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Assessing the Impact of Heat on alpha-tocopherol Content in Cold – Pressed Groundnut Oil

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HIGHLIGHTS

- Extraction efficiency of vitamin E using 90% methanol in oil sample was 100%.
- The saponification of the oil sample was optimized using sodium hydroxide, with a recovery of 99.8±0.55%.
- Quantitative analysis of vitamin E in 1 mlof oil sample yielded 782.04±55µg/ml or 78.2±5.5 mg/100 ml.
- Alpha-Tocopherol was highly heat sensitive, with major degradation beyond ~180°C, supporting strict thermal control in use and storage.

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Abbreviations
SD= Standard Deviation

ABSTRACT

Background: Vitamin E is a vital nutrient with strong antioxidant properties that plays an important role in maintaining various functions in the human body. Cooking processes such as frying, baking, and boiling can lead to the loss of alpha-tocopherol, significantly reducing its bioavailability in food products. The aim of the current study was to ascertain the actual alpha-tocopherol content in groundnut oil and evaluate its stability after heating to cooking temperature.

Methods: The wavelength maxima of alpha-tocopherol were evaluated using UV-Visible spectroscopy, and the oil sample was saponified and analyzed for alpha-tocopherol content. A method was developed for the extraction and quantification of alpha-tocopherol using UV-Visible spectroscopy. The oil sample was subjected to 100, 150, and 200°C and quantified for alpha-tocopherol content using the developed method**.**

Results: The absorption maximum (λmax) of alpha-tocopherol was 284.5 nm and obeyed the Beer-Lambert law. The calibration curve showed linearity between 50 and 350 µg/ml, and the limit of detection (LOD) and limit of quantification (LOQ) were found to be 8.24 µg/ml and 25 µg/ml, respectively. The method was validated according to ICH standards, and the percent recovery was 99–100% with a standard deviation (SD) of less than 1%. As the cooking temperature increased, the concentration of alpha-tocopherol declined, starting from 738.27 ± 9.26 µg/ml at 100°C, dropping to 680.67 ± 14.76 µg/ml at 150°C, and reaching 585.95 ± 16.30 µg/ml at 200°C. One-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics version 22.0, which demonstrated a statistically significant difference between the groups.

Conclusion: This study unequivocally establishes alpha-tocopherol as highly heat-sensitive, with degradation exacerbated by prolonged exposure to elevated cooking temperatures. A significant drop in the concentration of alpha-tocopherol at 100°C indicates early oxidative degradation, and more than 50% loss was noted at 200°C.

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Introduction

Vitamin E is a vital nutrient noted for its strong antioxidant properties and plays an important role in maintaining various functions in the human body. It was first identified as a vital nutrient for reproductive health by Evans and Bishop in 1922. Vitamin E is not a single compound but a group of naturally occurring substances collectively called tocochromanols, which include tocopherols and tocotrienols. Among these, alpha-tocopherol is the most biologically active and widely distributed form in the human body because of its higher affinity for the alpha-tocopherol transfer protein. The primary function of alpha-tocopherol is to act as a potent antioxidant, neutralizing free radicals and preventing lipid peroxidation in cell membranes. This protective role is critical in decreasing the risk of various diseases, including cardiovascular disease, cancer, and neurodegenerative diseases such as Alzheimer's disease (Rizvi et al., 2014). Alpha-tocopherol is also necessary for both males and females in the function and development of reproductive organs because of its major role in the modulation of antioxidant balance (Alta'ee, Majeed and Hadwan, 2018). Vitamin E, along with lansoprazole, shows synergistic effects in the treatment of ulcers (Mahurkar and Kumar, 2011).

Vitamin E refers to a group of eight similar compounds: four tocopherols (α, β, γ, and δ) and four tocotrienols (α, β, γ, and δ). Historically, only alpha-tocopherol has been recognized as "Vitamin E" in nutrition and medicine. This focus on alpha-tocopherol is because it is the most common in human tissues, has strong antioxidant properties, and is well-known for its biological functions and availability (Brigelius-Flohé and Traber, 1999).

Agricultural conditions and climate strongly affect alpha-tocopherol content in oilseeds and oils. In peanuts grown across regions, temperature, humidity, and geographic origin significantly influenced tocopherol levels. Higher temperatures and humidity increased tocopherol levels, including alpha-tocopherol, whereas cooler conditions favored sucrose and phenolic compounds. These findings indicate that climate, location, and genetics largely determine alpha-tocopherol content (Wang et al., 2025).

In cold-pressed sunflower oils, the climate during seed development also alters the antioxidant composition, including tocopherols. Thus, genotype and environmental conditions, especially temperature, sunlight, and humidity, are key drivers of tocopherol accumulation and oil nutritional and oxidative stability (Lužaić et al., 2026). Cold-pressed cactus pear seed oils are also rich in tocopherols, with one species (Opuntia robusta) ranking second after soybean oil. Marula, sunflower, canola, and sesame generally contain less alpha-tocopherol, and their composition is further influenced by processing and storage (Ahmed et al., 2024; Nematshema et al., 2026).

Unconventional oils, including camelina, black cumin, flaxseed, and milk thistle, also vary in tocopherol content, with total tocopherols often present at significant levels, contributing to their antioxidant properties and potential health benefits. Alpha-tocopherol dominance depends on the species and processing methods (Kamińska et al., 2025).

Groundnut oil is used in cooking processes such as frying. The oil is rich in triterpenic and aliphatic alcohols, triacylglycerols, waxes, phospholipids, sterols, pigments, tocopherols, phenolic compounds, tocotrienols, and volatile compounds. The phenolic compound content in peanuts varies depending on the type, product, and processing. Resveratrol levels in fresh peanuts range from 0.01 to 4.5 µg/g (Davis et al., 2016). Cinnamic acid, sinapic acid, vanillic acid, genistein, ferulic acid, catechin, apigenin, hydroxycinnamic acid, and epicatechin have all been identified in peanut seeds. The skin of the peanut also contains epigallocatechin, trans-resveratrol, protocatechuic acid, epicatechin, catechin, p-coumaric acid, and quercetin (Idrissi et al., 2022). The exact oil composition of cold-pressed groundnut oil, focusing on the detailed fatty acid profile, typically includes components such as saturated fatty acids like palmitic acid (8–14%) and stearic acid (2–5%); monounsaturated fatty acids like oleic acid (45–60%); polyunsaturated fatty acids like linoleic acid (20–35%) and alpha-linolenic acid (<1%); and other minor components such as trans fats, omega-3 fatty acids, omega-6 fatty acids, tocopherols, tocotrienols, and phytosterols (variable) (Senn, 1969).

Cooking processes such as frying, baking, and boiling can lead to the loss of alpha-tocopherol, significantly reducing its bioavailability in food products. The extent of degradation largely depends on the temperature, duration of heating, type of oil used, and presence of antioxidants. Studies have shown that vitamin E stability is better preserved in oils rich in monounsaturated fatty acids, such as olive and peanut oils, than in polyunsaturated oils, such as soybean and sunflower oils, which are more prone to oxidation. The thermal degradation of vitamin E occurs through autoxidation, where heat exposure accelerates the formation of lipid peroxides, leading to the breakdown of tocopherols. Among the many tocopherol isomers, alpha-tocopherol is more susceptible to oxidation than γ- and δ-tocopherols, which have potent antioxidant properties. Additionally, the cooking technique affects the stability of alpha-tocopherol. Because the oil is directly exposed to heat and oxygen, frying at high temperatures typically results in greater losses than boiling or baking. However, these losses can be reduced and the shelf life of vitamin E–

rich oils increased by adding synthetic stabilizers or natural antioxidants during processing. Vitamin E is an essential nutrient with several health benefits owing to its antioxidant properties. However, its stability during food preparation and heating continues to be a major obstacle (Pokorný et al., 2003). The aim of the current study was to determine the alpha-tocopherol content in groundnut oil and evaluate its stability after heating to cooking temperatures.

Materials and methods

Materials and instruments

The samples used were alpha-tocopherol and vitamin C (KNISS Laboratories Private Limited, Chennai). Cold-pressed groundnut oil was purchased from a local market in Othakuthirai, Erode district, Tamil Nadu, India. The oil was obtained by cold pressing, which involved mechanically pressing the groundnut seeds without the use of chemical solvents or deliberate heating. The pressing was performed under controlled low-temperature conditions below approximately 40–50°C to minimize heat-induced changes. All chemicals and reagents used were methanol (EMPLURA® grade, Merck, Germany), hexane (EMPLURA® grade, Merck, Germany), ethyl acetate (EMPLURA® grade, Merck, Germany), ethanol (EMPLURA® grade, Merck, Germany), sodium hydroxide (NaOH) (EMPLURA® grade, Merck, Germany), and sodium chloride (NaCl) (EMPLURA® grade, Merck, Germany). A UV-Visible spectrophotometer (Shimadzu, Japan) was used to measure the absorbance of the samples.

Preparation of standard stock solution of Vitamin E

Alpha-tocopherol (0.1 g) was transferred to a 100 ml amber-colored standard flask, and the volume was adjusted to 100 ml using 90% methanol. The mixture was then sonicated for 5 min to completely dissolve it and achieve a final concentration of 1000 µg/ml (ÖzgülArtuç, 2020).

Selection of wavelength

The λmax for the analysis was selected from the UV spectrum by scanning the sample between 200 and 400 nm using a standard solution of 100 µg/ml of alpha-tocopherol. The λmax was recorded (ÖzgülArtuç, 2020).

Preparation of calibration graph

To achieve a final concentration of 50–350 µg/ml of alpha-tocopherol, 0.5, 1, 1.5, 2, 2.5, 3, and 3.5 ml of the stock solution were taken and brought up to the mark with 90% methanol in separate 10 ml amber-colored standard flasks. The λmax was used to record the absorbance data for each sample, and the concentration against absorbance was plotted to create the calibration curve (ÖzgülArtuç, 2020).

Sample pretreatment (saponification)

An accurately measured volume of 1 ml of oil sample was introduced into a 10 ml amber-colored standard flask, and the quantity was adjusted to 10 ml using hexane. Then, 0.05 g of vitamin C was added to prevent oxidative degradation, and the mixture was shaken for 10 min to facilitate homogeneous mixing. Subsequently, 0.2 ml of 50% NaOH solution was added. The mixture was then transferred to a 100 ml volumetric flask. The flask was then exposed to controlled heating in a water bath at 70°C for 30 min. After heating, the mixture was allowed to cool for 10 min. Subsequently, 0.2 ml of 50% NaCl solution was added. In this way, alpha-tocopherol was isolated from the oil matrix and could be extracted effectively (Beldean-Galea, Horga and Coman, 2010).

Beldean-Galea, Horga, and Coman optimized the saponification process by systematically investigating the impact of NaOH concentration on alpha-tocopherol stability and recovery during extraction from vegetable oils. Alpha-tocopherol is highly susceptible to oxidative degradation under alkaline conditions, requiring precise optimization of NaOH concentration to attain effective saponification with minimal oxidative degradation of alpha-tocopherol. This study confirmed the recovery of alpha-tocopherol, demonstrating near-complete recovery with minimal losses. Approximately 99.6% of alpha-tocopherol was recovered with negligible loss when using a volume of 1.5 ml NaOH (Beldean-Galea, Horga and Coman, 2010).

Similarly, this study aimed to optimize the amount of NaOH required for saponification without significantly weakening alpha-tocopherol stability. Aliquots of 0.5, 0.4, 0.3, and 0.2 ml of 50% NaOH solutions were separately introduced into six 10 ml amber-colored volumetric flasks, each containing 100 µg/ml of alpha-tocopherol in hexane. Accurately weighed 0.05 g of ascorbic acid was added to all flasks. The mixture was stirred for 5 min to ensure uniform mixing. The solution was then transferred to a separating funnel, and 25 ml of 90% methanol was added. The mixture was shaken vigorously for 30 min and then left undisturbed for 10 min to allow phase separation. The methanolic layer was separated and evaporated to dryness at ambient temperature. The resulting residue was dissolved in a 90% methanol solvent mixture and transferred to a 10 ml amber-colored volumetric flask for further analysis.

Liquid-Liquid extraction

Liquid-liquid extraction presents numerous benefits in contrast to other extraction methods, particularly when juxtaposed with traditional solid-liquid extraction and

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modern non-conventional approaches. The benefits of liquid-liquid extraction encompass selectivity, efficiency, and sustainability, which render it an important technique in diverse chemical and biochemical applications (Pramanik et al., 2024).

The saponified sample mixture was extracted three times in a separating funnel using 25 ml of a 90% methanol solvent mixture. The methanolic layer was then carefully separated and allowed to evaporate until dry. The obtained residue was dissolved in 10 ml of a 90% methanol solvent mixture. The resulting solution was centrifuged at 1000 RPM for 10 min. The supernatant was analyzed using a UV-Visible spectrophotometer. Furthermore, interpolation of the absorbance data onto the calibration curve enabled the precise quantification of the analyte, that is, alpha-tocopherol, in terms of micrograms per milliliter. To identify the efficiency of the extraction procedure developed, a known quantity of alpha-tocopherol was dissolved in hexane and extracted using the same procedure as mentioned above and measured for absorbance and quantified (Lee and Kumar, 2009).

Thermal stability of alpha-tocopherol

To evaluate the thermal stability of alpha-tocopherol, the oil sample was subjected to systematic heating at three distinct temperature levels. Initially, 50 ml of oil was heated at 100°C for 30 min under controlled conditions. After heating, 1 ml of the heated oil sample was collected and diluted to 10 ml using hexane in an amber-colored volumetric flask, and the sample was then processed using the pretreatment protocol for further analysis. Subsequently, another 50 ml of oil sample was heated at 150°C for 30 min under controlled conditions, and another 50 ml of oil sample was heated at a higher temperature of 200°C for 30 min to assess the extent of thermal degradation. All samples were processed using the pretreatment protocol. All three samples were extracted using liquid-liquid extraction and analyzed (Fairoosa et al., 2019; Nagamalleswari et al., 2019; Steinhart and Rathjen, 2003). The statistical significance between temperature and concentration was determined using one-way ANOVA using IBM SPSS Statistics version 22.0.

Validation

The process primarily involves revealing that an analytical procedure is performed as planned. Therefore, the established method was validated according to International Council for Harmonisation (ICH Q2(R2)) standards, including factors such as linearity, accuracy, precision, limit of detection and limit of quantification, robustness, and ruggedness (Ravisankar et al., 2015; Rina, Baile and Jain, 2021; Sharma, Goyal and Chauhan, 2018).

Results and discussion

As shown in Figure 1, spectrophotometric analysis identified 284.5 nm as the ideal λmax for alpha-tocopherol quantification, exhibiting maximal absorbance and specificity, which almost matches previously reported data (Beldean-Galea, Horga and Coman, 2010). This selection aligns with the established spectral profiles of tocopherols, ensuring precise detection with minimal interference from other oil constituents. The robustness of this wavelength for alpha-tocopherol analysis reinforces its applicability in stability assessments.

Figure 2 presents the overlaid UV–Visible absorption spectra of alpha-tocopherol at five different concentrations ranging from 100 to 800 µg/ml. All spectra exhibit a similar spectral profile, and, importantly, the λmax remains at the same wavelength for all concentrations. This indicates that increasing the concentration of alpha-tocopherol does not cause any significant shift in its characteristic absorption maximum within the investigated concentration range.

As the concentration of alpha-tocopherol increases, a progressive increase in absorbance intensity is observed at the λmax. The spectra corresponding to the different concentrations therefore maintain the same characteristic absorption pattern while differing primarily in the magnitude of absorbance. This concentration-dependent increase in absorbance indicates that the analytical response is proportional to the concentration of alpha-tocopherol over the studied range.

The observed spectral behavior supports the applicability of the Beer–Lambert relationship, in which absorbance increases proportionally with analyte concentration when other experimental conditions, such as wavelength, optical path length, and solvent, are maintained constant. The corresponding calibration curve of absorbance versus alpha-tocopherol concentration was therefore used to establish the linearity of the analytical method.

Thus, Figure 2 confirms the characteristic λmax of alpha-tocopherol and demonstrates the progressive increase in spectral absorbance with increasing concentration, providing the spectral basis for the subsequent linearity assessment of the developed UV–Visible spectrophotometric method.

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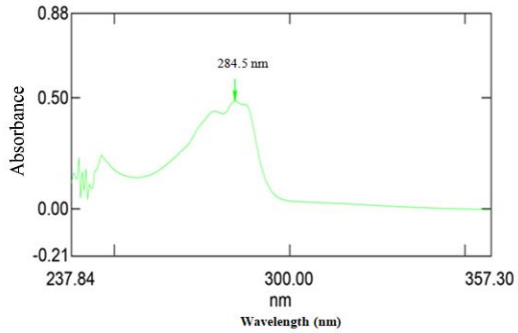


Figure 1: UV-Visible absorption spectrum and maximum absorption wavelength of alpha-tocopherol

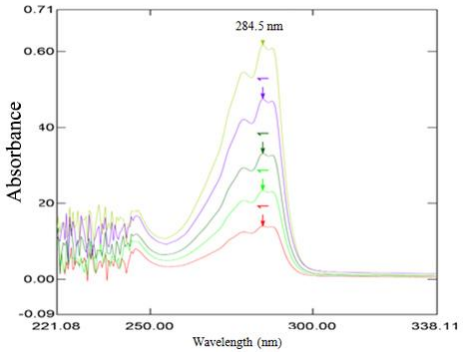


Figure 2: Overlay UV-Visible absorption spectra of alpha-tocopherol at different concentrations (100-800 µg)

The precision and reproducibility of the method were demonstrated by the calibration curve shown in Figure 3, which showed remarkable linearity throughout the concentration range of 50–350 µg/ml. These results confirm that the developed method is sensitive and accurate for measuring alpha-tocopherol content. The parameters of the calibration curve are listed in Table 1.

Table 1: Statistical parameters of the calibration curve for alpha-tocopherol determination

Maximum Wavelength (nm)	Slope	Intercept	Correlation coefficient (R^2)
284.5	0.0023	0.0058	0.9927

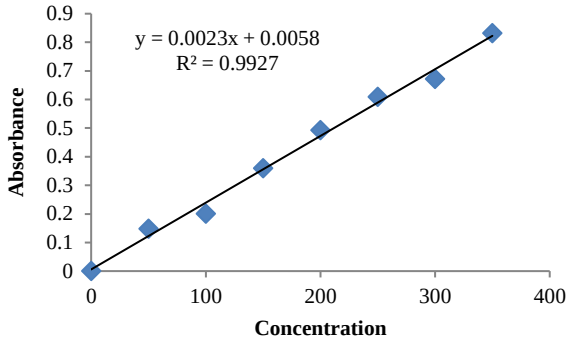


Figure 3: Calibration curve of alpha-tocopherol

Extraction efficiency

With a high extraction efficiency of 100% and minimal oxidative degradation, alpha-tocopherol was carefully separated from hexane using liquid-liquid extraction with 90% methanol. Hexane, the organic layer, maintained a consistent alpha-tocopherol profile, verifying the extraction process performance. Triglycerides were hydrolyzed by saponification with aqueous NaOH, which effectively released free alpha-tocopherol for accurate measurement. This method was also used to extract alpha-tocopherol from heated oil samples because it produced a 100% recovery of alpha-tocopherol from hexane.

Optimization of saponification

The results are presented in Table 2. The results showed an inverse relationship between the NaOH concentration and alpha-tocopherol retention. The volume of 50% NaOH used for saponification was determined to be 0.2 ml based on these results, guaranteeing efficient saponification while preserving the highest alpha-tocopherol stability. This concentration was chosen to reduce oxidative deterioration and avoid excessive loss of alpha-tocopherol. Furthermore, saponification of fatty materials requires more than 0.2 ml of 50% NaOH.

Table 2: Optimization of saponification in groundnut oil

Compound	Amount present (µg/ml)	Volume of 50% NaOH added (ml)	Amount found (µg/ml)	Mean ± SD (µg/ml)	Recovery (%)
alpha-tocopherol	100	0.2	100 98.8 99.9 100 99.7 100	99.8 ± 0.55	99.8 ± 0.55
		0.3	92.1 93.1 93.3 91.9 92.3 92.2	92.5 ± 0.57	92.5 ± 0.57

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Compound	Amount present (µg/ml)	Volume of 50% NaOH added (ml)	Amount found (µg/ml)	Mean ± SD (µg/ml)	Recovery (%)
		0.4	86.6 86.9 86.4 86.5 86.2 86.3	86.5 ± 0.24	86.5 ± 0.24
		0.5	81.8 81.6 81.7 82.1 81.5 81.9	82 ± 0.21	82 ± 0.21

Quantitative analysis

Effective sample pretreatment optimizes the extraction process by removing matrix interference. A concentration of 782.04 ± 55 µg/ml of alpha-tocopherol, or 78.2 ± 5.5 mg/100 ml, was obtained from a 1 ml oil sample that underwent sample pretreatment. The results of the quantitative analysis are presented in Table 3. Samples A–F represent six independent replicate preparations of the same sample, analyzed under identical experimental conditions to determine the average concentration of alpha-tocopherol in oil samples.

Thermal degradation kinetics of alpha-Tocopherol

Alpha-tocopherol showed strong vulnerability to heat-induced oxidation, and a distinct temperature-dependent degradation trend was observed. A significant drop at 100°C indicates early oxidative degradation. At 150°C, structural instability after prolonged heating is indicated by accelerated degradation. The thermal fragility of alpha-tocopherol in the oil matrix was confirmed by severe breakdown (>50% loss) at 200°C. The findings are presented in Table 4.

Table 3: Quantitative analysis of alpha-tocopherol in oil samples

Sample	Absorbance	Concentration (µg/ml)
A	1.690	732.26
B	1.920	832.26
C	1.689	731.82
D	1.691	732.69
E	1.918	831.39
F	1.919	831.82
Mean ± SD (µg/ml)		782.04 ± 55

Table 4: Thermal degradation of alpha-tocopherol in oil sample (1 ml) at three different temperatures

Sample	Temperature (ºC)	Absorbance	Concentration (µg/ml)	Mean ± SD (µg/ml)
1	100	1.725 1.707 1.687 1.732 1.693 1.679	747.47 739.65 730.95 750.52 733.56 727.47	738.27 ± 9.26
2	150	1.598 1.576 1.555 1.601 1.587 1.511	692.26 682.69 673.56 693.56 687.47 654.43	680.67 ± 14.76
3	200	1.378 1.391 1.369 1.354 1.285 1.344	596.60 602.26 592.69 586.17 556.17 581.82	585.95 ± 16.30

As the temperature increased, the concentration of alpha-tocopherol declined. The impact of temperature on the oil sample concentration was assessed using one-way ANOVA, which demonstrated a statistically significant difference between the concentration and three temperature levels ($p < 0.0001$). These results suggest that temperature significantly influences the concentration of the oil sample under study. The results are presented in Table 5.

Table 5: Effect of temperature on the concentration of oil sample

	Sum of squares	df	Mean square	F	Sig.
Between Groups	70979.158	2	35489.579		
Within Groups	2847.723	15	189.848	F(2,15) = 186.94	p < 0.001
Total	73826.881	17			

Ascorbic acid was added during pretreatment to stop oxidative deterioration. As ascorbic acid is a strong antioxidant, it can neutralize reactive oxygen species and stabilize alpha-tocopherol during the analysis. These findings imply that alpha-tocopherol degradation is driven by oxidative and thermolytic processes, with molecular breakdown becoming more intense at elevated temperatures. A key heat barrier, beyond which alpha-tocopherol bioactivity is significantly degraded, is highlighted by the dramatic drop beyond 180°C.

Validation

The calibration curve showed a good linear relationship over the range of 50–350 µg/ml for alpha-tocopherol, and the correlation coefficient was found to be 0.9927 at 284.5 nm, as shown in Figure 3 and Table 1.

Experiments were conducted to evaluate the accuracy, suitability, and reliability of the method. The conventional addition method was used to accomplish this at three levels: 80%, 100%, and 120%. The recovery rates were 100.28%, 100.77%, and 99.69%. This demonstrates the specificity of the developed method. The percentage relative standard deviation (SD) was < 2

The precision study of interday, intraday, and repeatability analysis was carried out as per the ICH standards, and the % RSD was found to be ≤2, which was within the limits. The LOD and LOQ were computed using the SD and slope and were found to be 8.24 and 24.99 (µg/ml), respectively.

Limitations of the Study

In this study, there was no discussion of other tocopherol isomers, such as β, γ, and δ tocopherols, or tocotrienols, such as α, β, γ, and δ tocotrienols. Future studies should include matrix-matched spike-and-recovery experiments and additional selectivity investigations in both fresh and thermally treated oil samples. Furthermore, optimization of cold-pressing conditions can also be performed by systematically varying the major processing parameters, such as pressing temperature, screw speed/pressing rate, pressure, moisture content of the groundnut kernels, and pretreatment conditions, and evaluating their influence on oil yield and quality.

Conclusion

This study clearly shows that alpha-tocopherol is extremely heat-sensitive, and prolonged exposure to high temperatures accelerates its malfunction. The interaction between thermolytic fragmentation and oxidative stress highlights the need for minimal thermal processing to maintain the nutritional and functional integrity of groundnut oil. These findings highlight the need for temperature-controlled processing and storage to prevent alpha-tocopherol reduction and preserve the antioxidant properties of the oil. The study also showed that oxidative interactions play a major role in alpha-tocopherol degradation, resulting in a series of thermolytic byproducts, and are not only a linear function of temperature. This emphasizes the importance of maintaining a regulated temperature during extraction and storage to avoid significant nutritional loss.

Furthermore, the consequences extend beyond simple preservation; the oxidative stability of the oil is jeopardized by alpha-tocopherol depletion, which could hasten rancidity and shorten its shelf life. Therefore, maintaining oil quality requires optimizing processing parameters such as cold pressing, inert atmosphere packaging, and refrigeration. Overall, our results support the need for cautious heat control in both home and commercial environments to preserve the useful, nutritious, and antioxidant properties of groundnut oil. In the future, the study needs to be extended to higher temperatures and times, and to different isomers of tocopherols and tocotrienols.

Author contributions

A. A., A. A. T., H. P. C., S. J., and S. K. conducted the experiments, analyzed the data, and wrote the manuscript; C. K. S. S. reviewed and edited the manuscript; I. K. B. supervised the research and reviewed the manuscript. All authors have read and approved the final manuscript.

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Conflicts of interest

The authors declare no conflicts of interest in this study.

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Ethical consideration

Not applicable.

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