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RESEARCH ARTICLE

Unveils the metabolic changes in groundnut CO 7 kernels stored under modified atmospheric conditions

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Abstract

Storing groundnut kernels is more challenging than storing pods due to increased oxidative damage. Pod storage requires more space and is not cost-effective for long-term storage. Therefore, an attempt was made to store kernels under modified conditions for six months to address these storage issues. An experiment was performed to evaluate the effects of different modified atmospheric storage conditions on the groundnut cultivar CO 7, in both shelled and unshelled forms. Changes in seed quality parameters were analyzed during storage at intervals of 30 days and up to 180 days. The kernels stored under nitrogen and vacuum conditions maintained their seed quality traits, including physical, physiological and biochemical characteristics. Metabolic profiling (GC-MS analysis was performed at the beginning of storage (fresh seeds) and after 180 days (aged seeds). Sixteen metabolic compounds were identified as responsible for seed viability and deterioration, with sucrose being the predominant compound. The sucrose area percentage in fresh seeds was 52.52%, which decreased during aging. At the end of the storage period, the highest sucrose area percentages were found in kernels stored under nitrogen (28.9%) and vacuum (26.95%) conditions. This study concludes that groundnut kernels stored under nitrogen conditions have good storage potential and excel at maintaining seed longevity.

Keywords

Groundnut; GC-MS; metabolic profiling; enzyme activity; seed quality; modified atmospheric storage

Introduction

Groundnut (*Arachis hypogaea* L.) kernels are rich in oil (48-50%) and protein (25-28%) and behave like recalcitrant seeds (1). They are intolerant to low temperatures and desiccation, challenging seed storage (2). After deshelling, maintaining kernel quality becomes even more difficult due to their sensitivity to biotic and abiotic environmental conditions (3). Seed deterioration is a major factor negatively impacting agriculture production (4). This process involves various metabolic, cellular and chemical changes, such as membrane disruption, lipid peroxidation, DNA damage and impairment of RNA, which

collectively lead to detrimental effects on the seed (5). The rate of deterioration primarily depends on the storage conditions and the initial quality of the seeds (6). Overall, seed deterioration is a complex process influenced by various biotic and abiotic factors during storage.

Recent studies have explored modified atmospheric storage (MAS) as an effective method for preserving the quality of oilseeds and reducing storage losses. MAS is achieved by introducing nitrogen (N_2) or carbon dioxide (CO_2) to lower the oxygen (O_2) concentration in the storage container (7). This approach has shown promising results in maintaining seed viability and extending storage potential. For instance, groundnut kernels stored in a mixture of 60% N_2 and 40% CO_2 exhibited better germination and vigor for up to 10 months compared to conventional storage (3). Furthermore, groundnut seeds stored under 100% N_2 at -5°C retained germination rates above 70% after 8 months, outperforming seeds stored under ambient conditions (8). Additionally, the N_2 storage of groundnut kernels demonstrated comparable enzyme activity and pest resistance compared to pod storage (9).

Research on other oilseeds, such as soybeans, has shown that hermetically sealed storage with low O_2 and high CO_2 levels can better maintain seed quality than ambient conditions (10). Similarly, hermetic packaging with CO_2 preserves the physiological quality of soybeans for up to 180 days compared to permeable packaging (11). These findings suggest that while MAS can serve as an effective alternative to conventional storage methods for oilseeds, its efficacy may vary depending on the specific crops. Additionally, MAS in seed storage offers a superior alternative to chemicals and insecticides, which can leave carcinogenic residues on treated produces (12). Therefore, this current investigation aims to study the impact of MAS conditions on seed longevity and the metabolic changes in stored kernels.

Materials and Methods

The research was conducted from 2023 to 2024 at the Department of Seed Science and Technology, Seed Centre, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India (Latitude: 11° 07' 3.36" N; Longitude: 76° 59' 39.91" E). The groundnut cultivar CO 7 was obtained from the Department of Oilseeds at TNAU, Coimbatore, for this study. The pods and kernels were dried to a moisture content of 6.1% and subjected to the following storage conditions before being kept under ambient for 180 days, as described by Punithavathi et al. (9) in groundnut and Divya et al. (13) in horse gram.

Storage conditions

M_1 - Pods stored in gunny bags

M_2 - Kernels stored in gunny bags

M_3 - Kernels under nitrogen storage

M_4 - Kernels under vacuum storage

Seed quality attributes

The kernel moisture content was estimated using the hot air oven method, as outlined by ISTA (14). The seed germination test was conducted using the sand method per ISTA guidelines (14), with four replicates of 100 seeds each. The seeds were placed in a germination chamber at a temperature of 25±2°C and a relative humidity (RH) of 95±2%. After 10 days (final count), the number of normal seedlings was recorded and the germination percentage was calculated using the formula:

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

The dry matter production and seed vigor were calculated according to the procedures described by Gupta (15) and Abdul-Baki and Anderson (16). The groundnut kernels were soaked in distilled water for 8 hours and the seed leachate's electrical conductivity (EC) was measured using an EC meter (17). The activities of dehydrogenase, catalase and peroxidase were assessed following the protocols provided by Kittock and Law (18), Aebi (19) and Malik and Singh (20), respectively.

Metabolic profiling

The changes in metabolic compounds during the storage period were analytically assessed through Gas Chromatography-Mass Spectrometry (GC-MS) at both the beginning (fresh seeds) and the end of the storage period (180 days).

Extraction process

Kernels were ground into a fine powder and 10 mg of the sample was used for metabolite extraction. The sample was mixed with 1000 µl of methanol and placed in a water bath at 70°C for 15 minutes, followed by centrifugation at 10,000 rpm for 20 minutes. 200 µl aliquots of the supernatant were collected and dried using a speed vacuum concentrator. To the dried sample, 50 µl of a 20 mg/ml methoxylamine solution in pyridine was added and the mixture was incubated in a water bath at 37°C for 2 hours. Subsequently, 80 µl of MSTFA solution was added and the sample was centrifuged at 10,000 rpm for 20 minutes.

GC-MS analysis

A 100 µl sample was injected into the GC-MS system at 280° C in split mode (20:1), using helium as the carrier gas (>99.9% purity) at a flow rate of 1 ml/min. The sample was separated using an HP-5 MS capillary column, following the protocol established by Wen et al. (21). Mass ranges from m/z 50 to 650 were analysed.

Statistical Analysis

The data were statistically analyzed using a factorial completely randomized design (FCRD) through AGRESS software. Prior to the analysis, the percentage values were transformed to arc-sine values. Graphs were created using GraphPad Prism version 5.8.

Results and Discussion

Seed quality attributes

Seeds are hygroscopic, meaning they absorb moisture from their surrounding storage environment. Storing seeds in MAS reduces the moisture absorption rate. Notably, the highest moisture absorption was observed in M_2 (7.1%), while the lowest moisture absorption was found in M_3 (6.4 %). Additionally, a gradual increase in seed moisture was noted as the storage period progressed (Table 1). The physiological attributes, such as germination, dry matter production and vigour index, declined during storage. Kernels stored under nitrogen (M_3) (74%) and vacuum (M_4) (70 %) maintained a germination rate of over 70% for up to 120 days and 90 days respectively. Due to the dormancy of groundnut CO 7, the overall mean germination percentage increased from 75% to 85% at 30 days after storage (Table 1). Furthermore, the physiological parameters of the seed, such as dry matter production and vigor index, followed a similar trend to that of germination potential (Table 2). These findings align with those of the findings of Gayathri et al. (8) in groundnut. Similarly, Doijode (22) in amaranth and Tahir et al. (23) in paddy found that MAS under N_2 conditions had comparable effects on seed viability and longevity. These studies suggest that MAS can effectively maintain seed quality across a number of species.

The storage potential of seeds is linked to physiological, biochemical and anatomical changes that occur within the seed during storage. In this study, kernels stored under nitrogen conditions (M_3) were more effective at maintaining seed physiological quality than other storage methods. The effectiveness is primarily due to the absence of CO_2 and O_2 in the storage environment, which may cause the seed to enter a quiescent or metabolically inactive state. Entering this state helps preserve the quality of reserve food materials and the integrity of the seed cell membranes in an O_2-free environment. This effect was evident in the electrical conductivity (EC) of the seed leachate, with M_3 storage recording a lower EC range (from 0.238 to 0.410 dS m^{-1}) over the storage period compared to other conditions (Fig. 1A).

Furthermore, the vitality of the stored groundnut seeds was indirectly evaluated by quantifying dehydrogenase enzyme activity. Among the different storage conditions, M_3 effectively maintained dehydrogenase activity (ranging from 1.627 to 1.085 OD) (Fig. 1B), as well as the antioxidant-activity related enzyme activities, including catalase (from 1.92 to 1.54 µmol H_2O_2 $min^{-1}g^{-1}$ of protein) (Fig. 2A) and peroxidase (from 2.15 to 1.69 U $mg^{-1}$of protein min^{-1}) (Fig. 2B). These elevated levels, in comparison to the other storage conditions employed in this experiment. The higher levels of antioxidant enzyme activities likely neutralized reactive oxygen species (ROS) generated in the M_3storage conditions, thereby supporting cell membrane integrity and viability to a greater extent (24).

Metabolic profiling

In metabolic profiling, 189 compounds were detected with peak intensities in the chromatogram obtained from GC-MS (Fig. 3; Supplementary Table 1). Among these, sixteen metabolic compounds were identified as related to seed viability and deterioration (Table 3). Kernels stored under nitrogen conditions retained vital metabolites, including sucrose, gamma-tocopherol, stigmasterol, campesterol, 1-monopalmitin, galactinol, elaidic acid and palmitic acid. Compared to other storage conditions tested in this study, these compounds play a crucial role in maintaining essential seed quality traits, such as kernel moisture content, germination, vigour and viability.

Table 1. Studies on kernel moisture content (%) and germination (%) of groundnut CO7 kernels under modified atmospheric conditions

Storage period (Days)	Moisture content (%)					Germination (%)				
	Modified atmospheric storage (M)					Modified atmospheric storage (M)				
	M_1	M_2	M_3	M_4	Mean	M_1	M_2	M_3	M_4	Mean
0	6.1^{a} (14.3)	6.1^{a} (14.3)	6.1^{a} (14.3)	6.1^{a} (14.3)	**6.1^{a} (14.3)**	75^{cd} (60.0)	75^{cd} (60.0)	75^{cd} (60.0)	75^{cd} (60.0)	**75^{b} (60.0)**
30	6.2^{ab} (14.4)	6.5^{cde} (14.8)	6.1^{a} (14.3)	6.1^{a} (14.3)	**6.2^{ab} (14.4)**	85^{a} (67.2)	84^{ab} (66.4)	86^{a} (68.0)	86^{a} (68.0)	**85^{a} (67.2)**
60	6.3^{abc} (14.5)	6.7^{efgh} (15.0)	6.2^{ab} (14.4)	6.2^{ab} (14.4)	**6.4^{b} (14.7)**	74^{d} (59.3)	70^{e} (56.8)	82^{b} (64.9)	78^{c} (62.0)	**76^{b} (60.7)**
90	6.4^{bcd} (14.7)	7.1^{ij} (15.3)	6.4^{bcd} (14.7)	6.5^{cde} (14.8)	**6.6^{c} (14.9)**	70^{e} (56.8)	62^{hi} (51.9)	78^{c} (62.0)	70^{e} (56.8)	**70^{c} (56.8)**
120	6.7^{efg} (15.0)	7.5^{kl} (15.9)	6.5^{cde} (14.8)	6.6^{def} (14.9)	**6.8^{d} (15.1)**	64^{gh} (53.1)	56^{j} (48.4)	74^{d} (59.3)	66^{fg} (54.3)	**65^{d} (53.7)**
150	6.9^{ghi} (15.2)	7.7^{l} (16.1)	6.7^{efgh} (15.0)	6.8^{fghi} (15.1)	**7.0^{e} (15.3)**	60^{i} (50.8)	52^{kl} (46.1)	68^{ef} (55.6)	62^{hi} (51.9)	**61^{e} (51.4)**
180	7.3^{jk} (15.7)	8.1^{m} (16.5)	7.0^{hij} (15.3)	7.1^{ij} (15.3)	**7.4^{f} (15.8)**	51^{l} (45.6)	48^{l} (43.9)	55^{jk} (47.9)	52^{kl} (46.1)	**52^{f} (46.1)**
Mean	**6.6^{b} (14.9)**	**7.1^{c} (15.3)**	**6.4^{a} (14.7)**	**6.5^{ab} (14.8)**		**68^{c} (55.6)**	**64^{d} (53.1)**	**74^{a} (59.3)**	**70^{b} (56.8)**	
	M		**P**		**M x P**	**M**		**P**		**M x P**
SEd	0.064		0.085		0.170	0.405		0.536		1.072
CD(P=0.05)	0.129		0.170		0.341	0.812		1.074		2.148

M_1- Pods stored in gunny bag; M_2- kernels stored in gunny bag;

M_3- kernels stored under nitrogen; M_4- kernels stored under vacuum.

Table 2. Studies on dry matter production (g seedlings^{-10}) and vigour index of groundnut CO7 kernels under modified atmospheric conditions

Storage period (Days)	Dry matter production (g seedlings^{-10})					Vigour index				
	Modified atmospheric storage (M)					Modified atmospheric storage (M)				
	M_1	M_2	M_3	M_4	Mean	M_1	M_2	M_3	M_4	Mean
0	3.679^{abc}	3.679^{abc}	3.679^{abc}	3.679^{abc}	**3.679^{ab}**	2213^{c}	2213^{c}	2213^{c}	2213^{c}	**2213^{b}**
30	3.720^{ab}	3.701^{abc}	3.768^{a}	3.753^{a}	**3.736^{a}**	2549^{a}	2503^{ab}	2597^{a}	2588^{a}	**2559^{a}**
60	3.629^{abcd}	3.557^{cde}	3.694^{abc}	3.648^{abcd}	**3.632^{b}**	2088^{d}	1897^{ef}	2427^{b}	2247^{c}	**2165^{c}**
90	3.471^{ef}	3.423^{efg}	3.575^{bcde}	3.516^{de}	**3.496^{c}**	1869^{ef}	1600^{h}	2230^{c}	1939^{e}	**1909^{d}**
120	3.220^{hi}	3.014^{jk}	3.442^{efg}	3.313^{gh}	**3.247^{d}**	1632^{h}	1338^{jk}	2057^{d}	1763^{g}	**1698^{e}**
150	3.154^{ij}	2.901^{kl}	3.320^{fgh}	3.235^{hi}	**3.153^{e}**	1470^{i}	1207^{l}	1809^{fg}	1581^{h}	**1517^{f}**
180	3.028^{jk}	2.772^{l}	3.209^{hi}	3.119^{ij}	**3.032^{f}**	1193^{l}	1057^{m}	1403^{ij}	1274^{kl}	**1232^{g}**
Mean	**3.414^{b}**	**3.292^{c}**	**3.527^{a}**	**3.466^{b}**		**1859^{c}**	**1688^{d}**	**2105^{a}**	**1943^{b}**	
	M	**P**	**M x P**			**M**	**P**	**M x P**		
SEd	0.029	0.038	0.076			18.027	23.847	47.695		
CD(P=0.05)	0.058	0.077	0.153			36.113	47.774	95.547		

M_1- Pods stored in gunny bag; M_2- kernels stored in gunny bag; M_3- kernels stored under nitrogen; M_4- kernels stored under vacuum.

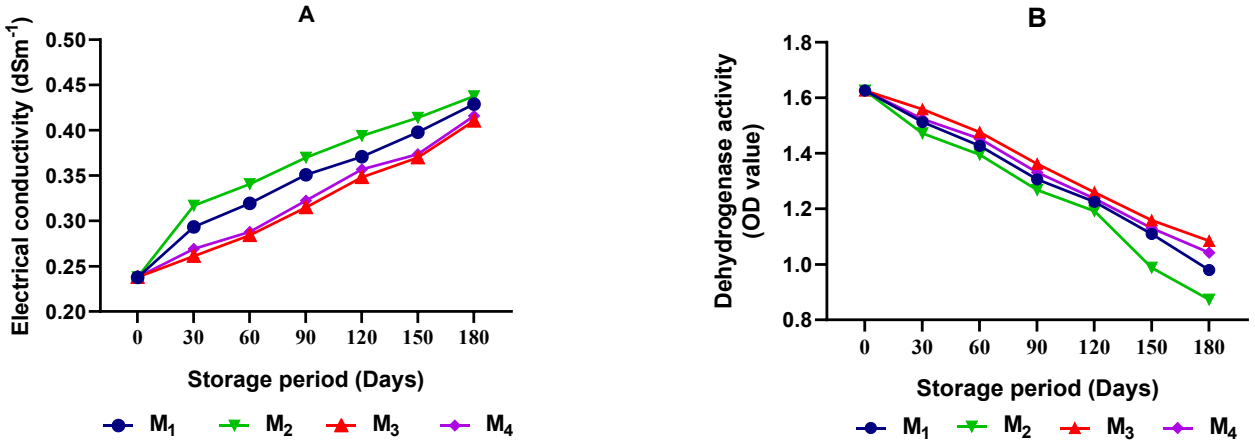

Fig. 1. Studies on (A) Electrical conductivity (dSm^{-1}) and (B) Dehydrogenase activity (OD value) of groundnut CO7 kernels under modified atmospheric conditions

M_1- Pods stored in gunny bag; M_2- kernels stored in gunny bag;M_3- kernels stored under nitrogen; M_4- kernels stored under vacuum.

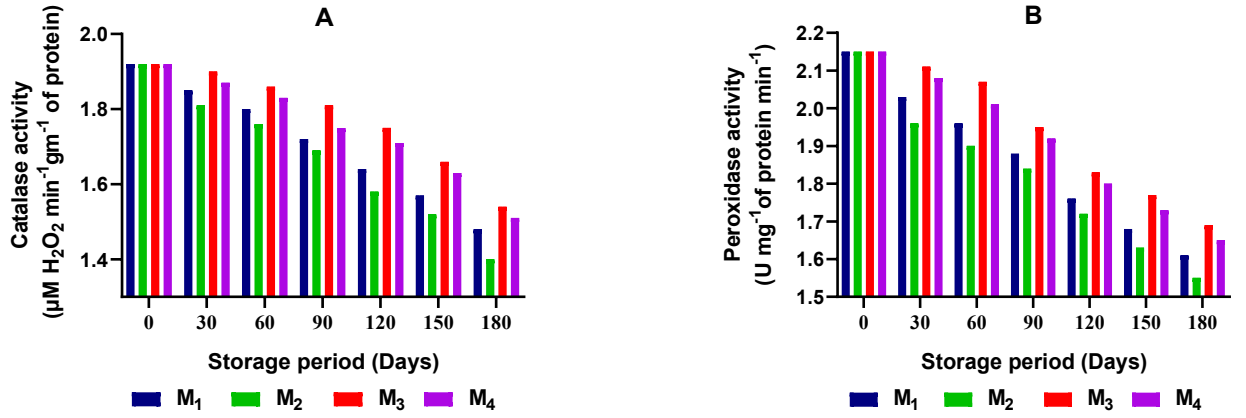

Fig. 2: Studies on (A) Catalase activity (µM H_2O_2 min^{-1}gm^{-1} of protein) and (B) Peroxidase activity (U mg^{-1} of protein min^{-1}) of groundnut CO7 kernels under modified atmospheric conditions

M_1- Pods stored in gunny bag; M_2- kernels stored in gunny bag; M_3- kernels stored under nitrogen; M_4- kernels stored under vacuum.

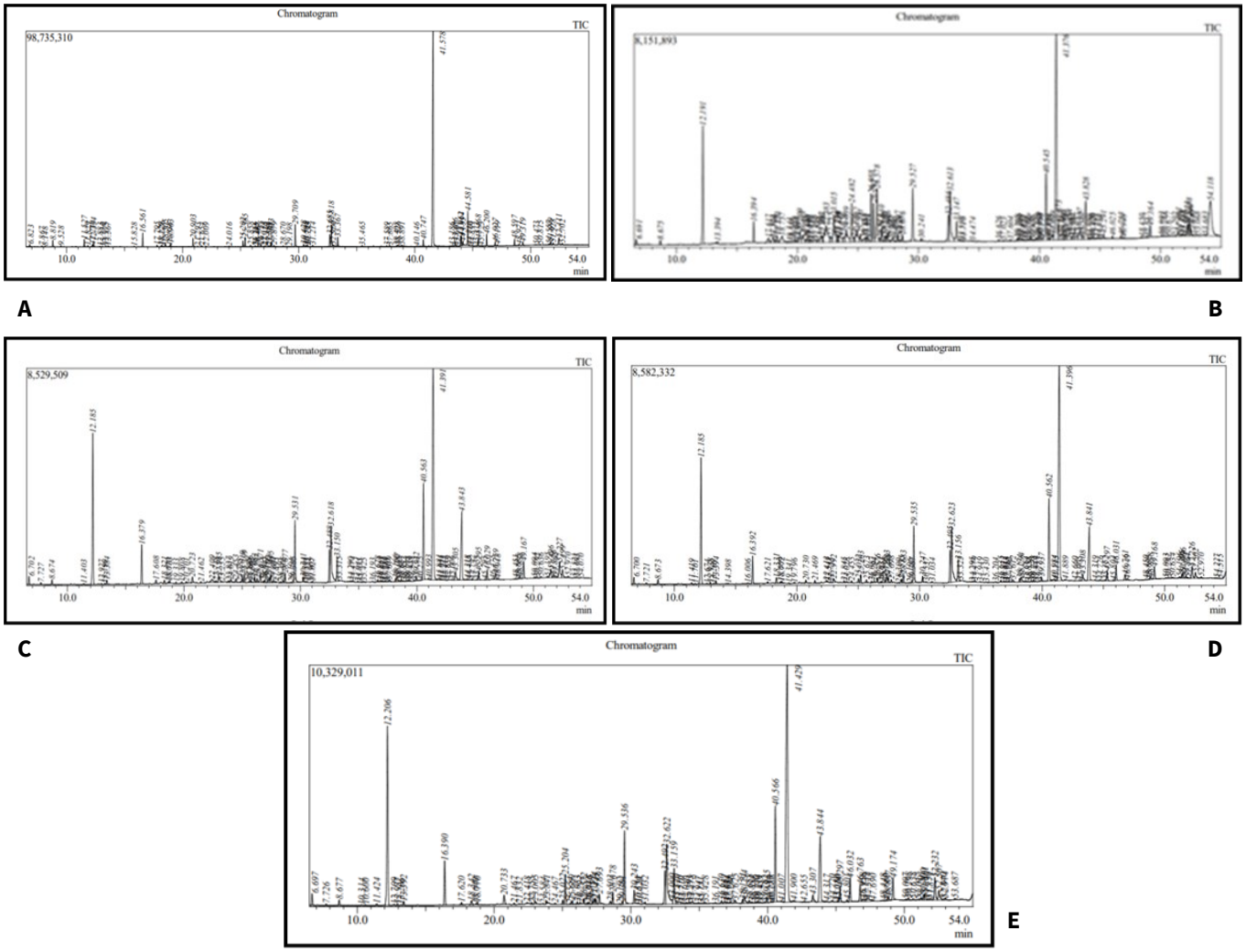

Fig. 3. GC-MS chromatogram of groundnut CO 7 kernels, (A) Freshly harvested seed; (B) Aged seed M_1; (C) Aged seed M_2; (D) Aged seed M_3; (E) Aged seed M_4

Table 3. Seed metabolites related to seed viability and deterioration in groundnut CO 7 kernels stored under modified atmospheric condition.

Storage period (Days)		0 (Fresh seeds)	180 (Aged seeds)			
Modified atmospheric storage (M)			M_1	M_2	M_3	M_4
S.No	Compound name	Area %	Area %	Area %	Area %	Area %
1	Sucrose	52.52	24.8	15.24	28.9	26.95
2	Gluconic acid	0	0	0.49	0	0
3	Urea	0	0	0.8	0.12	0.1
4	Fructose	0	1.46	0	0	0
5	Galactose	0	0.39	0.23	0.29	0
6	Glucose	0	3.56	0	0	0
7	Gamma.-Tocopherol	0.70	0.16	0.16	0.25	0.18
8	Phenol	0.34	0.93	1.3	0.47	0.85
9	Glycerol	0.72	12.48	13.4	6.26	11.8
10	Stigmasterol	0.12	0	0.13	0.16	0.14
11	Campesterol	0.9	0.15	0.1	0.6	0.17
12	1-Monopalmitin	1.1	6.5	4.11	7.95	7.73
13	Galactinol	1.85	0.75	0.23	1.52	1.37
14	Elaidic acid	0	5.99	3.85	7.11	6.2
15	Palmitic acid	5.11	3.3	3.28	5.9	4.76
16	4-Coumaric acid	0.3	0.61	0.63	0.41	0.55

M_1- Pods stored in gunny bag; M_2- kernels stored in gunny bag; M_3- kernels stored under nitrogen; M_4- kernels stored under vacuum.

Sucrose was recognized as the main compound in this investigation. In fresh seeds, it constituted 52.52% of the area; however, this percentage diminished with prolonged storage duration. The maximum area recorded was 28.9% in M_3, while the minimum was 15.24% in M_2 at the end of 180 day storage period. Sucrose serves as an antioxidant defense mechanism, helping mitigate oxidative damage caused by ROS during storage. Similar studies on the storability of rice found that sucrose was the major compound, with its concentration decreasing with aging in the IIYou998 hybrid cultivar (25).

Gluconic acid is detected during aging (180 days) in M_2, with an area of 0.49%. Aging seeds often experience increased oxidative stress, leading to the production of ROS. Gluconic acid, a product of glucose oxidation, indicates heightened oxidative processes within the seed. Excessive

ROS can damage cellular components, including DNA, proteins and lipids. An increase in gluconic acid is negatively correlated with seed germination, meaning that higher gluconic acid levels are associated with lower germination rates. This correlation suggests that gluconic acid could be a marker for seed aging and reduced vigor (26). Urea was also detected during aging, with a maximum area percentage of 0.12% recorded in M_3 after 180 days. During seed storage, urea accumulates and is later broken down by the enzyme urease to release nitrogen, which supports seedling growth during germination.

Reducing sugars, namely fructose, galactose, and glucose, were lacking in fresh seeds but were identified at maximum area percentages of 1.46%, 0.39% and 3.56%, respectively, in M_1 after 180 days of aging. During aging, reducing sugars accumulate and may trigger Maillard and Amadori reactions (27). The Amadori reaction, an initial stage of the Maillard reaction, involves reducing sugars reacting with amino groups in proteins to form Amadori products. These products can modify proteins, impacting the seed's metabolic functions. As the Maillard reaction progresses, Amadori products transform into advanced glycation end-products (AGEs), which lead to decreased seed vigor and viability, cause tissue browning, and ultimately reduce seed germination (28).

During storage, gamma-tocopherol, a vitamin compound, was detected in fresh and aged seeds (180 days). In fresh seeds, the area percentage was measured at 0.7, which diminished over time, with a lesser decline noted in M_3 at 0.25% area. Tocopherols, key components of vitamin E, play essential roles in plant stress tolerance and human nutrition. While alpha-tocopherol and gamma-tocopherol are predominant in leaves and seeds, gamma-tocopherol may be more effective than alpha-tocopherol in protecting against lipid peroxidation in both seeds and leaves (29).

Phenol was detected in fresh seeds at 0.34% area, and its content increased during aging (180 days), reaching a peak of 1.3% in M_2. Higher phenol concentrations inhibit seed germination. Glycerol was also detected in fresh seeds at 0.72% area and it increased significantly with aging, with the highest level in M_2 at 13.4%. Glycerol is a metabolic biomarker for seed aging and negatively correlated with seed germination. Being highly hygroscopic, glycerol attracts and retains moisture from the environment, which can lead to cellular damage and reduced viability by increasing moisture content (30). Low moisture levels are essential for seed longevity, while high moisture content accelerates seed deterioration (31).

In fresh seeds, stigmasterol was detected at 0.12% area, this increased during aging (180 days), reaching a maximum area of 0.16% in M_3. Campesterol showed a maximum area of 0.9% in fresh seeds but decreased with aging (180 days), with the most minor reduction observed in M_3 at 0.6% area. Secondary metabolites such as stigmasterol and campesterol are present in all oilseeds and contribute to seedling growth and development. Stigmasterol protects seeds and provides essential resources for developing seedlings in groundnuts (32). Campesterol, a precursor of brassinosteroids, is a plant hormone that promotes growth and development, including seed germination. Its presence can enhance hormone synthesis, thereby facilitating germination (33).

In fresh seeds, 1-Monopalmitin was identified with an area of 1.1 %. During storage (180 days), its level increased, reaching a maximum area of 7.95% in M_3. The compound 1-Monopalmitin has antioxidant properties that may help protect seeds from oxidative damage during storage. Antioxidants are critical in maintaining seed viability by preventing deterioration (34). Galactinol was detected in both fresh and aged seeds (180 days). In fresh seeds, it was recorded at 1.85 % area, decreasing slightly during aging, with a maximum of 1.55% area observed in M_3 at the end of the storage period. Galactinol is associated with seed germination, where higher galactinol content correlates with increased germination (35). Elaidic acid, an unsaturated fatty acid, was detected during aging (180 days) with a maximum area of 7.11% in M_3. Unsaturated fatty acids, such as elaidic, oleic and linoleic acids, play vital roles in seed storage and plant stress responses. They contribute to membrane permeability in seeds, which is essential for maintaining cellular integrity and function under stress conditions.

In fresh seeds, palmitic acid was detected with an area percent of 5.11%; however, this value decreased over the storage period (180 days), with the smallest reduction observed in M_3, where the area percentage remained at 5.9%. In groundnuts, saturated fatty acids, such as arachidonic acid and palmitic acid, play essential roles as facilitators in signal transduction and structural integrity (36). Saturated fatty acids contribute to the stability and shelf life of oils. However, a high intake of saturated fatty acids can increase the risk of heart disease and atherosclerosis (37). For health benefits, unsaturated fatty acids are recommended.

4-Coumaric acid was detected in fresh seeds with an area percentage of 0.3%; however, during storage (180 days), its content increased, with the highest level found in M_2, reaching 0.63% area. Coumarin, a plant allelochemical, inhibits seed germination by reducing GA4 production, consequently decreasing ROS accumulation (38).

Conclusion

The current investigation identified sixteen compounds linked to seed viability and deterioration during storage, as analyzed through GC-MS. This study demonstrates a clear relationship between the presence of specific metabolic compounds during storage and the viability and vigour of groundnut seeds, which in turn enhances their storage capacity. Findings concluded that kernels stored under nitrogen conditions preserved viability more effectively than those stored using other methods for up to four months (120 days), per the Indian Minimum Seed Certification Standards (IMSCS). Future research should examine different concentrations of N_2, alternative gases like CO_2, or combinations of these gases to optimize seed storage further.

For practical and large-scale applications, N_2-based storage can be scaled by incorporating N_2 generators or cylinders into existing storage facilities. This approach, while cost-effective over time compared to traditional methods, also minimizes seed deterioration and extends viability, resulting in significant savings on seed replacement and improved crop yields. Seed industries can integrate these findings into their protocols to enhance the longevity and quality of stored seeds, offering an efficient alternative to bulk pod storage and reducing associated costs in seed storage and transportation.

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Authors' contributions

KT carried out the experiment studies and data acquisition. PK and UR drafted the article. KR and BM analyzed and interpreted data. VV and KS critically reviewed important intellectual content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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Declaration of generative AI and AI-assisted technologies in the writing process

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