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# $^1$H NMR Spectroscopic Analysis of Lipid and Fatty Acid Modulations in Chironji Seed Oil Induced by Roasting and Auto Oxidation

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## Abstract
The study investigates the impact of roasting and auto- oxidation on the lipid composition of Chironji seed oil using $^1$H Nuclear Magnetic Resonance spectroscopy ($^1$H NMR). Chironji *(Buchanania lanzan Spreng.)* seeds, rich in fatty acids, proteins, and dietary fiber, hold significant potential in nutraceutical and food industries. The effect of roasting and auto oxidation on fatty acid contents were analysed. The roasting of Chironji seeds over the different time period exhibits the stability of fatty acids. In case of auto- oxidation of the seed oil, total increment in Free Fatty Acid (FFA) percentage was noted while Tri Acyl Glycerol (TAG) percentage reduced gradually with time of eight weeks. Auto - oxidation induces notable changes in lipid profiles, including TAG, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The study highlights the effectiveness of proton NMR spectroscopy for monitoring lipid changes during food processing, providing insights to optimize the storing conditions and preserve health-promoting compounds for applications in nutraceutical, food, and pharmaceutical sectors.

**Keywords**: Buchanania lanzan, Fatty acids, Roasting, Lipids, Metabolomics, $^1$H NMR.
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## Introduction
Nutraceuticals, a blend of 'nutrition' and 'pharmaceuticals refer to health enhancing products derived from food with benefits that include disease prevention and treatment. PUFAs especially have gained attention as nutraceuticals, for their roles in human health. These roles encompass regulating responses contributing to cell membrane flexibility and acting as precursors to bioactive lipid mediators [1][2]. The effectiveness of fatty acids as nutraceuticals can be impacted by temperature variations that influence their properties like availability and stability in the body [3].

Chironji (*Buchanania lanzan Spreng.*) a tree species, within the Anacardiaceae family holds value as it is adaptable and widely distributed across India, Burma, Bhutan and Nepal. The various parts of these occurring trees serve purposes related to addressing a range of health issues [4]. Chironji seeds contain a high calorific value of about 656 kcal per 100 g, due to their rich composition of fats, proteins and dietary fiber. Recent investigations have further demonstrated that *Buchanania lanzan* kernels are a rich source of lipids, proteins, carbohydrates, essential minerals, amino acids, and bioactive phytochemicals. The kernel oil is predominantly composed of oleic and linoleic acids, while the hydroalcoholic extracts exhibit considerable antioxidant activity and neuroprotective potential[5]. These seeds possess elements that can be utilized in the production of premium products such, as beverages, nutritious meals and supplements packed with oleic acid. Oleic acid plays a role in activating immune responses and mitigating inflammation effects [6]. Numerous studies highlight many health advantages of Chironji kernels, including its antidepressant, antioxidant, cough, leprotic, and anxiolytic qualities[7–9]. Chironji seeds are said to possess alkaloids, polyphenols, glycosides, phytosterols, sitosterol, and stigmasterol, which are responsible for their anti-inflammatory and antioxidant properties. These bioactive substances have a lot of potential uses in the food, pharmaceutical, and nutraceutical sectors[10].

A recent investigation systematically evaluated the nutraceutical potential of *Buchanania lanzan* seeds across six genetically distinct accessions, revealing significant variation in their nutritional composition, antioxidant activity, antidiabetic properties, mineral profile, and physicochemical characteristics of the seed oil. The study demonstrated that *B. lanzan* seeds are a rich source of proteins, lipids, essential minerals, vitamin C, and bioactive phytochemicals, underscoring their potential as a functional food with promising nutraceutical and industrial applications[11].

Deep frying is a widely favoured method of cooking in many regions, where it is a common practice for preparing numerous traditional dishes. Globally, it is not

uncommon for oils to be reheated and reused multiple times in homes, despite the significant health risks associated with this practice [12]. When oils are heated, various chemical reactions take place, such as the oxidation of different fatty acids and triacylglycerols, the creation of polymers or cyclic compounds, the evaporation of volatile substances, and the generation of polycyclic aromatic hydrocarbons. Additionally, during the heating process, there is typically a reduction in the PUFA content of vegetable oils, while the level of SFAs tends to rise [13].

In this context, the aim of this study is to analyse the fatty acid profile in Chironji seeds exposed to roasting temperature for different intervals of time, and the changes in fatty acids percentage of Chironji seeds oil, upon auto - oxidation[14]. The unique benefits of NMR spectroscopy-based metabolomics are demonstrated by its ability to simultaneously detect and measure several tiny molecule metabolites in a single experiment, eliminating the requirement for customized standards. Interestingly, NMR spectroscopy is widely used for lipid profiling of edible and non-edible oils, demonstrating its adaptability and usefulness in a variety of biochemical research fields[15,16].

To the best of our knowledge, this study is the first to systematically use $^{1}H$ NMR spectroscopy to examine how roasting and auto - oxidation affect Buchanania lanzan seed oil's lipid composition. The current work uses a quick and non-destructive NMR-based method for the simultaneous quantification of major lipid constituents and assessment of processing-induced lipid changes, in contrast to other research that mainly concentrated on fatty acid profiling using chromatographic techniques. The findings provide new insights into the thermal and oxidative stability of Chironji seed oil and its potential implications for food quality assessment and nutraceutical applications.

# Materials and methods

## Reagents used

Deuterated Chloroform $CDCl_3$, Deuterated Dimethyl sulfoxide DMSO (Cambridge Isotope Laboratories Inc.,USA), Carbon tetrachloride $CCl_4$, Hexane (AR. grade, LOBA)

## Extraction of Chironji (*Buchanania lanzan Spreng.*) seed oil

The seeds used in this study were purchased from the local market and then allowed to air dry until a consistent weight was reached. The seeds were ground in liquid nitrogen with a pre-cooled pestle and mortar to produce a 10 g sample of dry seeds. The resulting pulverized seeds then placed inside a 60 ml Soxhlet apparatus connected to a 100 ml round-bottom flask containing hexane, and kept in an electric mantle maintained at 50 – 55 $^{o}$C. About six hours were spent on the extraction procedure, which involved 25 to 30 solvent circulation cycles. A rotary evaporator was used to separate the seed oil once the extracted oil had been collected in the solvent. After being extracted from the rotary evaporator, the solvent-free oil was carefully transferred into a glass sample vial, sealed with black polythene bags, and kept at -20 °C for further examination[17].

## Sample oxidation

For roasting method, each sample had a precise weight of 10 grams, which were then put onto stainless steel plates with an 80 mm diameter. The plates were subsequently placed in a convection oven without lids to ensure the maximum exposure to air for different time intervals 15, 30, 45 and 60 minutes (**Figure 1**), which maintained at constant temperature of 180°C with an accuracy of ±0.5%. Each sample underwent the oxidation process in triplicates for each time interval before the extraction[18–20].

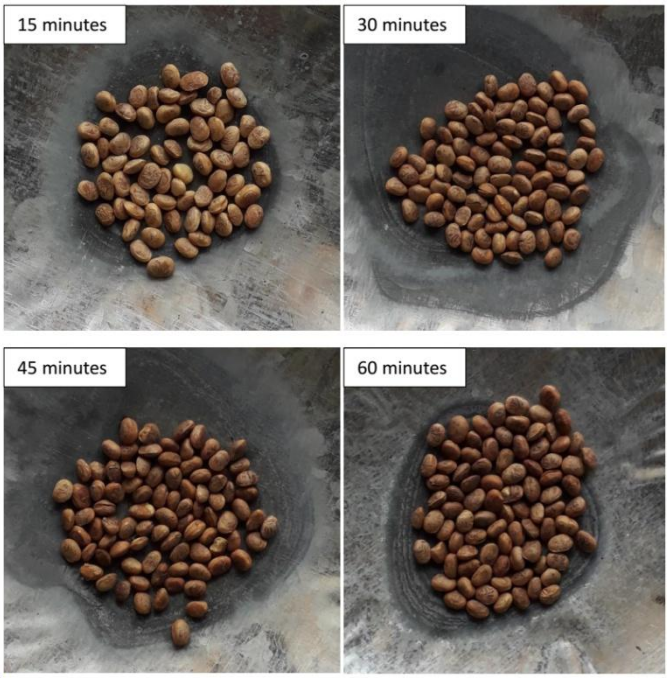


**Figure 1**: Roasted Chironji (*Buchanania lanzan Spreng.*) seed at different time intervals

Similarly, for auto - oxidation the same amount of dry sample was taken and subjected to Soxhlet extraction. The obtained oil placed in four different petri dishes covered with perforated filter paper under ambient laboratory conditions. The oil samples collected in two weeks of interval and subjected to NMR analysis in triplicates from each petri dishes.

**Table 1**: Percentage composition of fatty acids in the Unroasted and Roasted Chironji (*Buchanania lanzan Spreng*) seed at different time intervals

| Group | SFA (%) | PUFA (%) | MUFA (%) | USFA (%) | FFA (%) | TAG (%) |
|---|---|---|---|---|---|---|
| **Unroasted** | 39.42 ± 0.14$^{a}$ | 3.93 ± 0.14$^{a}$ | 56.65 ± 0.09$^{a}$ | 60.58 ± 0.14$^{a}$ | 1.17 ± 0.58$^{a}$ | 98.83 ± 0.58$^{a}$ |
| **15 min** | 39.06 ± 0.39$^{ab}$ | 4.48 ± 0.16$^{b}$ | 56.47 ± 0.31$^{a}$ | 60.94 ± 0.39$^{ab}$ | 1.83 ± 0.76$^{a}$ | 98.17 ± 0.76$^{a}$ |
| **30 min** | 38.55 ± 0.21$^{b}$ | 4.81 ± 0.20$^{b}$ | 56.64 ± 0.16$^{a}$ | 61.45 ± 0.21$^{b}$ | 1.17 ± 0.29$^{a}$ | 98.83 ± 0.29$^{a}$ |
| **45 min** | 38.25 ± 0.29$^{b}$ | 4.56 ± 0.41$^{b}$ | 57.20 ± 0.52$^{a}$ | 61.75 ± 0.29$^{b}$ | 1.50 ± 0.87$^{a}$ | 98.50 ± 0.87$^{a}$ |
| **60 min** | 38.61 ± 0.74$^{ab}$ | 4.43 ± 0.40$^{b}$ | 56.96 ± 1.01$^{a}$ | 61.39 ± 0.74$^{ab}$ | 1.17 ± 0.76$^{a}$ | 98.83 ± 0.76$^{a}$ |

* Values are expressed as mean ± SD (n = 3). Different superscript letters within a column indicate significant differences ($p < 0.05$, Tukey's HSD). SFA: Saturated Fatty Acid, PUFA: Poly Unsaturated Fatty Acid, MUFA: Mono Unsaturated Fatty Acid, USFA: Un-Saturated Fatty Acid, FFA: Free Fatty Acid, TAG: Tri Acyl Glycerol.

## NMR sample preparation

The resultant extracted oil (120 mg) dissolved in the corresponding deuterated solvent or solvent mixture (550 µL). $CDCl_3$ and mixture of $CCl_4$/DMSO-$d_6$ used as deuterated solvents[17,21]. The chemical shift of chloroform at 7.26 ppm and dimethylsulphoxide at 2.50 ppm used for referencing, respectively for solvent and solvent mixture.

## NMR spectroscopy

Hexane extracts of all the samples were subjected to $^1$H NMR spectroscopy, utilizing a JEOL advanced ECZ400S spectrometer, which was outfitted with a 5 mm TH5 Broadband Direct Probe and operated at a proton frequency of 400 MHz. One-dimensional one-pulse $^1$H NMR studies were carried out. A spectral width of 20-ppm, 16K time domain data points, a flip angle of 45 degrees, a 5-second relaxation delay, a 32K spectrum size, 64 scans, and a 0.2 Hz line broadening for the exponential window function were the experimental settings. All measurements were performed to obtain comprehensive $^1$H NMR spectra for the hexane extracts.

Results and discussion

## Identification and quantification of fatty acids in the unroasted and roasted conditions at different time intervals

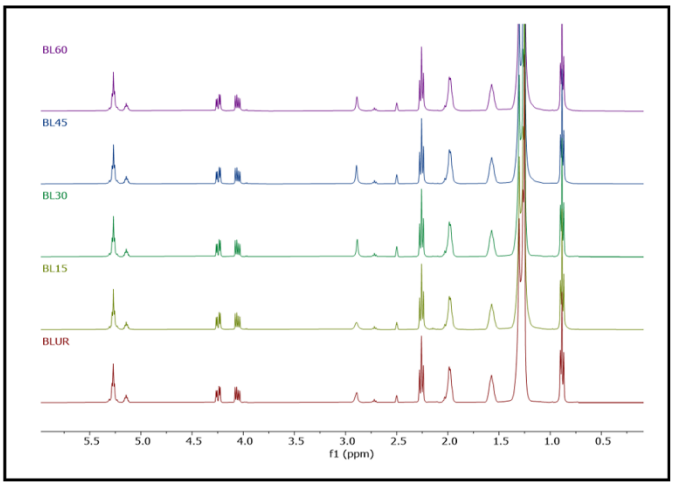


**Figure 2:** $^1$H NMR spectra of Chironji (***Buchanania Lanzan Spreng.***) seeds at Unroasted (BLUR) and different roasted timings

The representative $^1$H NMR spectra of hexane extract of *Buchanania lanzan Spreng.* seed under unroasted and roasted conditions are shown in **Figure 2**. Characterization and quantification of lipid components at different time intervals were done using the reported methods [22–29].

$^1$H NMR spectra revealed the presence of linolenic acid (18:3), linoleic acid (18:2), oleic acid (18:1) saturated fatty acids were detected. The results obtained from the spectral data are in good agreement with the previously published GC-MS based studies, demonstrating the reliability of the present analytical approach [30]. The percentage contribution of FFA, TAG, SFA, USFA MUFA and PUFA were estimated using $^1$H NMR spectra of different time intervals of roasted *Buchanania lanzan Spreng.* seed by measuring integral areas of the respective peaks. Area under the curve at 4.05 - 4.30 ppm of Sn1 and Sn3 was used for the estimation of TAG. The FFAs were then determined by extrapolating percentage contribution of TAG. The percentage estimation of SFA and USFA was calculated using allylic methyl signal at 2.02 ppm. MUFA and PUFA was estimated using diallylic proton signal at 2.71 ppm. The **Table 1** shows the percentage contribution of lipids at unroasted and four different time intervals of roasted stages.

PUFA levels remained higher than those in the unroasted samples, although a slight decline was observed at extended roasting durations (45 to 60 minutes). This pattern implies that the relative amount of PUFA may have increased by moderate roasting, perhaps as a result of structural changes or the stability of fatty acid classes at varying heat. In all roasting circumstances, the MUFA content varied from 56.47 ± 0.31% to 57.20 ± 0.52%, with no statistically significant differences. This suggests that in comparison to polyunsaturated fatty acids, MUFAs were comparatively stable at the applied roasting conditions, which is consistent with their known thermal resistance. The USFA content increased significantly with roasting time, peaking at 45 minutes (61.75 ± 0.29%). This rise is consistent with the noted drop in SFA,

indicating a change in the overall saturation profile of the oil. However, overlapping statistical categories showed that the variations between roasting intervals were moderate. **Figure 3** illustrates a bar graph showing the percentage variations of PUFA, MUFA, SFA and USFA across different roasting time intervals in minutes.

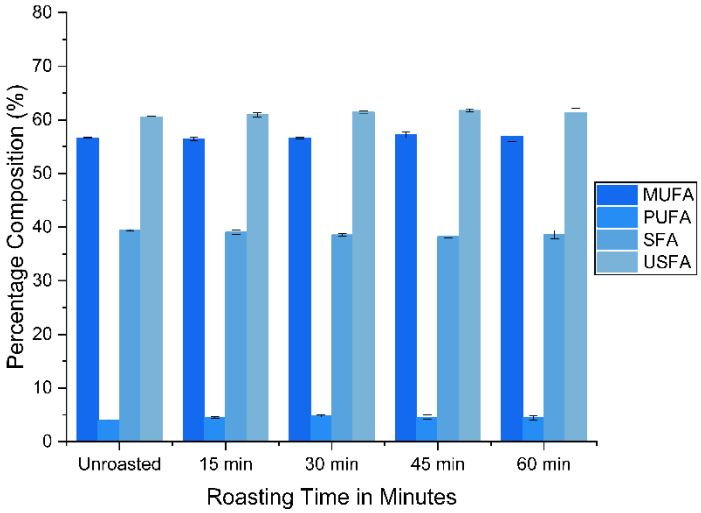


**Figure 3:** Percentage variations of PUFA, MUFA, SFA and USFA of Chironji seed oil against roasted time intervals in minutes

The range of FFA values was 1.17 ± 0.58% to 1.83 ± 0.76%, and there were no significant variations between roasting conditions ($p > 0.05$). The lack of statistical significance suggests that roasting under the investigated conditions does not significantly increase the hydrolytic breakdown of lipids, despite minor variations being noted. TAG content remained comparatively stable throughout all the samples (98.17- 98.83%) with no statistically significant fluctuation ($p > 0.05$). This implies that the primary lipid structure was largely maintained during roasting, with no significant degradation of triacylglycerols within the studied time range. **Figure 4** represents the percentage variations of TAG and FFA with respect to roasting time intervals in minutes.

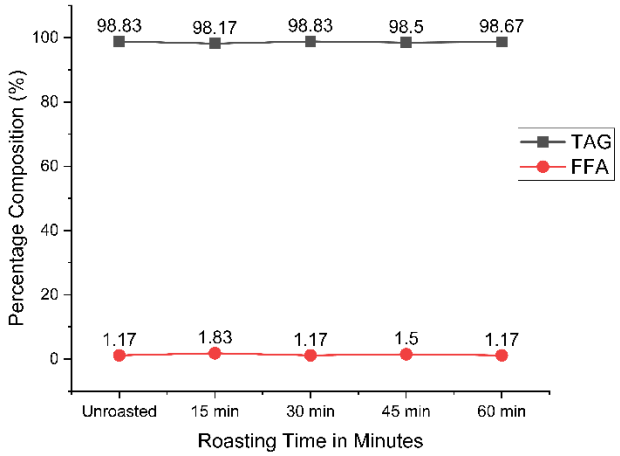


**Figure 4:** Percentage variations of TAG and FFA against roasted time intervals in minutes.

The results show that roasting modifies the fatty acid saturation profile in a moderate but statistically significant way, especially influencing the proportions of SFA, PUFA, and USFA. A shift toward a more unsaturated lipid profile with longer roasting times is suggested by the drop in SFA and matching rise in USFAs. The overall stability of the lipid matrix, even during prolonged heat treatment, highlighted by the notable changes in MUFA, FFA, and TAG. From a nutritional and industrial standpoint shows inverse relation, this stability is especially significant because it implies that roasting does not negatively impact the basic lipid integrity of *Buchanania lanzan* seed oil. Notably, the degree of difference across groups was rather small, despite the fact that statistical significance was noted for certain measures. This suggests that roasting mostly results in minor compositional changes rather than significant changes, which is in line with the regulated roasting parameters used in this investigation.

The observed patterns show how sensitive the used NMR based technique is to even the smallest changes in lipid content. The efficacy of this method for tracking the effects of thermal processing in edible oils is demonstrated by the capacity to record statistically significant changes in SFA, PUFA, and USFA. From the unroasted sample (39.42 ± 0.14%) to the 45-minute roasting sample (38.35 ± 0.29%), the SFA concentration gradually decreased until slightly increasing at 60 minutes (38.61 ± 0.74%). When compared to the unroasted sample, the decrease in SFA at intermediate roasting intervals (30-45 minute) was statistically significant. Thermal degradation or change of saturated lipid fractions during extended heating could be the cause of this decline. After roasting, the PUFA content rose dramatically, from 3.93 ± 0.14 % in unroasted samples to a high of 4.81± 0.20% at 30 minutes (USFA) ($p < 0.05$). In contrast, MUFA, TAG, and FFA did not show statistically significant variation ($p > 0.05$).

## Identification and quantification of fatty acids in the auto - oxidation conditions at different time intervals

$^1$H NMR spectra of samples obtained (**Figure 5**) from auto - oxidation study showed an inverse relationship in percentage contribution of TAG and FFA from two weeks to eight weeks, as represented in the **Figure 6**. TAG values undergone gradual reduction from 98.83% to 92.83% as the Chironji seed oil subjected to air and sunlight for longer duration of 8 weeks.

**Table 2:** *Percentage composition of fatty acids subjected to auto oxidation of Chironji (*Buchanania lanzan Spreng.*) seed at different time intervals in weeks

| Week | SFA (%) | PUFA (%) | MUFA (%) | USFA (%) | FFA (%) | TAG (%) |
|---|---|---|---|---|---|---|
| **0 Week** | 39.42 ± 0.14$^{a}$ | 3.93 ± 0.14$^{a}$ | 56.65 ± 0.09$^{a}$ | 60.58 ± 0.14$^{a}$ | 1.17 ± 0.58$^{a}$ | 98.83 ± 0.58$^{a}$ |
| **2 Week** | 35.58 ± 0.54$^{c}$ | 5.59 ± 0.38$^{c}$ | 58.83 ± 0.34$^{ab}$ | 64.42 ± 0.54$^{c}$ | 2.00 ± 0.00$^{b}$ | 98.00 ± 0.00$^{a}$ |
| **4 Week** | 35.97 ± 1.29$^{c}$ | 5.07 ± 0.24$^{b}$ | 58.96 ± 1.33$^{ab}$ | 64.03 ± 1.29$^{bc}$ | 4.83 ± 0.76$^{c}$ | 95.17 ± 0.76$^{b}$ |
| **6 Week** | 36.50 ± 0.38$^{bc}$ | 4.52 ± 0.47$^{ab}$ | 58.98 ± 0.73$^{ab}$ | 63.50 ± 0.38$^{b}$ | 4.00 ± 0.50$^{c}$ | 96.00 ± 0.50$^{b}$ |
| **8 Week** | 37.36 ± 0.13$^{b}$ | 4.51 ± 0.26$^{ab}$ | 58.13 ± 0.39$^{ab}$ | 62.64 ± 0.13$^{b}$ | 7.17 ± 0.29$^{d}$ | 92.83 ± 0.29$^{c}$ |

*Values are mean ± SD (n = 3). Different superscript letters indicate significant differences ($p < 0.05$, Tukey's HSD). SFA: Saturated Fatty Acid, PUFA: Poly Unsaturated Fatty Acid, MUFA: Mono Unsaturated Fatty Acid, USFA: Un-Saturated Fatty Acid, FFA: Free Fatty Acid, TAG: Tri Acyl Glycerol.

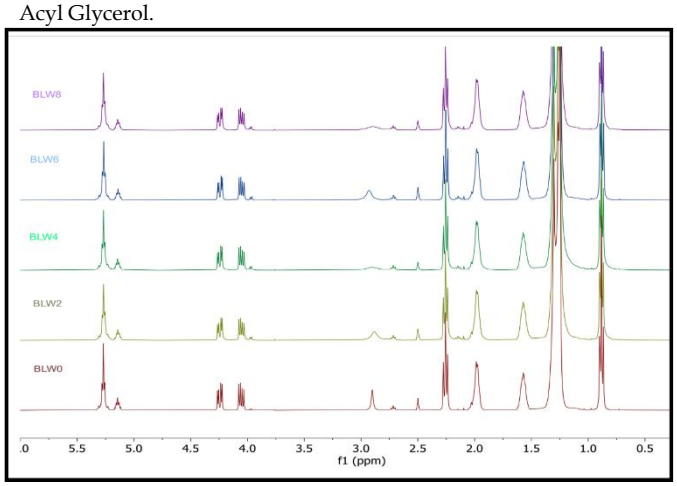


**Figure 5:** $^{1}$H NMR spectra of Chironji (***Buchanania Lanzan*** *Spreng.*) seeds subjected to auto oxidation at different time intervals in weeks

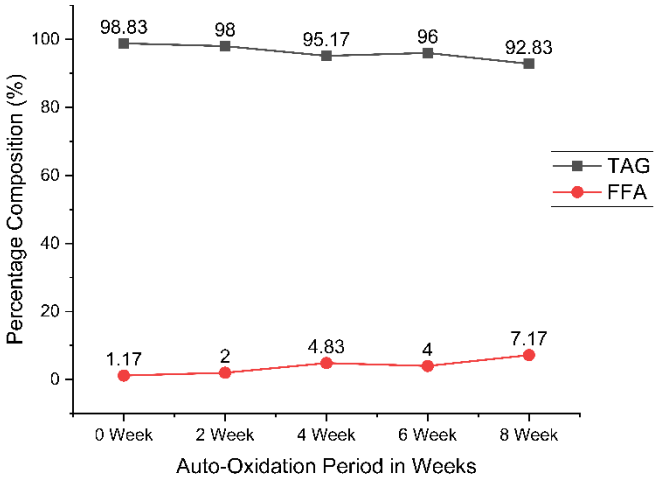


**Figure 6:** Percentage variations of TAG and FFA against auto - oxidation, time intervals in weeks

However, percentage of free fatty acid noted to be raised from 1.17 to 7.17 as the oil exposed to ambient conditions for the period of 8 weeks. Regression analysis demonstrated a strong linear relationship between the storage time and FFA levels. When the oil was subjected to oxidative storage, the TAG present in it, may undergo ester hydrolysis, resulting in generation of free fatty acids. It automatically raises overall FFA percentage in oil. On the other hand, the effect of auto- oxidation on PUFA and MUFA found to be remained largely unchanged as depicted in **Figure 7**. It represents the stability of PUFA and MUFA towards air and light. In case of saturated fatty acid, a gentle rise in its percentage has been observed from two weeks to eight weeks of duration. In contrast, a tiny decrease in percentage of USFA was noted (**Table 2**); this oxygen-induced degradation may have reduced the stability, nutritional quality and shelf life.

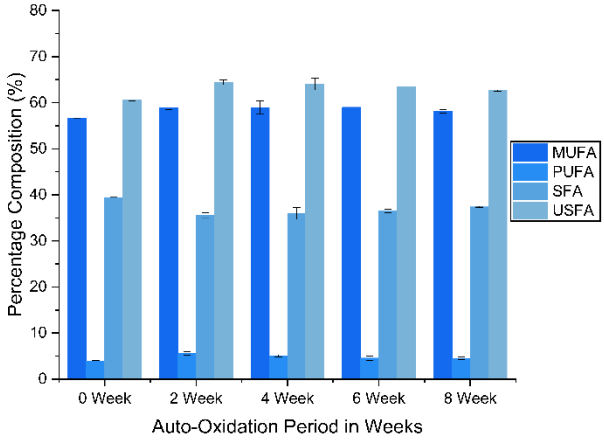


**Figure 7:** Percentage variations of PUFA, MUFA, SFA and USFA of Chironji seed oil against auto - oxidation, time intervals in weeks

Roasting induced only a mild variation in the fatty acid compositions, whereas storage under aerial condition resulted in a pronounced oxidative degradation. The rise in free fatty acid and reduction in triacylglycerols during the aerobic storage significantly greater than those observed during the thermal processing, which highlights that aerobic oxidation has significant impact on the stability of lipids than roasting. Consequently, storage under aerial condition accelerates the oxidative degradation, leading to increased rancidity.

## Kinetic Comparison of Lipid Oxidation

The kinetics of lipid degradation during storage were further studied by comparing the time-based changes in the FFA and TAG. Regression analysis revealed the clear positive linear relationship between the storage time and FFA levels, suggesting a steady increment in the hydrolytic degradation products (**Figure 8a**). TAG, on the other hand, showed a negative linear trend, indicating a

gradual disintegration of intact lipid molecules (**Figure 8b**). The concurrent rise in FFA and fall in TAG, demonstrates that during aerial exposure, lipid oxidation happens via hydrolysis and subsequent triacylglycerol breakdown. The rate of FFA formation was noticeably higher than the rate of TAG depletion, indicating that secondary processes such as oxidation and fatty acid release play a major role in the observed alterations. These results demonstrate a coordinated kinetic link between oxidation and lipid degradation, supporting the susceptibility of *Buchanania lanzan* seed oil to storage - induced deterioration.

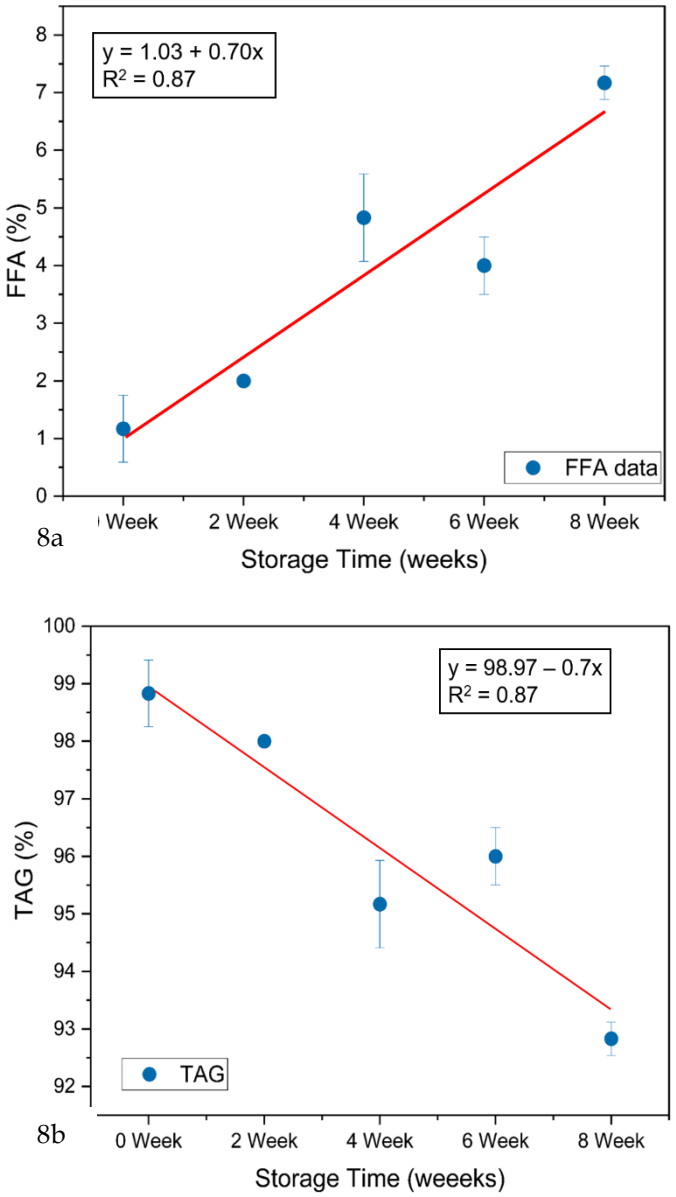


**Figure 8. (a)** linear increase in FFA content with storage time, indicating progressive lipid hydrolysis, **(b)** linear decrease in TAG content with storage time, reflecting gradual lipid degradation

The current study has a number of advantages. $^1$H NMR spectroscopy may be used for simultaneous lipid profiling without derivatization or significant sample preparation. It is a quick, non-destructive, and repeatable method. A thorough analysis of the stability of Chironji seed oil under processing and storage circumstances is provided by the comparison of roasting and auto-oxidation. Lastly, the observed consistency between the current results and previously published chromatographic investigations highlights the usefulness of the NMR-based analytical approach for evaluating the quality of edible oils while also supporting its dependability.

## Conclusions

This study demonstrates that roasting *Buchanania lanzan* seeds at 180 °C for up to 60 min induces only minor changes in the lipid composition, indicating that the major fatty acid classes remain largely stable under the investigated roasting conditions. In contrast, prolonged auto - oxidation resulted in a progressive increase in FFA content accompanied by a gradual decline in TAGs, indicating oxidative deterioration during storage. The results further demonstrate that $^1$H NMR spectroscopy is a rapid, non-destructive, and reliable analytical technique for the simultaneous characterization and quantification of lipid constituents in Chironji seed oil. The close agreement of the obtained lipid profile with previously reported chromatographic studies further validates the applicability of the NMR-based approach. These findings provide valuable insights into the thermal and oxidative stability of *B. lanzan* seed oil and may contribute to quality assessment, optimization of storage conditions, and its potential utilization in food and nutraceutical applications.

## Limitations of the study

The present study has certain limitations. The investigation was conducted using seed oil obtained from a single Chironji seed source and primarily focused on lipid compositional changes determined by $^1$H NMR spectroscopy. Complementary oxidation indices, such as peroxide value, anisidine value, and volatile oxidation products, were not evaluated. Future studies involving multiple geographical accessions, complementary analytical techniques, and extended storage conditions would provide a more comprehensive understanding of the oxidative stability, quality, and shelf life of *Buchanania lanzan* seed oil.

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## Declaration of competing interest

No competing interests were disclosed

## Funding sources

The author(s) declared that no grants were involved in supporting this work.

## Authorship contribution statement

P M: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft. S A: Project administration, Writing – review & editing. S S: Formal analysis, Investigation, Methodology. J K: Data interpretation, Writing – review & editing.

"All authors read and approved the final manuscript."

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