage. Among the 52 volatile compounds, ethanol, 1-butanol, 1-hexanol, acetaldehyde, hexanal, Nonenal, 9,12,15-octadecatrionic acid, acetic acid and 3-methyl acetate were found to be the highly negatively correlated volatiles with seed germination. So these volatile components could be considered as the signature components of seed deterioration for assessing the groundnut seed quality.

Conflict of interest

G.P. Chinnasamy, carried out, generated, analyzed the experiment interpreted the data and written the original draft. Dr. S. Sundareswaran, Dr. K. Raja, Dr. P.R. Renganayaki, Dr. K.S. Subramaniyan, Dr. S. Marimuthu and Mr. D. Pradeep an designed the experiment and corrected the manuscript as an advisory committee.

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