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The Impact of Volatile Organic Compounds on Assessing Soybean Seed Quality during Storage

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ABSTRACT

Background: Maintaining soybean seed quality during storage is crucial for sustaining profitable seed production. The weak seed coat and abundant fatty acid content in soybean causes seeds to lose viability more quickly and are very sensitive to the storage environment. The kinds of chemical reactions that take place during storage have been linked to emissions of volatile organic compounds (VOCs), suggesting that these reactions might be used as indicators of the seed quality. Hence, the present study was conducted to profile the VOCs emitted during storage and their relationship to the physiological and biochemical quality of soybean seeds.

Methods: Soybean seeds were subjected to VOC profiling using gas chromatography-Mass Spectrometry (GC-MS/MS) and physiological and biochemical quality assessments at monthly intervals over an eight-month storage period.

Result: GC-MS/MS identified sixty-eight volatile compounds in the soybean seeds stored over an eight-month period. The aldehyde (37.84%) contributed to the 1/3 of total emission of different groups of volatiles emitted from stored soybean seeds. Concurrently, seed germination, seedling dry weight and vigour declined, indicating deterioration in physiological quality. Biochemical analysis showed increased seed leachate electrical conductivity, lipoxygenase activity and lipid peroxidation, alongside decreased catalase, peroxidase and dehydrogenase enzyme activity, suggesting increased oxidative stress and lipid peroxidation in soybean seeds. The strong link between increased VOC emissions and the decline in seed quality parameters underscores the critical role of VOC in assessing the loss of seed viability of soybean during storage.

Key words: GC-MS/MS, Seed deterioration, Soybean, VOCs.

INTRODUCTION

Seeds are one of the most notable desiccation-tolerant organisms capable of surviving prolonged periods without water. As metabolism slows in desiccated states, seeds with water content below 15% exhibit minimal measurable metabolic responses (Walters *et al*., 2005). Despite their limited chemical activity dry organisms clearly undergo certain reactions resulting seed ageing. This aging process driven by both internal and external factors results in physiological, cytological and biochemical changes that signal a decline in seed viability and quality (Nadarajan *et al*., 2023).

For soybean seeds (*Glycine max* L.), maintaining high vigour and viability during storage is particularly challenging due to their relatively fragile seed coat and high fatty acid content, which make them highly susceptible to oxidation and environmental factors (Shaban, 2013). Lipid auto-oxidation in oilseeds during storage damages essential molecules including lipoproteins and cell membranes accelerates seed deterioration (Tatić *et al*., 2012). Environmental conditions notably temperature and moisture also play significant roles in lipid peroxidation and consequently in seed aging (Murthy *et al*., 2002).

Recent research has found a link between the types of chemical reactions that occur during storage and VOCs emissions, implying that they may serve as markers of seed quality (Mira *et al*., 2010; Chinnasamy *et al*., 2022). GC-MS technology has become an effective tool for analyzing VOC profiles offering detailed information on chemical reactions associated with seed deterioration (Aldini *et al*., 2011). Some VOCs may even exhibit phytotoxic effects potentially accelerating seed aging (Akimoto *et al*., 2004). This study was therefore designed to characterize VOCs released from soybean seeds during storage and evaluate their impact on physiological and biochemical seed quality.

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MATERIALS AND METHODS

Seed material

Genetically pure, freshly harvested seeds of the soybean variety JS 335 were stored in multiple glass vials each with a septum and a screw top to enable collecting gas samples from the bottles. Each bottle was filled with 300 grams of seeds and stored at room temperature for eight months. Every month, three vials were taken, subjected to VOC profiling and then seeds in each vial were taken for germination test, vigour and biochemical analysis at monthly interval. The storage studies and VOC profiling was done at Department of Seed Science and Technology, TNAU, Coimbatore and NABL-accredited Analytical Technical Laboratory in Coimbatore, respectively during the year 2022-2023.

VOC collection and GC-MS/MS analysis

Every month, three separate vials were selected as replicates, allowing for fresh samples each time. Solid-phase micro-extraction fiber was then inserted into each of these vials to collect VOCs individually for 45 minutes. Next, a direct injection of SPME fiber with air sample was made into a GC-MS/MS (Agilent 7000D chromatograph system, coupled to an III quadruple mass spectrometer) in a split less mode. Helium was used as a carrier gas at a flow rate of 1.0 mL/min at a pressure of 15 Psi. A 5% -phenyl-methyl poly siloxane column (HP-5ms ultra inert column) was used to extract the volatile chemicals from the air sample. To ensure optimal separation of different chemicals, the injector and detector temperatures were consistently maintained at 250°C and 260°C, respectively throughout 45 min (Mathure *et al*., 2011).

Physiological seed quality characteristics

Germination tests were conducted with six replicates, each consisting of 100 seeds on germination tray. After that, the seeds were incubated in a germination chamber at 25°C ± 3°C with 1000 lux light for 7 d. The root and shoot length of the normal seedlings were measured and expressed in cm. Based on the number of germinated seeds, germination % and the vigour index were calculated as follow (ISTA, 2015; Abdul-Baki and Anderson, 1973):

$$G\% = \Sigma(Gt/Gi)$$

Where,
Gt= Number of germinated seeds on day 8 .
Gi= Total number of seeds sown.

$$VI = G\% \times SL$$

Where,
SL= Seedling length.

Biochemical seed quality characteristics

The leachate conductivity of the seeds was measured with a digital conductivity meter (Presley, 1958) and expressed as dSm^{-1}. Lipoxygenase activity and lipid peroxidation was assayed by the method of Hildebrand *et al*. (1993) and Bernheim *et al*. (1948), respectively. Dehydrogenase activity was measured by the method given by Kittock and Law (1968). The catalase and peroxidase enzyme activity calculated based on Aebi (1984) and Malik and Singh (1980), respectively.

Statistical analysis

The volatiles emitted (area %) from soybean seeds during storage were plotted using Origin software version 2024b. Physiological and biochemical quality parameters analysis were conducted using completely randomized design with three replication. The mean values were evaluated using the least significant difference (LSD) test at a significance level of p = 0.05 in AGRESS software. Before analysis, percentage values were converted into arcsine values.

RESULTS AND DISCUSSION

Profiling of VOCs emitted from soybean seeds during storage

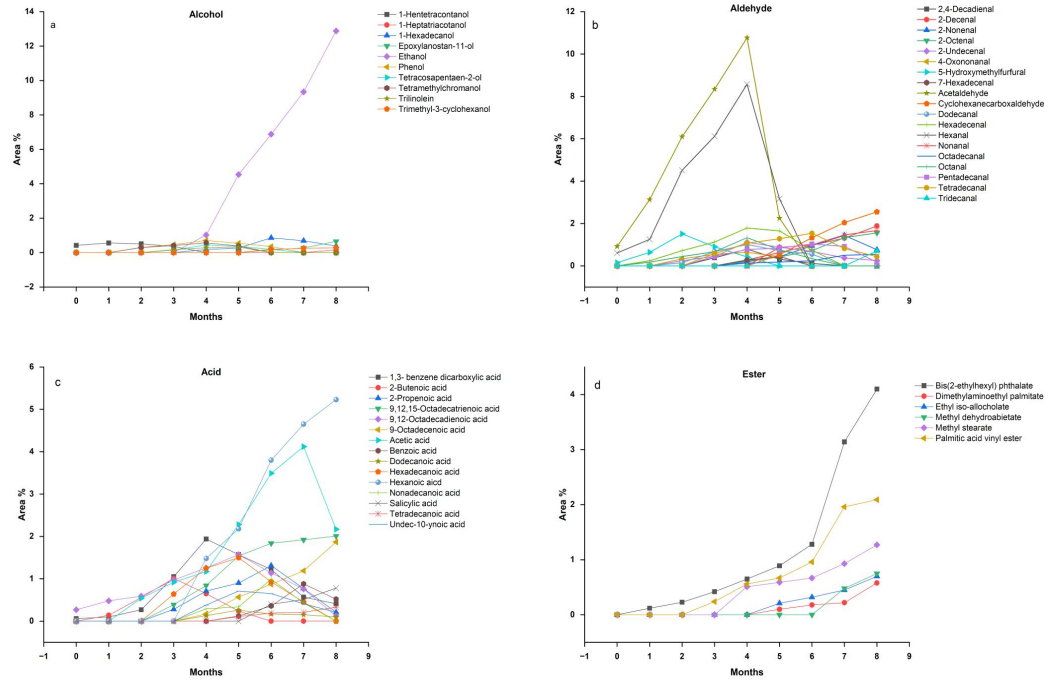
GC-MS/MS profiling of VOCs in soybean seeds which was stored in 25±2°C, 40% relative humidity throughout the storage period results in eight groups. 68 volatile compounds encompass 19 aldehydes, 15 acids, 4 alkanes, 10 alcohols, 8 ketones, 6 esters, 4 alkenes and 2 amides were found. Ethanol, 1-hexadecanol and phenol were found to be the most prevalent alcohol and over the course of storage, their proportions gradually expanded. After four months, the starting strength of ethanol (1.02%) increased dramatically to 12.88%, whereas 1-hexadecanol peaked at 0.86% after six months and then began to decline. After four months, phenol showed a rise to 0.70% and then a significant decline (Fig 1a). Seeds release a wide range of volatile compounds during storage and some of these compounds may affect or result from the aging process (Lee *et al*., 2015). As seeds get older, the amount of these compounds released increases with more types and higher amounts being emitted when stored at 25°C compared to 10°C (Zhang *et al*., 1993). The volatiles ethanol, 1-hexadecanol, phenol and other alcoholic compounds emission might be due to anaerobic metabolism, lipid peroxidation and glycolysis pathway. Ethanol is released during storage in canola seeds due to anaerobic metabolism (Buckley and Buckley, 2009). Glycolytic processes caused dry seeds of lettuce, carrot and soybean to release ethanol and other alcoholic compounds when they were stored (Zhang *et al*., 1993). During storage, dry sunflower seeds released 1-hexanol and ethanol due to glycolytic processes and lipid peroxidation (Meenakshi, 2020). These chemicals are also released when the integrity of the mitochondrial membrane is denatured (Colville *et al*., 2012).

During the initial five months of storage, acetaldehyde and hexanal were continually released. Acetaldehyde exhibited the highest area percentage among the aldehydes, with its contribution increasing substantially from 0.92% initially to 10.77% before declining at the fourth month. Hexanal followed a similar trend, increasing from 0.61% to 8.57% before decreasing. The most prevalent

aldehydes were acetaldehyde and hexanal followed by cyclohexane carboxaldehyde, hexadecenal and tetradecanal (Fig 1b). Lipid peroxidation is responsible for emission of majority of aldehydes (Grotto *et al*., 2009). The oxidation of oleic acid, linoleic acid and linolenic acid results in the origination of aliphatic aldehydes (Solina *et al*., 2007). Acetaldehyde is released from stored seeds due to the breakdown of linoleic poly unsaturated fatty acids (PUFA) caused by enzymatic oxidation or autooxidation and mitochondrial degradation (Colville *et al*., 2012). Acetaldehyde emission in dry seeds may be caused by lipid membrane oxidation during storage (Zhang *et al*., 1993). Degradation of linoleic acid causes emission of hexanal, 2, 4-nonadienaland nonanalin stored seeds (Colville *et al*., 2012). Hexanal is associated with unsaturated fatty acid oxidation resulting from autoxidation, photo-oxidation, thermal oxidation or isozyme-assisted oxidation (Gardner, 1996). The oxidative deamination-decarboxylation of amino acids such as leucine, valine and isoleucine *via* Strecker degradation is thought to be the primary mechanism for the generation of branched aldehyde (Ardo, 2006). Lipid peroxidation, which occurs through the action of the enzyme lipoxygenase plays a key role in stored seeds. This process is a major contributor to the release of volatile aldehydes (Frankel *et al*., 1981). During seed deterioration, hexanal builds up which serves as a marker for lipid peroxidation (Frankel, 1983).

The volatile 1,3-Benzenedicarboxylic acid and 9,12-octadecadienoic acid were continuously released over the storage period. The two acids that were most prevalent and their concentrations increases with time were acetic acid and hexanoic acid. In the beginning, acetic acid recorded 0.54% in the second month and hexanoic acid 1.48% in the fifth. But as storage time increases, their proportions exceed noticeably, peaking at 4.12% and 5.23% in the seventh and eighth months, respectively (Fig 1c). The release of esters showed a distinct pattern over time in stored soybean seeds. Bis (2-ethylhexyl) phthalate and palmitic acid vinyl ester were the most abundant, starting at 0.12% and 0.24% at 1st month and 3th months, respectively, peaking at 4.10% and 2.09% by the 8th month after storage (Fig 1d).

Among alkanes, pentaoxacyclopentadecane were the most abundant, starting at 0.0% at initial month peaking at 2.87% by the 5th month after storage and then gradually decreased (Fig 2a). Octylfuran and furan were the predominant volatiles among alkenes, recording concentrations of 0.72% and 0.44% at the 5th month, respectively and continued to increase to 1.47% and 1.26% by the 8th month (Fig 2b). The dehydration of carbohydrates



a: Alcohol - Ethanol showed a significant increase among alcohols, peaking at approximately 12% by the eighth month; b: Aldehyde - Aldehydes exhibited a sharp peak in emissions, particularly hexanal and acetaldehyde, around the fourth month before stabilizing; c: Acid - varied widely with hexanoic acid rising notably after the sixth month; d: Ester - Bis (2-ethylhexyl) phatlate showed an increase in emissions throughout the storage period.

Fig 1: Profile of VOCs emitted from soybean seeds during storage.

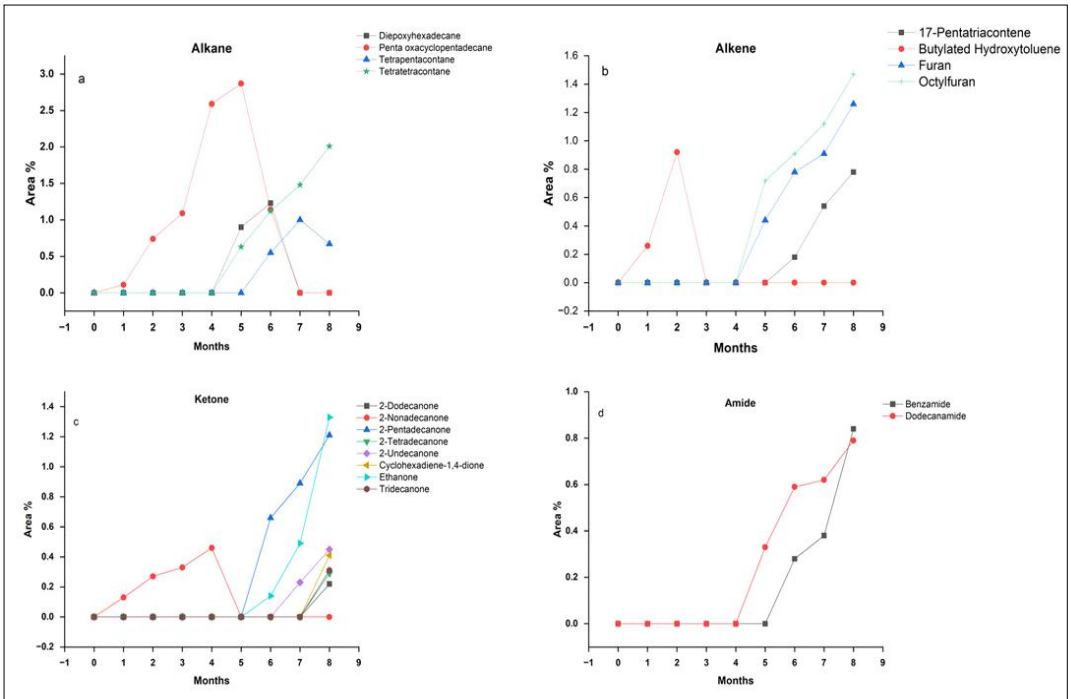
through the maillard reaction leads to the formation of furan in seeds (Monforte *et al*., 2015). This compound has been utilized as a marker for distinguishing aging in long-duration rice, aiding in its identification and classification (Wang *et al*., 2020).

2-pentadecanone and ethanone dominated, with concentrations of 0.66% and 0.14% at the 6th month, respectively, gradually increasing to 1.21% and 1.33% by the 8th month in ketones. Meanwhile, 2-dodecanone exhibited the lowest volatile strength, recording 0.22% (Fig 2c). Only amides, such as decanamide and benzamide were noted in the fifth (0.33%) and sixth month (0.28%), respectively and lasted until the eighth month, at 0.79% and 0.84%. which was dominated by dodecanamide (Fig 2d).

Total VOCs strength emitted from soybean seeds during storage were 15.07% alcohol, 37.84% aldehyde, 26.03% acid, 8.14% ester, 5.84% alkane, 3.32% alkene, 2.52% ketone and 1.23% amide (Fig 3). The Strecker degradation of Maillard reaction, non-enzymatic degradation of macromolecules, glycolysis and lipid bi-layer cell membrane oxidation produce acid, alkene, alkane, ketones, esters and ethers (Mira *et al*., 2016). Linoleic auto-oxidation in seeds results in the emission of esters (methyl formate, *etc*.), alcohols (butanol, propanol, pentanol, etc.), ketones (2-heptanone, *etc*.), aldehydes (propanal, pentanal, hexanal, butanal, *etc*.) and three to six carbon alkanes (propane, pentane, butane, *etc*.) (Knutson *et al*., 2000). Lipid peroxidation in stored pea seeds produces alcohols, ketones, esters and alkanes (Bhattacharjee, 2019). Volatile aldehydes emission from heated soybean might be due to the thermal breakdown of lipid hydroperoxides (Hailstones and Smith, 1989). Weathering-related deterioration of soybean seeds also results in the emission of volatile aldehydes (Tyagi, 1992).

Understanding the relationship: VOC emission and physiological seed quality

The current study found a clear association between the amounts of volatile organic compounds (VOC) emitted and seed germination. Germination started at 76% but dropped to 59% after the eighth month of storage. During the first three months, there was small decline in germination, which coincided with a VOC emission strength of 29.12%. However, there was a substantial decrease in germination during the fourth month, when VOC emission strength exceeded by 47.60%. The sharp decline in germination during the fourth month coincided with a peak in VOC emissions, particularly from aldehydes. By the end of the 8th month, germination had reached 59%, resulting in a VOC emission strength of 60.36%. Root length reduced



a: Alkane - There is a notable increase in compounds like Diepoxyhexadecane and Tetratetracontane, particularly peaking around 4 to 6 months; b: Alkene - The levels of Octylfuran increase steadily, with 17-Pentatriacontene showing a significant peak at 2 months; c: Ketone - Compounds like ethanone and 2-Tetradecanone rise gradually from 5 months; d: Amide - Both Benzamide and Dodecanamide increase steadily over the months.

Fig 2: Profile of VOCs emitted from soybean seeds during storage.

from 17.6 cm to 12.5 cm after eight months of storage, while shoot length decreased from 15.7 cm to 11.8 cm with in the same time period (Table 1).

Seedling dry weight decreased from 1.189 to 0.755 g per 10 seedlings and the vigour index considerably reduced from 2531 to 1434 during the first and eighth month of storage (Table 1). In the first month of storage, total volatiles accounted for only 2.43%, but over the course of eight months, this figure surged to 60.36%. This increase coincided with a significant increase in volatile groupings' individual strengths. In the initial month, alcohol, aldehyde and acids had values of 0.42%, 1.68% and 0.33%, respectively, but by the eighth month, those numbers had risen to 14.35%, 10.52% and 13.96%, respectively. Esters, alkanes, alkenes, ketones and amide grew from 0.0% in the initial month to 9.49%, 2.68%, 3.51%, 4.22% and 1.63% by 8th month respectively (Fig 4). This substantial rise demonstrates the dynamic nature of volatile compound generated from soybean seeds over the storage period.

Volatile compounds produced through fermentation and lipid oxidation of the lipid bilayer membrane are likely responsible for the decline in physiological parameters, such as reduced mitochondrial activity, which leads to decreased seed germination and seedling vigour. Studies have reported a negative correlation between seed quality and volatile emissions during storage (Mira *et al*., 2010; Colville *et al*., 2012). Aldehydes, alcohols and ketones have been shown to adversely impact the germination and vigour of sunflower seeds (Balesevic *et al*., 2005). Similarly, volatile aldehydes released from stored dry seeds have been associated with reduced germination and vigour in pea and soybean seeds (Harman *et al*., 1982). A decline in the viability of *Pyrus communis* and *Sorbus aucuparia* seeds has been linked to fermentation-related volatiles such as ethanol, acetaldehyde, methyl acetate, acetic acid and ethyl acetate (Michalak *et al*., 2021). Additionally, soybean seed germination has been negatively affected by ethanol and acetaldehyde emissions during storage

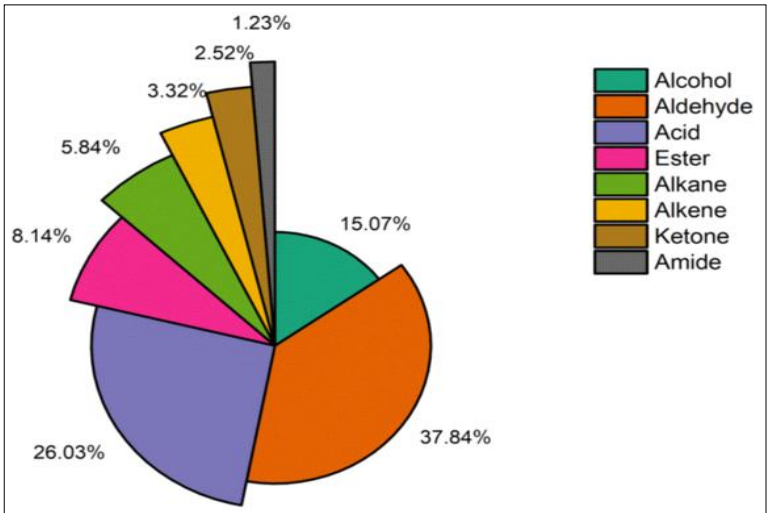


Fig 3: Total VOCs strength emitted from stored soybean seeds.

Table 1: VOCs emission levels of soybean seeds in relation to physiological seed characteristics.

Months of storage	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling dry weight (g/ 10 seedlings)	Vigour index	Total VOC (%)
0	76±1.316	17.6±0.304	15.7±0.271	1.189±0.020	2531±44	2.43
1	76±1.160	16.7±0.255	15.1±0.230	1.145±0.075	2417±37	7.33
2	74±1.540	15.9±0.331	14.6±0.303	1.065±0.022	2257±47	19.22
3	72±1.498	15.1±0.314	14.1±0.293	0.944±0.019	2102±44	29.12
4	70±1.852	14.7±0.388	13.7±0.365	0.901±0.023	1988±53	47.60
5	67±1.160	14.2±0.246	13.1±0.226	0.863±0.014	1829±32	44.42
6	64±1.108	13.8±0.239	12.6±0.218	0.821±0.014	1690±29	47.18
7	62±0.715	13.1±0.151	12.0±0.138	0.789±0.009	1556±18	52.60
8	59±1.021	12.5±0.216	11.8±0.204	0.755±0.013	1434±25	60.36

Values were expressed as mean ± standard error.

Table 2: VOCs emission levels of soybean seeds in relation to biochemical seed characteristics.

Months of storage	Electrical conductivity of seed leachate (dSm^{-1})	Dehydrogenase activity (OD value)	Catalase activity ($\mu mol\ H_2O_2\ min^{-1}\ g^{-1}$ protein)	Peroxidase activity (U mg^{-1} protein min^{-1})	Lipid peroxidation (µmol MDA/ g of FW)	Lipoxygena activity (OD value)
0	0.499±0.009	0.766±0.013	1.329±0.023	0.970±0.017	0.529±0.009	0.403±0.007
1	0.513±0.008	0.741±0.011	1.311±0.020	0.905±0.014	0.580±0.008	0.420±0.006
2	0.526±0.011	0.698±0.015	1.292±0.026	0.844±0.018	0.657±0.014	0.437±0.010
3	0.549±0.011	0.656±0.014	1.276±0.027	0.785±0.016	0.713±0.015	0.470±0.013
4	0.571±0.015	0.626±0.017	1.255±0.033	0.719±0.019	0.772±0.020	0.516±0.010
5	0.600±0.010	0.603±0.010	1.231±0.021	0.667±0.012	0.832±0.014	0.530±0.010
6	0.626±0.011	0.582±0.010	1.211±0.021	0.613±0.011	0.883±0.015	0.587±0.007
7	0.657±0.008	0.554±0.006	1.184±0.013	0.561±0.015	0.925±0.011	0.600±0.007
8	0.690±0.012	0.481±0.008	1.156±0.020	0.502±0.014	0.969±0.017	0.680±0.012

Values were expressed as mean ± standard error.

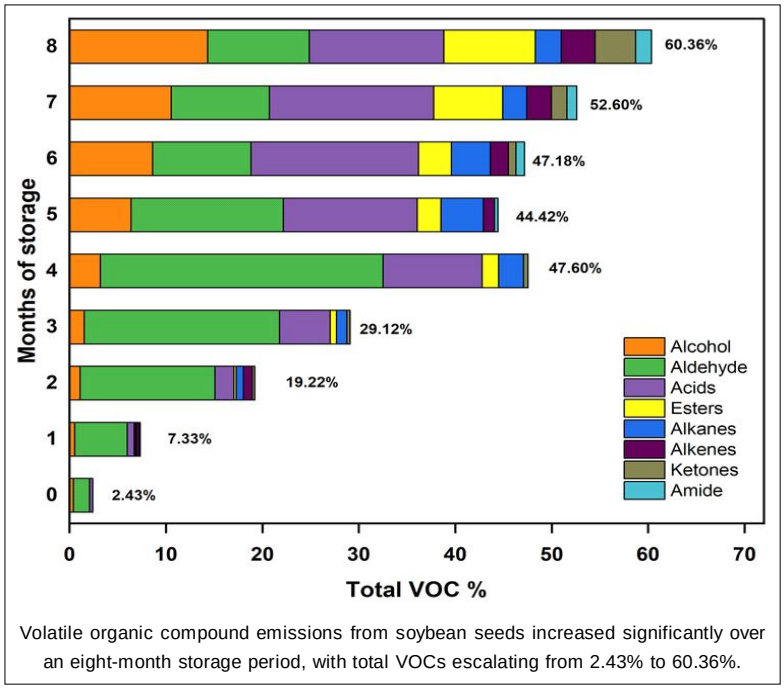


Fig 4: Individual VOCs emitted from stored soybean seeds.

(Zhang *et al*., 1994). Studies indicate that the seed quality of a variety of crop species is decreased by volatile organic compounds (VOCs) especially ethanol and methanol (Rutzke *et al*., 2008). Seed viability is greatly reduced by volatile aldehydes particularly malondialdehyde generated as a result of lipid peroxidation as observed in *Ammopiptanthus mongolica* and hazelnuts (Pastorelli *et al*., 2006; Yi *et al*., 2010).

Understanding the relationship: VOC emission and biochemical seed quality

The increased VOCs emissions positively correlated with increased electrical conductivity of seed leachate, lipoxygenase activity and lipid peroxidation and negatively correlated with catalase, peroxidase and dehydrogenase enzyme activity (Table 2).The biochemical parameters of seeds were closely associated with elevated levels of VOCs, likely due to the damaging effects of free radicals and the catabolic processes affecting the cell membrane. Volatiles emitted from stored seeds have been shown to reduce biochemical quality attributes in cabbage Bicanic *et al*. (2003) and pine Tammela *et al*. (2003). Volatile compounds such as aldehydes, alkanes, carboxylic acids, ketones and other polymerization products can readily diffuse through and penetrate biological membranes, affecting both cellular and extracellular matrix components which leads to a decline in the biochemical quality of seeds (Bhattacharjee, 2019).

CONCLUSION

VOC profiling can serve as a valuable tool for assessing soybean seed quality during storage. A significant increase

in VOC emissions, particularly aldehydes, correlates with the decline in seed germination, vigour and biochemical quality including increased oxidative stress and lipid peroxidation. These findings suggest that VOC profiling could be used as an indicator of seed viability and deterioration offering a novel approach for real-time monitoring soybean seed quality during storage complementing traditional methods.

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Conflict of interest

The authors of this research state that they have no conflicting interests with regard to the publication of this work.

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