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Research Article

Volatile organic compound analysis as advanced technology to detect seed quality in groundnut

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Abstract
An experiment was conducted to profiling the volatile organic compounds emitted from groundnut seeds during storage and also to assess the volatiles emission level during seed deterioration. Volatile organic compounds profiling of stored groundnut seeds was done through GC-MS at monthly intervals. The results showed that several volatile compounds were released from stored groundnut seeds and all the compounds are falling into eight major groups *viz.,* alcohols, aldehydes, acids, esters, alkanes, alkenes, ketones and ethers. The study clearly demonstrated the influence of volatile organic compounds emission level on physiological and biochemical properties during storage. There was a significant decrease in physiological and biochemical quality attributes noted due to an increase in the strength of volatiles released during ageing. When the release of total volatile strength reached more than 50%, a significant reduction in physiological attributes such as germination, root and shoot length, dry matter production and vigour index were observed. With respect to biochemical properties, a significant increase in electrical conductivity of seed leachate, lipid peroxidation and lipoxygenase activity, and a decrease in dehydrogenase, catalase and peroxidase activities were observed. However, the highest reduction in all these properties was recorded when the total volatile strength reached 92.72%. The study concluded that the volatiles released during seed deterioration could be considered the signature components for detecting the seed quality during storage.

Keywords: Groundnut, Seed deterioration, Seed quality, Volatile organic compounds

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a “King of oilseed crops” and belongs to the family Fabaceae. It has seeds with poor storage life and storing seeds after harvest till the next cropping season without impairing the quality is crucial for successful seed production. The problem of loss of seed viability is more severe in groundnut harvested in the summer season and about 50 per cent viability could be lost within 5 to 6 months of storage (Nautiyal *et al*., 1991). Seed storage in groundnut is inescapable for the sensitivity of seed to environment, seasonal demand, dormancy, specificity of planting time, the necessity of carrying over and

need for buffer seed stock. The longevity of stored seed is affected by a number of factors and the four major factors are seed moisture content, temperature, relative humidity and oxygen concentration in the storage environment. High temperature and high relative humidity cause rapid deterioration of viability and vigour of groundnut seeds (Begum *et al*., 2014).

Seeds with high oil content appear to lose their germination and vigour in a very short time in spite of the precaution taken during harvesting and drying.The lipids in groundnut seeds are at risk of auto-oxidation, which contains 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. Degree of unsaturation has a significant influence on the degree of lipid degradation. Lipid peroxidation and the products resulting from these processes lead to DNA denaturation, prevent translation and protein transcription and cause oxidation of the most reactive amino acids. 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). When these types of damages occur in seed, they may cause a decrease in seed germination and vigour (Zhang *et al*., 1993). During storage, major losses of seeds are caused by various biological and non-biological factors, including fungi, temperature, humidity and seed moisture level, which play a major role in the deterioration of seeds, including seed rots, molding of seeds, pre and post-emergence damping-off, low seed viability and poor seedling growth. Hence, there is a need to examine reasonable factors of these storage losses, which ultimately affect the market value and quality of the seeds (Mira *et al*., 2010).

Stored seeds produce increased volatile organic compounds that lead to seed deterioration. Many volatile organic compounds (VOCs) are reactive and toxic, perpetuating reactions that lead to deterioration and accelerating the rate at which seeds lose viability for high concentrations of ethanol and acetaldehyde (Zhang *et al*., 1995; Akimoto *et al*., 2004). VOCs are the major by-product of catabolic reactions and volatile alka(e)nes and aldehydes are major by-products of lipid peroxidation (Grotto *et al.*, 2009). Saturated aldehydes such as hexanal are the potential biomarkers of lipid peroxidation during the storage of seeds. These volatile organic compounds are toxic to seeds and give a path to free radical production and, finally, deterioration of seeds during storage (Aldini *et al*., 2011).

A conventional technique developed in the early 1960's is now used to determine the quality of seeds. The evaluation process is complicated and requires a simple, innovative approach. Volatile organic compound analysis is one of the advanced methods to assess the quality of seeds in recent years. The main objective of the study was to profiling the volatile organic compounds and also to assess the effect of volatile emission on physiological and biochemical seed quality in groundnut during storage.

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 was carried out at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

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

Profiling of volatile organic compounds

The air sample was taken at monthly intervals from the glass bottle using solid-phase microextraction (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 and 400-700 Kpa. Volatile compounds from air sample were separated by phenyl methyl silicon fused-silica capillary column (TG-5 MS, 30m in length, 0.25µm and 0.25µm film thicknesses).

Volatiles were extracted and concentrated using SPME manual holder assembly equipped with SPME fiber conditioned at 250ºC for 30 min. The fiber was desorbed at 250ºC injector temperature in splitless mode. The GC oven was programmed as 1 min hold at 50ºC, ramped to 100ºC at the rate of 4ºC/min and was further ramped to 240ºC at the rate of 50ºC/min with final hold of 2 min. An injection volume of 1µl was taken in split less mode. The injector and detector were constantly maintained at temperature of 250ºC and 260ºC, respectively with a total run time of 1hr for good separa-

tion of the diverse compounds. Then the volatile organic compounds were identified by the fragmentation pattern of the individual compound and confirmed with the NIST (National Institute of Standards) Library database (Mathure *et al.* 2011).

Evaluation of physiological and biochemical seed quality attributes

The groundnut seeds were tested for different physiological seed quality parameters such as germination percentage, shoot and root length, dry matter production and vigour index as per ISTA seed testing protocols (ISTA, 2019) and the biochemical seed quality parameters such as electrical conductivity of the seed leachate (Presley, 1958), dehydrogenase enzyme activity (Kittock and law, 1968), catalase activity (Aebi, 1984), peroxidase activity (Malik and Singh, 1980), lipid peroxidation activity (Bernheim *et al*., 1948) and lipoxygenase activity (Hildebrand *et al*., 1993).

Statistical analysis

Data obtained from the experiments were analysed using an analysis of variance (ANOVA) as a factorial combination of treatments. Mean values were separated on the basis of least significant difference (LSD) only if F test of ANOVA for treatments was significant at 0.05 probability level. Values in per cent data were arcsine transformed before analysis. If the F test is non-significant it was indicated by the letters NS.

RESULTS AND DISCUSSION

Profiling of volatile organic compounds

Volatile organic compounds profiling of stored groundnut seeds was done through GC-MS. The results of the analyses of volatiles at monthly intervals showed that several volatile components were released from stored groundnut seeds and all the components fall into eight major groups such as alcohols, aldehydes, acids, esters, alkanes, alkenes, ketones and ethers.

In the alcohol group, methanol, 1-butanol, 1-propanol, 1-pentanol and 1-hexanol were emitted from first month of ageing to 5-6 months after storage. Ethanol and 2-nonen-1-ol were released 5-6 months after storage and continued till the end of the storage period. Among the volatile alcohol compounds, ethanol, 1-butanol and 1-hexanol were the most abundant volatiles, and the area percentage was progressively increased during the storage period. The area percentage/strength of ethanol at initial was 1.68 per cent and increased upto 9 months after storage (12.83%) followed by 1-hexanol, which started from 0.93 per cent and increased up to 5 months after storage (6.83 %), thereafter decreased; whereas, 1-butanol started from 0.18 per cent and increased upto 4 months after storage (5.11 %), thereafter decreased significantly (Table 1). The most abundant release of ethanol, 1-hexanol and 1-butanol might be due to lipid peroxidation process, anaerobic metabolism and also the glycolysis pathway both are involved. This may also happen because of loss in mitochondrial membrane integrity (Colville *et al*., 2012). Buckley and Buckley (2009) reported the emission of ethanol in canola seeds during ageing due to anaerobic metabolism. According to Zhang *et al.* (1993), the ethanol was emanated in very slow rate and detected in dry stored seeds of soybean, sunflower, lettuce and carrot, and they stated that the glycolytic reactions in stored seeds produce the ethanol and other alcoholic compounds.

In aldehyde group, hexanal, acetaldehyde and benzaldehyde were emitted from first month of ageing to 6 months after storage. Nonenal and 2,4-nonadienal found to be released from 7 months after storage and continued till the end of storage period. Among aldehydes, hexanal was observed in higher area percentage, its contribution was substantially increased from 0.49 per cent at first month to 6.95 per cent at 5th month followed by acetaldehyde, nonenal and 2,4-nonadienal. Benzaldehyde was the least component released in aged seeds compared to other volatile aldehydes (Table 1). The release of acetaldehyde from aged seeds might be due to the disintegration of linoleic poly unsaturated fatty acids by auto-oxidation or by enzymatic oxidation and also attributed to the mitochondrial damage (Colville *et al*., 2012). Zhang *et al.* (1993), detected acetaldehyde from dry seeds of 47 species during ageing and they demonstrated that the oxidation of lipid membrane produced the aldehyde. Hexanal is an aldehyde that was found to be accumulated in dry seeds during storage (Leufven *et al.,* 2010). Aldehydes are the major volatiles emitted as a result of lipid peroxidation, hexanal, Nonenal and 2,4-Nonadienal were the volatiles associated with the degradation of linoleic acid (Colville *et al*., 2012). Grotto *et al.* (2009) reported t a higher quantity of aldehydes in stored seeds due to oxidation of lipid membrane.

In acid group, acetic acid, hexadecanoic acid and octadecanoic acid were released throughout the storage period. 9,12-octadecatrionic acid appeared in 3-month-aged seeds; thereafter, they continued to release until the end of the storage period. 9,12,15-octadecatrionic acid and benzoic acid were found to release from 5 months after storage and continued till the end of storage period. Acetic and 9,12,15-octadecatrionic acids were the dominant acids and their concentrations increased with storage time. Initially, the percentage contribution of acetic acid and 9,12,15-octadecatrionic acid was 0.44 and 1.03 per cent, respectively. Afterwards, it increased with the advancement in storage time at 9th month they accounted for about 11.82 and 10.93

Table 1. Area percentage (strength) of volatile organic compounds (VOCs) profile in groundnut seeds during 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	1.72	6.83	3.94	0.61	0	0	0	0	0	0
Ethanol	0	0	0	0	1.68	2.51	9.96	11.07	12.83	12.06
1-Butanol	0.18	0.92	3.82	5.11	2.21	0.18	0	0	0	0
1-Propanol	0.08	0.47	2.18	0.93	0.27	0	0	0	0	0
1-Pentanol	0.35	0.73	2.93	3.05	0.65	0	0	0	0	0
1-Hexanol	0.93	1.08	4.18	4.29	6.83	2.84	1.51	0.28	0	0
1,4-Benzenediol	0	0	0	0.12	0.28	0.72	0.91	2.95	0	0
Phenol	0	0	0	0	0.18	0.52	0	0	0	0
2-Nonen-1-ol	0	0	0	0	0	0	0.83	1.78	1.93	0.74
1-Monolinoleoyl glycerol	0	0	0	0	0	0	0	0.38	0.62	0
Total	3.26	10.03	17.05	14.11	12.10	6.77	13.21	16.46	15.38	12.80
Aldehydes (5)										
Hexanal	0.49	1.02	2.23	4.1	6.95	4.08	3.06	1.15	0	0
Acetaldehyde	3.46	4.74	3.06	1.64	0.85	0.16	0	0	0	0
Benzaldehyde	0.17	1.36	2.61	1.29	1.18	0.93	0	0	0	0
Nonenal	0	0	0	0	0	0	1.02	1.96	2.63	5.41
2,4-Nonadienal	0	0	0	0	0	0	0	1.04	2.95	4.02
Total	4.12	7.12	7.90	7.03	8.98	5.17	4.08	4.15	5.58	9.43
Acids (15)										
Acetic acid	0.44	2.83	5.27	7.64	8.47	8.94	9.73	11.05	11.82	9.83
Butonoic acid	0	0	0	0	0.97	1.52	1.84	0	0	0
Proponoic acid	0	0	0	0	1.91	2.85	2.94	0	0	0
Hexadecanoic acid	0.11	0.94	1.73	3.29	3.75	5.93	4.03	2.05	1.17	1.02
Octadecanoic acid	0.27	1.28	3.29	3.72	4.03	6.08	3.02	1.92	1.12	0.83
9,12-Octadecatrionic acid	0	0	1.83	1.94	4.05	5.42	7.06	10.08	7.83	6.94
9,12,15-Octadecatrionic acid	0	0	0	0	1.03	1.86	6.82	9.02	10.93	9.07
Benzoic acid	0	0	0	0	0.92	2.23	3.02	6.82	8.97	7.32
Docosanoic acid	0	0	0	0	0	0	0.27	0.51	0.96	1.48
Decanoic acid	0	0	0	0	0	0	0.22	0.91	1.05	1.26
Tetra decanoic acid	0	0	0	0	0	0	0.11	0.28	0.72	0.95
2,4,6-Decatrienoic acid	0	0	0	0	0	0	1.29	1.72	1.94	2.07
Arsenous acid	0	0	0	0	0	0	0	0.87	1.18	0.92
Glutaranoic acid	0	0	0	0	0	0	0	0.26	0.63	0.83
Tetracontanedioic acid	0	0	0	0	0	0	0	0	1.38	1.62
Total	0.82	5.05	12.12	16.59	25.13	34.83	40.35	45.49	49.70	44.14
Esters (9)										
Methyl ester	0.28	1.39	1.63	4.1	4.95	7.2	7.52	3.84	1.72	1.38
Ethyl ester	0.16	1.26	1.83	2.88	3.07	3.86	1.05	0.24	0	0
Butyl ester	0	0	0	0	0.82	1.57	1.94	0.93	0.88	0.37
Propyl ester	0	0	0	0	0	0	0	0.95	1.84	0.72
Dimethyl ester	0	0	0	0	0	0	0	0	1.38	1.72
Trimethylsilyl ester	0	0	0	0	0	0.84	0.98	1.92	4.05	4.98
1,2-Ethanediyl ester	0	0	0	0	0	0	0	0	0.07	0.69
1,2,3-Propanetriyl ester	0	0	0	0	0	0	0	0	1.01	1.29
3-Methyl acetate	0.13	0.72	2.18	3.85	5.12	3.93	1.02	0	0	0
Total	0.57	3.37	5.64	10.83	13.96	17.4	12.51	7.88	10.95	11.15
Alkanes (7)										
Butane	0.22	1.16	1.2	1.98	2.94	4.03	1.62	0	0	0
Hexane	0	0	0	0	0.63	0.98	1.17	0	0	0
Octadecane	0	0	0	0	0.72	0.91	1.12	0.57	0.89	1.06
9-Octadecane	0	0	0	0	1.05	1.82	1.95	0.82	0.53	0.82
3,3,3-Hexafluropropane	0	0	0	0	0	0.72	0.78	0.96	2.62	5.61
Tetratetracontane	0	0	0	0	0	0	0	0	1.06	1.53
17-Pentatriacontane	0	0	0	0	0	0	0	0	1.04	1.86
Total	0.22	1.16	1.20	1.98	5.34	8.46	6.64	2.35	6.14	10.88

Contd…...

Table 1. Contd…...

Compounds	Storage period (Months)									
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS
Alkenes (3)										
1-Pentene	0.28	0.93	1.85	2.1	0.79	0.98	0	0	0	0
Benzene	0	0	0	0.26	0.64	0.96	0.28	0.15	0.12	0.04
à-Pinene	0	0	0	0.12	0.35	0.72	0.17	0.12	0.07	0.05
Total	0.28	0.93	1.85	2.48	1.78	2.66	0.45	0.27	0.19	0.09
Ketones (2)										
2,6-Dihydroxy acetophenone	0	0	0	0	0	0.93	5.02	6.73	2.01	1.84
Ethanone	0	0	0	0	0	0	1.06	3.83	0	0
Total	0	0	0	0	0	0.93	6.08	10.56	2.01	1.84
Ether (1)										
Trimethylsilyl ether	0	0	0	0	0	0	0	0.38	1.92	2.39
Total	0	0	0	0	0	0	0	0.38	1.92	2.39
Grand Total	9.27	27.66	45.76	53.02	67.29	76.22	83.32	87.54	91.87	92.72

MAS: Months after storage

per cent respectively, followed by 9,12-octadecatrionic acid and benzoic acid. Even though some of the other acid compounds were released during the storage period, their area percentage was low for the whole period of storage (Table 1).

In ester group, methyl ester was released throughout the storage period. Ethyl ester and 3-methyl acetate appeared in first month of aged seeds and thereafter they continued to release up to 7-8 months of storage. Initially the percentage contribution of methyl ester was 0.28 per cent, afterwards it was increased up to 7 months of storage (7.52 &) followed by ethyl ester and 3-methyl acetate (Table 1).

In the alkane group, butane was found to be released in the first month of aged seeds and thereafter continued to release up to 7 months after storage. Octadecane, 9-octadecane and 3,3,3-hexafluropropane appeared in 5-6 months of aged seeds; thereafter, they continued to release until the end of the storage period. Among the alkane compounds, butane registered a higher area percentage; it was 0.22 per cent at the first month of ageing thereafter it was increased up to 6 months after storage (4.03 %). In the alkene group, 1-Pentene was released in first month of aged seeds and thereafter continued to release up to 6 months after storage. Benzene and à-pinene appeared in 4 months old seeds; thereafter, they were released until the end of the storage period. But all the three alkene compounds recorded very minimum area per cent. 2,6-dihydroxy acetophenone and ethanone belong to ketone group and trimethylsilyl ether belonged to ether group were released during the storage period, but their area percentage was very low during the whole period of storage (Table 1). The release of these volatiles during ageing might be attributed to oxidation of lipid bi-layer cell membrane and also non-enzymatic degradation of macromolecules. Various chemical reactions such as glycolysis, auto-oxidation or non-enzymatic oxidation and strecker degradation of Maillard reaction in aged seeds produce acids, alkanes, alkenes, esters, ketones and ethers (Mira *et al*., 2016). Lipid oxidation in stored pea seeds produced a range of volatile organic compounds *viz*., ketones, alcohols, alkanes and esters (Colville *et al*., 2012) and they also stated that these compounds are likely source for alcohols *viz*., pentanol, butanol and propanol, and also for *n*-hexylformate and 2-butanone. According to Aldini *et al*. (2011) the oxidation of polyunsaturated fatty acids (PUFA) like linoleic and linolenic acids release volatile alkane compounds.

VOCs emission and physiological seed quality

Seeds evolve volatile organic compounds that are directly deteriorating the physiological seed qualities and there is a strong correlation between VOCs release and seed quality deterioration during storage (Mira *et al*., 2010). All the seed-emitted VOCs have harmful effects on germination and seed vigour of stored seeds.

In the present study, the results demonstrated the effect of VOCs emission level on physiological seed quality attributes. The germination, root and shoot length, dry matter production and vigour index were significantly reduced during storage, corresponding with the increase in release of volatile organic compounds. The germination was decreased from 91 per cent at first month to 42 per cent at 10th month of ageing. Initially (up to 2 months), there was not much germination reduction and during that time, VOCs release strength was 27.66%. When VOCs strength increased beyond 45 per cent, significant decrease in germination was noted at 3rd month. At the end of the storage period, the germination was 42 per cent. At that time VOCs re-

Table 2. Effect of VOCs emission levels on physiological quality of groundnut seeds during storage

Storage period (Months)	Germination (%)	Root length (cm)	Shoot length (cm)	VOCs strength (%)		
				Strength of individual VOC group	Dominant individual VOC	Total VOCs strength
1 MAS	91 (72.54)	10.1	15.4	Alcohol (3.26), Aldehyde (4.12), Acids (0.82), Esters (0.57), Alkanes (0.22), Alkenes (0.28), Ketones (0), Ether (0)	Acetaldehyde (3.46) Methanol (1.72)	9.27
2 MAS	88 (69.73)	10.3	15.0	Alcohol (10.03), Aldehyde (7.12), Acids (5.05), Esters (3.37), Alkanes (1.16), Alkenes (0.93), Ketones (0), Ether (0)	Methanol (6.83) Acetaldehyde (4.74)	27.66
3 MAS	83 (65.65)	9.5	14.6	Alcohol (17.05), Aldehyde (7.90), Acids (12.12), Esters (5.64), Alkanes (1.20), Alkenes (1.85), Ketones (0), Ether (0)	Acetic acid (5.27) 1-Hexanol (4.18) Acetaldehyde (3.06)	45.76
4 MAS	80 (63.43)	9.1	13.8	Alcohol (14.11), Aldehyde (7.03), Acids (16.59), Esters (10.83), Alkanes (1.98), Alkenes (2.48), Ketones (0), Ether (0)	Acetic acid (7.64) 1-Butanol (5.11) Acetaldehyde (1.64)	53.02
5 MAS	76 (60.66)	9.3	13.4	Alcohol (12.10), Aldehyde (8.98), Acids (25.13), Esters (13.96), Alkanes (5.34), Alkenes (1.78), Ketones (0), Ether (0)	Acetic acid (7.64) Hexanal (6.95)	67.29
6 MAS	71 (57.41)	9.5	13.5	Alcohol (6.77), Aldehyde (5.17), Acids (34.83), Esters (17.40), Alkanes (8.46), Alkenes (2.66), Ketones (0.93), Ether (0)	Acetic acid (8.94) Octadecanoic acid (6.08)	76.22
7 MAS	64 (53.13)	8.8	12.4	Alcohol (13.21), Aldehyde (4.08), Acids (40.35), Esters (12.51), Alkanes (6.64), Alkenes (0.45), Ketones (6.08), Ether (0)	Ethanol (9.96) Acetic acid (7.64)	83.32
8 MAS	57 (49.02)	8.2	12.8	Alcohol (16.46), Aldehyde (4.15), Acids (49.49), Esters (7.88), Alkanes (2.35), Alkenes (0.27), Ketones (10.56), Ether (0.38)	Ethanol (11.07) Acetic acid (11.05)	87.54
9 MAS	51 (45.57)	7.5	11.9	Alcohol (15.38), Aldehyde (5.58), Acids (52.70), Esters (10.95), Alkanes (6.14), Alkenes (0.19), Ketones (2.01), Ether (1.92)	Ethanol (12.83) Acetic acid (11.82)	91.87
10 MAS	42 (40.39)	7.1	10.2	Alcohol (12.80), Aldehyde (9.43), Acids (48.14), Esters (11.15), Alkanes (10.88), Alkenes (0.09), Ketones (1.84), Ether (2.39)	Ethanol (12.06) Acetic acid (9.83)	92.72
Mean	70 (56.79)	8.94	13.3			
SEd	1.60	0.20	0.22			
CD (P=0.05)	3.34	0.42	0.46			

MAS: Months after storage (Figures in parenthesis indicate arcsine values)

lease strength reached 92.72 per cent and the individual VOCs released in higher strength are Alcohol (12.80 %), Aldehyde (9.43 %), Acids (48.14 %), Esters (11.15 %), Alkanes (10.88 %), Alkenes (0.09 %), Ketones (1.84 %) and Ether (2.39 %). Likewise, the root length decreased from 10.1 cm in first month to 7.1 cm at 10th month of storage. Similarly, a significant reduction in shoot length from 15.4 cm at first month to 10.2 cm at 10th month was also recorded. The dry matter production was decreased from 3.125 g 10 seedling^{-1} in first month to 1.936 g 10 seedling^{-1} at 10th month and the vigour index was also significantly reduced from 2321 in first month to 727 in 10th month of ageing (Table 2, 3).

The deterioration in physiological parameters might be due to the toxic volatiles emitted from lipid oxidation of lipid bi-layer membrane and fermentation, which decline the mitochondrial activity resulting in reduced ger-

Table 3. Effect of VOCs emission levels on dry matter production and vigour index of groundnut seeds during storage

Storage period (Months)	Dry matter production (g 10 seed-lings^{-1})	Vigour index	VOCs strength (%)		
			Strength of individual VOC group	Dominant individual VOC	Total VOCs strength
1 MAS	3.125	2321	Alcohol (3.26), Aldehyde (4.12), Acids (0.82), Esters (0.57), Alkanes (0.22), Alkenes (0.28), Ketones (0), Ether (0)	Acetaldehyde (3.46) Methanol (1.72)	9.27
2 MAS	2.863	2226	Alcohol (10.03), Aldehyde (7.12), Acids (5.05), Esters (3.37), Alkanes (1.16), Alkenes (0.93), Ketones (0), Ether (0)	Methanol (6.83) Acetaldehyde (4.74)	27.66
3 MAS	2.902	2000	Alcohol (17.05), Aldehyde (7.90), Acids (12.12), Esters (5.64), Alkanes (1.20), Alkenes (1.85), Ketones (0), Ether (0)	Acetic acid (5.27) 1-Hexanol (4.18) Acetaldehyde (3.06)	45.76
4 MAS	2.836	1832	Alcohol (14.11), Aldehyde (7.03), Acids (16.59), Esters (10.83), Alkanes (1.98), Alkenes (2.48), Ketones (0), Ether (0)	Acetic acid (7.64) 1-Butanol (5.11) Acetaldehyde (1.64)	53.02
5 MAS	2.848	1725	Alcohol (12.10), Aldehyde (8.98), Acids (25.13), Esters (13.96), Alkanes (5.34), Alkenes (1.78), Ketones (0), Ether (0)	Acetic acid (7.64) Hexanal (6.95)	67.29
6 MAS	2.492	1633	Alcohol (6.77), Aldehyde (5.17), Acids (34.83), Esters (17.40), Alkanes (8.46), Alkenes (2.66), Ketones (0.93), Ether (0)	Acetic acid (8.94) Octadecanoic acid (6.08)	76.22
7 MAS	2.172	1357	Alcohol (13.21), Aldehyde (4.08), Acids (40.35), Esters (12.51), Alkanes (6.64), Alkenes (0.45), Ketones (6.08), Ether (0)	Ethanol (9.96) Acetic acid (7.64)	83.32
8 MAS	2.191	1197	Alcohol (16.46), Aldehyde (4.15), Acids (49.49), Esters (7.88), Alkanes (2.35), Alkenes (0.27), Ketones (10.56), Ether (0.38)	Ethanol (11.07) Acetic acid (11.05)	87.54
9 MAS	2.063	989	Alcohol (15.38), Aldehyde (5.58), Acids (52.70), Esters (10.95), Alkanes (6.14), Alkenes (0.19), Ketones (2.01), Ether (1.92)	Ethanol (12.83) Acetic acid (11.82)	91.87
10 MAS	1.936	727	Alcohol (12.80), Aldehyde (9.43), Acids (48.14), Esters (11.15), Alkanes (10.88), Alkenes (0.09), Ketones (1.84), Ether (2.39)	Ethanol (12.06) Acetic acid (9.83)	92.72
Mean	2.543	1601			
SEd	0.0477	41.65			
CD (P=0.05)	0.0995	86.88			

MAS: Months after storage

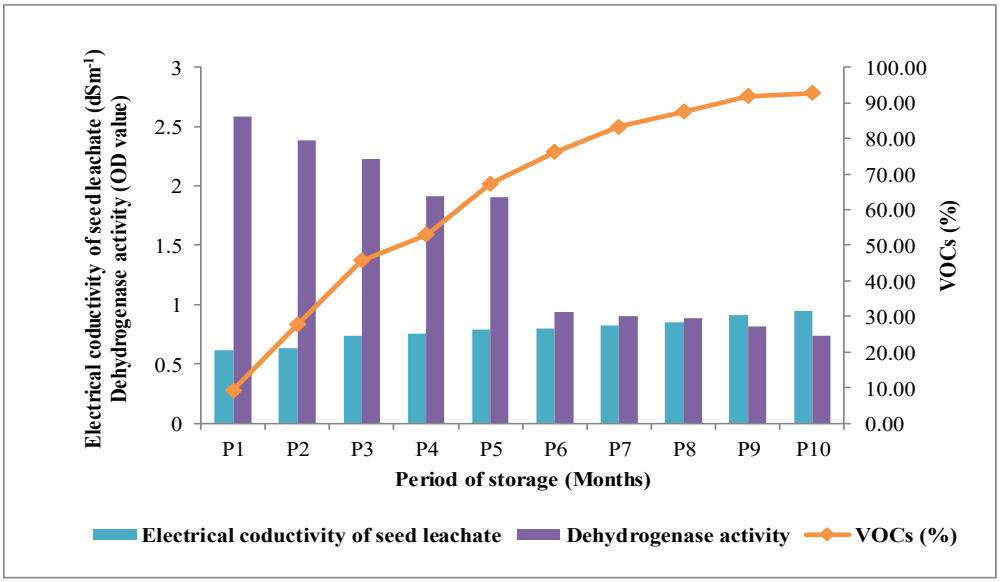

Fig.1. *VOCs emission levels on electrical conductivity of seed leachate and dehydrogenase activity of groundnut seeds during storage*

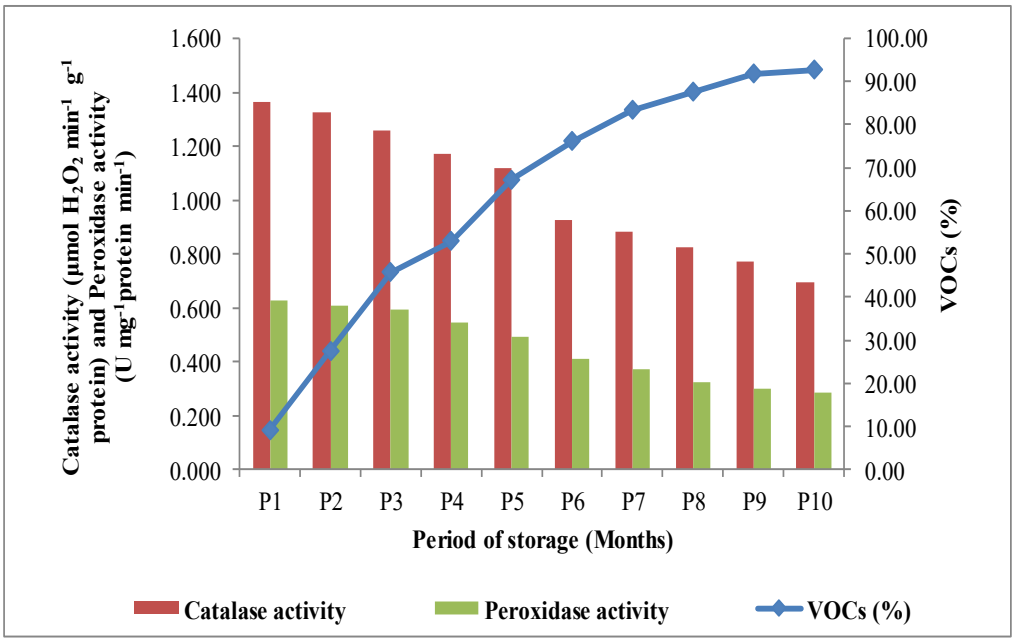

Fig. 2. *VOCs emission levels on catalase and peroxidase enzyme activity of groundnut seeds during storage*

mination and seedling vigour. Balesevic *et al.* (2005) stated that the production of toxic volatiles like aldehydes, alcohols and ketones reduced the germination, seedling length, dry matter production and seedling vigour in sunflower. Volatile organic compounds, especially the aldehydes emanated from stored dry seeds, have reduced the germination and seedling vigour of pea and soybean seeds (Harman *et al.*, 1982). According to Woodstock and Taylorson (1981), soybean seeds' germination and seedling vigour were reduced due to the deleterious effect of toxic volatiles (acetaldehyde and ethanol) evolved by seeds during ageing and these compounds knockout the mitochondria and reduces the seed quality. 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 regardless of storage environment and all the volatile organic compounds were negatively correlated with seed germination and seedling vigour tested over a period of storage.

VOCs emission and biochemical seed quality

The biochemical seed quality attributes *viz.,* electrical conductivity of seed leachate, lipid peroxidation and lipoxygenase activity increased and dehydrogenase, catalase and peroxidase activities decreased due to an increase in the release of volatile organic compounds during storage.

The electrical conductivity of seed leachate increased

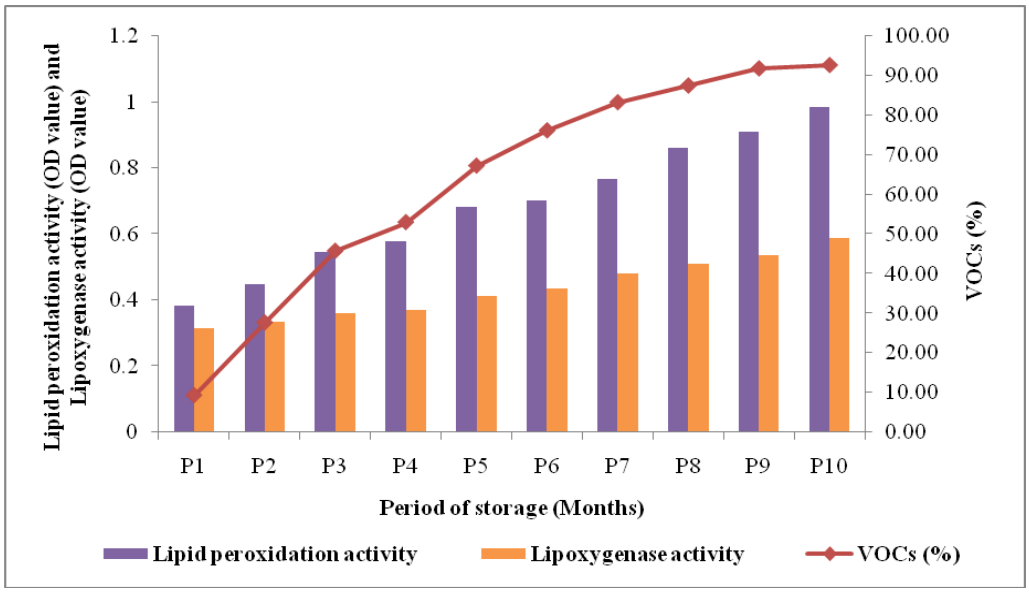

Fig. 3. *VOCs emission levels on lipid peroxidation and lipoxygenase activity of groundnut seeds during storage*

from 0.611 dSm^{-1} at first month to 0.947 dSm^{-1} at 10^{th} month of ageing. The lipid peroxidation was increased from 0.384 OD value in first month to 0.984 OD value in 10^{th} month of ageing. Similarly, the lipoxygenase activity increased from 0.315 OD value in the first month of storage to 0.588 OD value in the 10^{th} month of storage. In contrast, the dehydrogenase activity was decreased from 2.588 OD value in first month to 0.739 OD value in 10^{th} month of ageing and the catalase activity was reduced from 1.362 µmol H_2O_2 min^{-1} g^{-1} protein in first month to 0.695 µmol H_2O_2 min^{-1} g^{-1} protein at 10^{th} month of storage. Similarly, the peroxidase activity lso reduced from 0.626 U mg^{-1} protein min^{-1} in first month to 0.284 U mg^{-1}protein min^{-1} at 10^{th} month of storage. At the same time, the strength of total volatiles was 9.27 per cent in first month and it was increased to 92.72 per cent in 10^{th} month of storage. The individual strength of volatile groups *viz.,* Alcohols (3.26%), Aldehydes (4.12%), Acids (0.82%), Esters (0.57%), Alkanes (0.22%), Alkenes (0.28%) were higher at first month of storage which further increased to 12.80 (Alcohols), 9.43 (Aldehydes) 48.14 (Acids), 11.15 (Esters), 10.88 (Alkanes), 0.09 (Alkenes), 1.84 (Ketones) and 2.39 per cent (Ether) at 10^{th} month of storage (Figs. 1, 2 and 3). The loss of biochemical seed quality characteristics might be due to the increased level of VOCs, which might have been produced by the cell membrane's catabolic event and also due to the toxic effect of free radicals. Volatiles emitted from stored seeds were found to reduce the biochemical quality parameters in pine (Tammela *et al.,* 2003) and cabbage (Bicanic *et al.,* 2003). According to the report of Min *et al.* (2017), Oenel *et al.* (2017) and Bhattacharjee (2019), the release of aldehydes, ketones, alkanes, carboxylic acids and other polymerization products produced due to lipid peroxidation process were 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 seed quality.

Conclusion

The present study clearly demonstrated the influence of volatile organic compounds emission levels on the physiological and biochemical properties of seeds during storage. There was a significant decrease in physiological and biochemical quality attributes due to an increase in the strength of volatiles released during ageing. When the release of total volatile strength reached more than 50 per cent, there was a significant reduction in physiological attributes such as germination, root and shoot length, dry matter production and vigour index. With respect to biochemical properties, the significant increase in electrical conductivity of seed leachate, lipid peroxidation and lipoxygenase activity, and a decrease in dehydrogenase activity, catalase activity and peroxidase activity was observed. However, the highest reduction in all these properties were recorded when the total volatile strength reached 92.72 per cent. Finally, the study concluded that eight different categories of volatiles were profiled in groundnut seeds during ageing. Among all the volatile organic compounds, ethanol, 1-butanol, acetaldehyde, hexanal and acetic acid were the most closely associated volatiles with seed deterioration. It indicated that these components could be considered the signature components for assessing the seed quality during storage.

Conflict of interest

The authors declare that they have no conflict of interest.

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