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RESEARCH ARTICLE

Modified atmospheric storage: A cost effective method for preserving biofuel feedstock quality in tropical condition

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Abstract

The storage of biodiesel feedstock presents significant challenges for the biodiesel industry due to the deterioration of fatty acid constituents, which compromises both oil and biodiesel quality. Seed viability plays a critical role in preserving the quality of oil within biodiesel feedstock during storage. Recognizing the need for an affordable and efficient storage solution, this study aimed to develop a method for preserving the physiological and fuel characteristics of groundnut seeds (biodiesel feedstock) under tropical conditions. Groundnut seeds with a moisture content of 6.4% and an initial viability of 91% were stored in 3 different types of container: cloth bags, evacuated aluminum foil pouches and CO_2-infused plastic containers for 7 months under ambient tropical conditions (34 ± 2 °C, 56 ± 4% RH). The study assessed the impact of storage conditions on seed viability, antioxidant enzyme activity and oil quality parameters, including free fatty acid (FFA), acid value (AV), peroxide value (PV), saponification value (SV), iodine value (IV) and cetane number (CV). Results indicated that CO_2-infused plastic containers effectively maintained seed viability (72%), enhanced antioxidant enzyme activity and minimized oil deterioration. Furthermore, the feedstock stored in these containers complied with international biodiesel standards and remained suitable for alkali-based trans-esterification processes. This cost-effective storage method offers a viable solution for preserving biodiesel feedstock storage in tropical climates, thereby ensuring year-round biodiesel production and supply.

Keywords

antioxidant enzymes; biofuel feedstock; free fatty acid; groundnut; viability

Introduction

Vegetable oils serves as the primary feedstock for biodiesel production, accounting for more than >90% of total biodiesel output. Currently, oil extracted from over 350 crop species has been identified as suitable for biodiesel production (1). However, the major contributors primarily fall under edible oil sources.

Generally, a huge quantity of oilseeds is collected during harvesting season, followed by = oil extraction and storage, an essential upstream activity in most biodiesel production industries. Since oilseed production is seasonal, biodiesel producers attempt to store and utilize extracted oil throughout the

year, ensuring a continuous supply of biodiesel as needed. However, the deterioration of extracted oil/biodiesel during storage poses a significant threat, negatively impacting the agriculture sector and the companies involved in production of biodiesel (2-4).

The ability to store vegetable oil or biodiesel is significantly influenced by environmental factors (5). A key challenge faced in the biodiesel production sectors is the rapid decline in fuel quality during the storage of raw oil derived from oilseeds or biodiesel itself. Several factors contribute to oil degradation, including its initial quantity, chemical composition and the amount of moisture in the atmosphere. Additionally, non-viable oilseeds, often referred to as grains, are commonly diverted for biodiesel production, resulting in poor initial oil quality in most of the case.

Storage conditions such as temperature, relative humidity, moisture content in the oil and duration of storage may significantly affect the characteristics of stored oil or biodiesel (6-8). Oils with a higher degree of unsaturation are particularly susceptible to oxidative degradation. Therefore, preserving unsaturated oil with desirable biodiesel production is often difficult (9).

Biodiesel quality and recovery largely depend on oil properties, especially FFA content. Other factors affecting biodiesel production include high moisture content and the presence of solids impurities (10). Among these parameters, FFA content plays a vital role in determining the appropriate biodiesel production methods. The alkali-based transesterification process is widely preferred due to its lower cost and higher reaction rates. However, when an alkali catalyst is used, feedstocks with high FFA levels promote soap formation, which hinders biodiesel production. To solve this problem, the feedstock with lower FFA content should be utilized for the production of biodiesel (11).

The quality of the oilseed used for extraction is the primary determinant of feedstock quality. To comply with biodiesel standards set by the American Society for Testing and Materials (ASTM), the oil industry employs chemical processing methods to reduce FFA content and other contaminants (12, 13). However, these processes ultimately reduce biodiesel yield during transesterification and increase production costs.

Fatty acid composition serves as a reliable marker of oilseed quality. The types of fatty acids present in oilseeds and their bonding patterns influence the chemical reactions that may occur during storage (14, 15). Unlike grains, seeds are living biological entities that can regulate metabolic activity and maintain oil quality under suitable storage conditions through their self-defense mechanisms (16).

For long-term storage without compromising seed viability and oil quality, oilseeds are typically dried to a moisture content of 6-8% (17). Under low moisture conditions, the rate of lipid metabolism and the consumption of lipid molecules during respiration are significantly reduced in seeds compared to grains (18). Consequently, oilseeds maintain superior fatty acid profiles, oil content and protein levels when compared to grains after harvest and during storage.

Given the high cost of oil storage, including the use of synthetic antioxidants and low-temperature storage, it was hypothesized that storing biodiesel feedstock in seed form could provide a cost-effective and feasible alternative. Therefore, this study aimed to develop an economical and practical methodology for storing biodiesel feedstock (oilseeds) while preserving seed viability and fuel characteristics until oil extraction begins.

Materials and Methods

Genetically pure groundnut seeds (*Arachis hypogaea* L.) of the TMV 7 variety, with a moisture content of 6.4% and viability of 91%, were procured from Tamil Nadu Agricultural University, Coimbatore, India. All chemicals and reagents used in this study were of analytical grade. Potassium hydroxide (KOH), methanol and hexane were purchased from Precision chemicals (India) for oil extraction and acid value determination.

For the assessment of antioxidant enzyme activities, reagents including nitro blue tetrazolium (NBT), reduced glutathione (GSH) and hydrogen peroxide (H_2O_2) were procured from Sigma-Aldrich. Deionized water was used throughout the study, and all glassware was thoroughly cleaned and rinsed with ethanol before use. Gas chromatography-mass spectrometry (GC-MS) analysis was conducted using high-purity helium gas (99.99%) as the carrier.

The fatty acid profile of the procured groundnut seeds was analyzed using GC-MS (19) prior to the commencement of storage as a biodiesel feedstock. A bulk quantity (10kg) of the feedstock was packaged in three different containers viz., cloth bag (C1), evacuated aluminum foil pouch (C_2) and plastic containers infused with 30% CO_2 (C_3). All three containers were stored under ambient conditions (34 ± 2 °C and 56 ± 4% RH) for a period of 7 months. The initial storage period was designated as P_0, while the remaining months of storage are abbreviated as P_1 to P_7 in the results. Containers were re-arranged every month for uniform ageing.

Properties of biodiesel feedstock

Oil extraction: Biodiesel feedstock samples were collected separately from each of the 3 storage containers at monthly intervals throughout the storage period. Oil was extraction was carried out using the Savaliya Oil Maker Machine, which is specifically designed for cold-pressing processes. This machine operates without heating, thereby preserving of the natural nutrients and quality of the oil. The model is durable with a stainless-steel body, making it ideal for small-scale or laboratory-based oil extraction (20).

Qualitative analysis: The chemical properties of the oil extracted from groundnut seeds (biodiesel feedstock) stored in the 3 different containers were estimated. The parameters assessed included acid value (AV), free fatty acid (FFA), saponification value (SV), peroxidase value (PV) and iodine value (IV), following the established protocol (21). Additionally, the cetane number (CN) was calculated based on SV and IV values (22-24).

Physiological quality of biodiesel feedstock

The biodiesel feedstock stored in 3 different containers was tested through germination tests (25) and dehydrogenase enzyme activity assays (26).

Assessment of antioxidant enzymes activity of biodiesel feedstock

At monthly intervals, enzyme extracts were prepared from the biodiesel feedstock stored in each of the 3 containers to quantify the activity of key antioxidant enzymes, including catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) (16).

Statistical Analysis

The study employed a factorial completely randomized design (FCRD) for the storage experiment. Data were analyzed using Tukey's multiple range test with the Statistical Analysis System (SAS) Version 9.2, SAS Institute Inc., USA.

Results and Discussion

To assess the effect of oilseed physiology on biofuel feedstock quality during long-term storage at room temperature, samples were collected at monthly intervals and various quality parameters were assessed. The findings revealed that as the storage period extended, seed viability gradually declined. Groundnut seeds stored in the C_3 container maintained the highest germination percentage (72%) after 7 months of storage, followed by C_2 (70%) and C_1 (53%) (Table 1). Prolonged storage was associated with seed deterioration, characterized by reductions in germination rate, emergence, seedling length, dry matter accumulation and vigor index. Similar trends in physiological decline during extended storage have been reported in sunflower seeds (27).

Storage conditions had a significant influence on seed germination across all storage periods, underscoring its importance as a key indicator of seed quality and viability. Seeds stored in CO_2-enriched atmospheres, regardless of concentration or exposure period, maintained their germination potential without adverse effects (28).

The tetrazolium test is a rapid test to evaluate viability and vigour of the seeds based on color changes in living tissue upon exposure to 2,3,5 triphenyl tetrazolium chloride. This test reflects the activity of the dehydrogenase enzyme system, which is closely related to respiration and viability of the seeds. On the advancement of the storage period, the dehydrogenase activity was reduced significantly. It was significantly reduced at the end of the storage period. The findings of this study align with previous research, demonstrating that groundnut seeds stored in the C_3 container exhibited the highest dehydrogenase activity (0.517 OD), followed by C_2 (0.494 OD), while the lowest value was observed in C_1 (0.296 OD) (Fig. 3b). The similar result was also reported in onion (29). Additionally, a decline in dehydrogenase activity with seed aging in *Brassica* spp was noted (30).

During the evaluation of fuel properties in oil extracted from groundnut seed samples, it was observed that feedstock stored in the C_3 container had the lowest free fatty acid content (1.5%), followed by C_2 (1.7%) and C_1 (2.6%) (Fig. 1a). The conversion of triglyceride to FFAs by saponification (hydrolysis of glyceride, releasing glycerol and free fatty acids) normally occur during storage of oilseeds (31).

Another cause of loss of seed viability and vigor during rapid ageing may be free fat acidity, i.e., the increase in free fatty acids level in the seeds. The hydrolysis of ester linkage between fatty acid chains and glycerol in triacylglycerols liberates fatty acids (32). Furthermore, the FFA content present in the oil extracted from the seeds increase with storage duration, leading to a corresponding decrease in seed germination. There will be more chances for increasing FFA content of oil under storage. Extended storage further increases the likelihood of FFA accumulation in oil, which in turn affects oilseed viability.

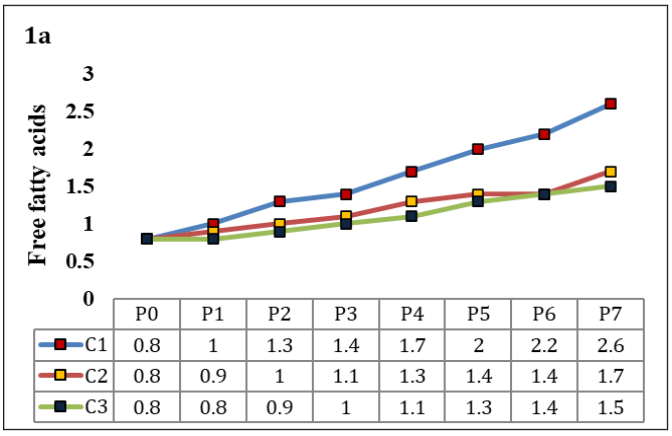
The amount of free fatty acids was measured by using acid value. The acid value is an indication for deterioration of the oils. The feedstock stored in C_3 recorded the lowest acid value (0.75 value mg KOH g^{-1}) followed by C_2 (0.85 value mg KOH g^{-1}), while the highest acid value was recorded in C1 (1.31 value mg KOH g^{-1}) (Fig. 1b). Seeds absorbing moisture promote hydrolysis of ester, which also increased the acid value (33).

The PV, which measures rancidity levels, also indicated significant differences among storage conditions. The feedstock stored in C_3 recorded the lowest value for peroxide (16.5 mq peroxide/kg sample), followed by C_2 (17 mq peroxide/kg sample), while the highest PV was observed in C_1 (19 mq peroxide/kg sample) after 7 months of storage (Fig. 2a). The PV is used as a measure of the extent to which rancidity reaction has occurred during storage. It could be

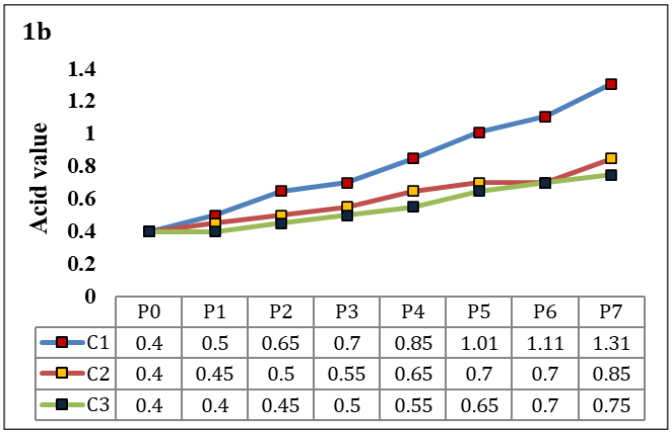
Table 1. Germination (%) of feedstock (groundnut seeds) stored under 3 different containers

Storage period (P)	Storage containers			
	C_1	C_2	C_3	Mean
P_0	91 (72.6)	91 (72.6)	91 (72.6)	91 (72.6)
P_1	85 (67.3)	88 (69.7)	89 (70.7)	87 (69.2)
P_2	78 (62.0)	86 (68.2)	86 (68.2)	83 (66.1)
P_3	74 (59.4)	82 (64.9)	84 (66.4)	80 (63.6)
P_4	70 (56.8)	78 (62.0)	81 (64.2)	76 (61.0)
P_5	64 (53.1)	75 (60.0)	78 (62.0)	72 (58.1)
P_6	60 (50.8)	73 (58.7)	75 (60.0)	69 (56.5)
P_7	53 (46.7)	70 (56.8)	72 (58.1)	65 (53.9)
Mean	**72 (58.1)**	**80 (64.1)**	**82 (64.9)**	**78 (62.0)**
	P	**C**		**PxC**
SEd	**0.86**	**0.53**		**1.49**
CD(P=0.05)	**1.73 ****	**1.06 ****		**3.00 ****

(Figures in parenthesis indicate arcsine transformed values)



	P0	P1	P2	P3	P4	P5	P6	P7
C1	0.8	1	1.3	1.4	1.7	2	2.2	2.6
C2	0.8	0.9	1	1.1	1.3	1.4	1.4	1.7
C3	0.8	0.8	0.9	1	1.1	1.3	1.4	1.5



	P0	P1	P2	P3	P4	P5	P6	P7
C1	0.4	0.5	0.65	0.7	0.85	1.01	1.11	1.31
C2	0.4	0.45	0.5	0.55	0.65	0.7	0.7	0.85
C3	0.4	0.4	0.45	0.5	0.55	0.65	0.7	0.75

Fig. 1. (1a) Free fatty acids (%) and **(1b)** Acid value (mg KOH g^{-1}) content of oil extracted from feedstock (groundnut seeds) stored under 3 different containers.

used as an indication of the oil quality and stability of fats and oils (34). Similar trends have been reported in Jatropha oil (20) as well as sesame oil and peanut oil (31). Increased storage period or accelerated ageing elevates the peroxide value and lower the oxidation stability of the oil making (35).

The saponification value is an indication of the molecular weight of fats and is inversely proportional to it, also serving as an indication of adulteration (31). The SV of various oil samples decreased with increasing temperature and moisture during storage period (36). Generally, the SV value of many vegetable oils would give an idea about the presence of a higher or lower amount of fatty acids. The feedstock stored in C_3 recorded a lesser saponification value (179.45 mg KOH g^{-1}), followed by C_2 (182.32 mg KOH g^{-1}), whereas the highest SV was recorded in C_1 (189.20 mg KOH g^{-1}) (Fig. 2b). The similar kind of observations recorded in Jatropha (20) and in sunflower and mustard oils (37).

The feedstock stored in C_3 recorded the highest iodine value (56.14), followed by C_2 (54.56), while the lowest value was recorded in C_1 (38.76) (Fig. 3a). The degree of unsaturation, represented by the IV, is an important consideration when selecting feedstock for biodiesel production. A certain level of unsaturated fatty acids in methyl esters is necessary to prevent solidification. However, excessive unsaturation renders the methyl esters unsuitable for biodiesel production, as they react with oxygen, forming peroxide that can induce cross- linkage at unsaturation sites. It's possible that the substance will polymerize into a plastic-like body. The process can be accelerated at high temperatures, such as those within an internal combustion engine, potentially leading to engine gumming due to polymerized methyl esters.

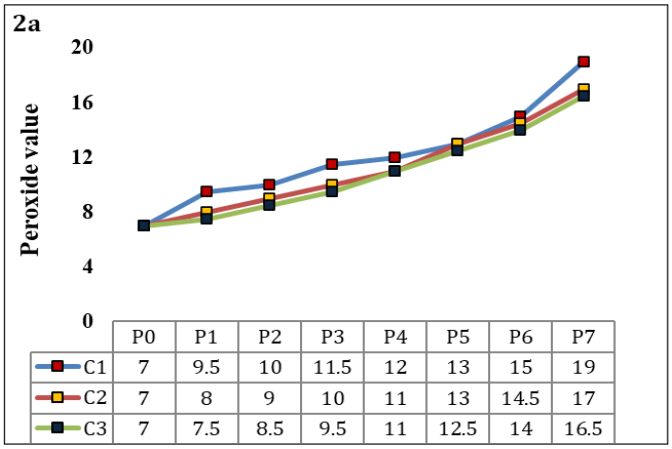
To avoid this problem, biodiesel standards specify a minimum limit of IV. In this study, all feedstock samples had IV value below 115, aligning with the lowest permitted value set by the European Organization (EN 14214) (Fig. 3a) (27).

The highest CN value was recorded in the feedstock stored in C1 (66.43), followed by C_3 (64.08) and C_2 (63.96) after 7 months of storage. The CN value of the oil reflects its ignition quality. Oil with a higher CN value will have better ignition properties. This constitutes one of the most significant oil properties to consider while choosing feedstock for the production of biodiesel.

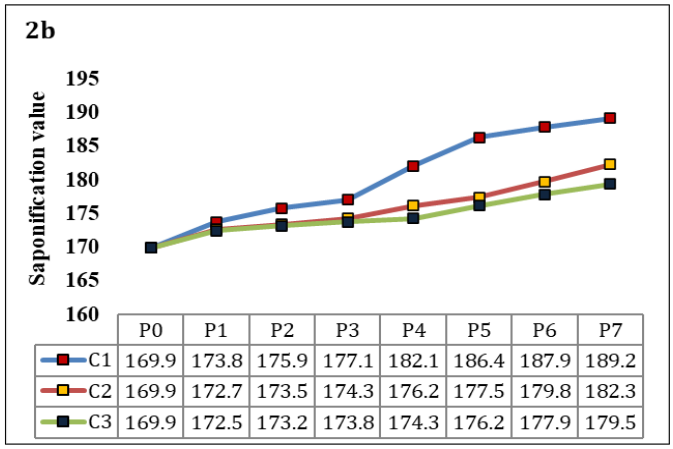
The standards for the CN value vary by country. Biodiesel standards in the United States (ASTM D 6751): CN ≥ 47 , Germany (DIN V51606): CN ≥ 49 and the European Organization (EN 14214):CN ≥ 51 (38). In the present investigation, oil extracted from feedstock stored for 7 months in all 3 containers exceeded the minimum CN value requirement of 51 (Table 2).

However, as CN increases, the value of IV decreases, indicating that the degree of unsaturation decreases, resulting in the consolidation of methyl esters at elevated temperatures. To resolve this issue, the US biodiesel standards (ASTM D 6751-99 & ASTM PS 121-99) set the highest limit of CN of 65 (39, 40).

By the sixth month of storage, exceeded the maximum CN limit (65.25), rendering it less suitable for biodiesel production. Consequently, feedstock stored for 5 months in



	P0	P1	P2	P3	P4	P5	P6	P7
C1	7	9.5	10	11.5	12	13	15	19
C2	7	8	9	10	11	13	14.5	17
C3	7	7.5	8.5	9.5	11	12.5	14	16.5



	P0	P1	P2	P3	P4	P5	P6	P7
C1	169.9	173.8	175.9	177.1	182.1	186.4	187.9	189.2
C2	169.9	172.7	173.5	174.3	176.2	177.5	179.8	182.3
C3	169.9	172.5	173.2	173.8	174.3	176.2	177.9	179.5

Fig. 2. (2a) Peroxide value (mq peroxide/kg sample) and **(2b)** Saponification value (mg KOH g^{-1}) of oil extracted from feedstock (groundnut seeds) stored under 3 different containers.

all three containers was deemed optimal for biodiesel production, ensuring compatibility across various climatic conditions. Despite seven months of storage, feedstock stored in C_2 and C_3 containers retained optimal fuel properties (Table 1).

Current research suggests maintaining biodiesel at 20 °C or mixing an artificial antioxidant with it to extend the shelf life to a maximum of 120 days (41-43).

To determine the biochemical underpinnings of the oil derived from feedstock (groundnut seed) stored in three different containers, antioxidant enzyme activity was analyzed monthly throughout the storage period.

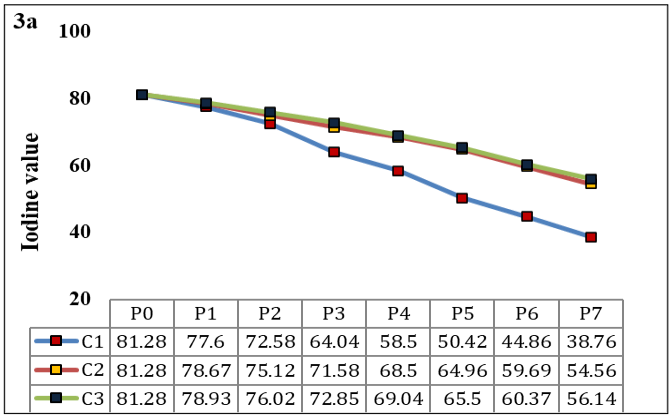
The SOD activity in the feedstock packed in C_3 ranged from 0.80 - 0.73 enzyme unit/mg protein, the highest throughout the storage period, followed by C_2 (0.80 -0.72 enzyme unit/mg protein) (Table 3). Similarly, compared to the other 2 storage conditions viz., C_1 and C_2, C_3exhibited the highest catalase (CAT) activity, ranging from 1.44 – 1.40 µM H_2O_2 reduced/min/mg protein. Feedstock with higher viability (91%) exhibited the highest CAT activity (1.44 µM H_2O_2 reduced/min/mg protein), whereas feedstock with lower viability (0.69 µM H_2O_2 reduced/min/mg protein) had correspondingly lower CAT activity (Table 4). The initial feedstock samples (P_0C1, P_0C_2, P_0C_3) exhibited the highest GR activity(1.18 µM), while the lowest GR activity was recorded in C_1 after 7 months of storage (P_7C1; 0.90 µM) (Table 5).

Generally, seed viability protection is governed by free radical and peroxide-scavenging enzymes, which endorse the oxidation- reduction cycle in biological ecosystem. The levels of SOD, CAT and GR present in the feedstock were strongly correlated with viability potential in the current experiment, which involved examining biodiesel feedstock stored in three different containers. The experimental results of this work reveal a relationship between seed viability and antioxidative defense mechanisms, which play a crucial role in maintaining the quality of biodiesel feedstock during storage. A decline in seed germination from 91% (C1P0) to 53% (C1P7) was strongly associated with reduced activity of GR, CAT, and SOD (Fig. 4), leading to a deterioration in biodiesel feedstock quality.

Furthermore, the unsaturated fatty acid content of the groundnut seed (feedstock) used in this experiment was 72.29% (Table 7). Feedstock with more than 50% unsaturated fatty acid content is highly amenable for quicker deterioration of oil if we could make an attempt to store the feedstock under ambient temperature conditions, such as those in Thiruchirappali, Tamil Nadu, India (Table 6). However, in this study, feedstock stored in either evacuated aluminum foil pouches (C_2) or 30% CO_2-infused plastic containers (C_3) at room temperature (Table 6) for seven months retained biodiesel feedstock quality parameters.

The cost of producing biodiesel is dependent on the quality of the source oils, particularly in terms of FFA, CN and IV values, which are crucial parameters in the trans-esterification process.

A higher FFA content not only reduces biodiesel yield but also complicates the biodiesel separation process. The findings of this study indicate a strong correlation between the chemical properties of oils and the physiological characteristics of oilseeds (feedstock). Notably, superior groundnut seed viability was associated with lower FFA concentrations in the oil, and conversely, lower seed viability corresponded with higher FFA levels.



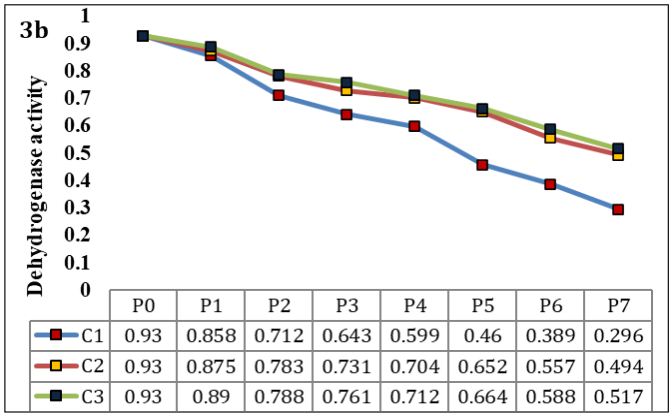


Fig. 3. (3a) indicates iodine value and **(3b)** Dehydrogenase activity (OD Value) of oil extracted from feedstock (groundnut seeds) stored under 3 different containers.

Table 2. Cetane number of oil extracted from feedstock (groundnut seeds) stored under 3 different containers

Storage period (P)	Storage containers			
	C_1	C_2	C_3	Mean
P_0	60.13	60.13	60.13	60.13
P_1	60.25	60.20	60.18	60.21
P_2	61.00	60.87	60.57	60.81
P_3	62.70	61.51	61.31	61.84
P_4	63.10	61.86	62.08	62.35
P_5	64.24	62.44	62.54	63.07
P_6	65.25	63.22	63.40	63.96
P_7	66.43	63.96	64.08	64.82
Mean	62.89	61.77	61.79	62.15
	P	**C**		**PxC**
SEd	**0.456**	**0.279**		**0.790**
CD(P=0.05)	**0.909****	**NS**		**1.574****

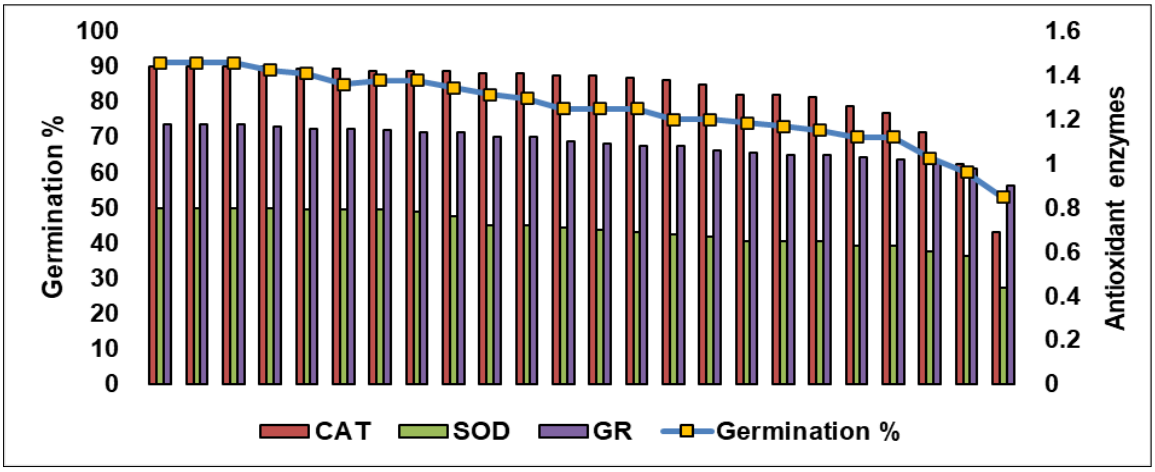


Fig. 4. Effect of Germination (%) on antioxidant enzymes activity of feedstock (groundnut seeds) during storage.

Table 3. SOD activity (enzyme unit mg of protein^{-1}) of feedstock (groundnut seeds) stored under 3 different containers

Storage period (P)	Storage containers			
	C_1	C_2	C_3	Mean
P_0	0.80 ± 0.020	0.80 ± 0.020	0.80 ± 0.020	0.80
P_1	0.78 ± 0.015	0.80 ± 0.018	0.79 ± 0.025	0.79
P_2	0.70 ± 0.016	0.79 ± 0.020	0.79 ± 0.018	0.76
P_3	0.65 ± 0.023	0.72 ± 0.020	0.76 ± 0.015	0.71
P_4	0.63 ± 0.018	0.71 ± 0.022	0.72 ± 0.016	0.69
P_5	0.60 ± 0.018	0.68 ± 0.028	0.69 ± 0.025	0.66
P_6	0.58 ± 0.025	0.65 ± 0.016	0.67 ± 0.022	0.63
P_7	0.44 ± 0.023	0.63 ± 0.025	0.65 ± 0.020	0.57
Mean	0.65	0.72	0.73	0.70
	P	**C**	**PxC**	
SEd	**0.006**	**0.004**	**0.011**	
CD(P=0.05)	**0.013****	**0.008***	**0.022****	

Table 4. CAT activity (μM H_2O_2 reduced min.$^{-1}$ mg of protein^{-1}) of feedstock (groundnut seeds) stored under 3 different containers

Storage period (P)	Storage containers			
	C_1	C_2	C_3	Mean
P_0	1.44 ± 0.028	1.44 ± 0.028	1.44 ± 0.028	1.44
P_1	1.42 ± 0.025	1.43 ± 0.018	1.44 ± 0.027	1.43
P_2	1.39 ± 0.022	1.42 ± 0.020	1.43 ± 0.015	1.41
P_3	1.31 ± 0.030	1.41 ± 0.025	1.42 ± 0.023	1.38
P_4	1.23 ± 0.035	1.40 ± 0.028	1.41 ± 0.022	1.35
P_5	1.14 ± 0.020	1.36 ± 0.019	1.40 ± 0.025	1.30
P_6	1.00 ± 0.018	1.30 ± 0.026	1.38 ± 0.038	1.23
P_7	0.69 ± 0.024	1.26 ± 0.030	1.31 ± 0.025	1.09
Mean	1.20	1.38	1.40	1.33
	P	**C**	**PxC**	
SEd	**0.008**	**0.005**	**0.014**	
CD(P=0.05)	**0.017****	**0.010****	**0.029****	

Values are mean ± standard error (n = 3)

Table 5. GR activity (μM reduced glutathione formed min.$^{-1}$ mg of protein^{-1}) of feedstock (groundnut seeds) stored under 3 different containers

Storage period (P)	Storage containers			
	C_1	C_2	C_3	Mean
P_0	1.18 ± 0.020	1.18 ± 0.020	1.18 ± 0.020	**1.18**
P_1	1.14 ± 0.016	1.16 ± 0.018	1.17 ± 0.015	**1.16**
P_2	1.10 ± 0.019	1.15 ± 0.025	1.16 ± 0.018	**1.14**
P_3	1.08 ± 0.024	1.12 ± 0.020	1.14 ± 0.016	**1.11**
P_4	1.04 ± 0.018	1.08 ± 0.016	1.12 ± 0.030	**1.08**
P_5	1.01 ± 0.010	1.06 ± 0.017	1.09 ± 0.022	**1.05**
P_6	0.98 ± 0.039	1.04 ± 0.025	1.05 ± 0.019	**1.02**
P_7	0.90 ± 0.043	1.02 ± 0.030	1.03 ± 0.025	**0.98**
Mean	**1.05**	**1.10**	**1.12**	**1.09**
	P	**C**	**PxC**	
SEd	**0.009**	**0.005**	**0.016**	
CD(P=0.05)	**0.018****	**NS**	**0.031****	

Values are mean ± standard error (n = 3)

Table 6. Mean weather data recorded during storage period of feedstock

Month	Temperature (˚C)		RH (%)		Sunshine hours
	Max.	Min.	Morning (7.30 AM)	Afternoon (2.30 PM)	
October, 2023	33.6	24.2	87	63	4.6
November, 2023	30.7	24.3	89	64	6.8
December, 2023	30.9	22.0	87	69	4.8
January, 2024	31.2	20.8	87	54	8.2
February, 2024	33.7	22.6	82	49	8.5
March, 2024	37.3	24.5	80	36	9.2
April, 2024	39.6	25.7	73	30	9.3

Table 7. Fatty acid profile of feedstock (groundnut seed) used in this experiment

S. No	Fatty acid name	Type	Area (%)
1.	Methyl octadecenoate and Methyl elaidate	Mono unsaturated fatty acid (MUFA)	41.43
2.	Methyl eicosenoate	Mono unsaturated fatty acid (MUFA)	2.51
		Sub total	**43.940**
3.	Methyl linolelaidate and Methyl octadecadienoate	Poly unsaturated fatty acid (PUFA)	28.35
		Sub total	**28.35**
4.	Methyl palmitate	Saturated fatty acid (SAT)	13.2
5.	Methyl stearate	Saturated fatty acid (SAT)	4.28
6.	Methyl arachidate and Methyl eicosanoate	Saturated fatty acid (SAT)	
7.	Methyl behenate	Saturated fatty acid (SAT)	5.03
8.	Methyl lignocerate	Saturated fatty acid (SAT)	2.77
		Sub total	**27.70**

Since oilseed crop production is a seasonal process, biodiesel feedstock (oilseeds) must be stored and supplied as needed to facilitate continuous biodiesel production and supply throughout the year. This study highlights that providing favorable storage conditions, such as using evacuated aluminum foil pouches (C_2) or 30% CO_2-infused plastic containers (C_3), ensures that feedstock stored for over seven months under tropical ambient conditions remains suitable for biodiesel production, meeting the biodiesel quality standards set by various countries.

Conclusion

The study demonstrated that storage conditions significantly impact the physiological and biochemical properties of groundnut seeds, which serve as biodiesel feedstock. Among the 3 storage methods evaluated, the CO_2-infused plastic container (C_3) consistently outperformed the others, maintaining higher seed viability (72%), maintaining enhanced antioxidant enzyme activities (SOD, CAT, and GR) and sustaining optimal dehydrogenase activity over a 7-month storage period.

Furthermore, biodiesel feedstock stored in C_3 exhibited superior fuel quality parameters, including lower free fatty acid content, acid value and peroxide value, alongside higher iodine and cetane numbers, making it suitable for biodiesel production as per international standards. The results highlight that prolonged storage in C_3 minimizes the detrimental effects of lipid oxidation, thereby preserving both seed viability and oil quality.

This cost-effective storage method offers a sustainable alternative to conventional practices, enabling the biodiesel industry to maintain a steady supply of high-quality feedstock under tropical conditions.

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Authors' contributions

TE carried out the experiment and drafted the original manuscript. KNN provided supervision, validation, reviewed and edited the manuscript. VV supplied the necessary resources. VKM carried out the experiment. JA participated in the analysis and interpretation of results. TNK participated in data validation. SB was in charge of visualization. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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