

HARNESSING NATURAL ANTIOXIDANTS: COMPARITIVE STUDY AND FORMULATION OF A NOVEL EDIBLE OIL

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INTRODUCTION

Lipid peroxidation is a critical issue in the food industry, leading to oxidative degradation of edible oils, decreased nutritional quality, and the formation of harmful secondary oxidation products.^[1] This oxidative degradation is primarily driven by the presence of oxygen, light, heat, and moisture, accelerating the breakdown of unsaturated fatty acids into peroxides, aldehydes, ketones, and free radicals.

Sunflower oil widely used in commercial cooking due to its high unsaturated fatty acid content, is particularly susceptible to oxidative deterioration, which negatively impacts its shelf life and sensory properties.^[2] To counteract this, antioxidants are widely used to inhibit lipid oxidation and extend oil stability.

While synthetic antioxidants such as **butylated hydroxytoluene (BHT)** and **butylated hydroxyanisole (BHA)** are commonly

employed, concerns over their potential health risks have led to increased interest in natural antioxidants.^[3] Natural plant-derived antioxidants, such as rosemary (*Rosmarinus officinalis*) extract, avocado (*Persea americana*) seed extract, and vitamin E (tocopherols), have been shown to exhibit significant antioxidative properties in edible oils.^[4] Castor oil (*Ricinus communis*), which contains ricinoleic acid, also possesses antioxidant potential, though its effect on lipid peroxidation remains less explored.

This study aims to comparatively evaluate the antioxidant efficacy of rosemary leaves, avocado seed, vitamin E, and castor oil in reducing lipid peroxidation of sunflower oil. Oxidation levels will be assessed using peroxide value (PV), thiobarbituric acid reactive substances (TBARS), and other lipid oxidation parameters.^[5] Understanding the relative effectiveness of these antioxidants could provide valuable insights for the food industry in selecting natural preservatives for enhancing oil stability.^[6]

MATERIALS AND METHOD

Refined sunflower oil was used as the base oil for the study. Natural antioxidants selected for comparison included rosemary leaves, avocado seed, Vitamin E, and castor oil. All chemicals and reagents used were of analytical grade and obtained from standard suppliers.

➤ Preparation of Antioxidant Extracts

Rosemary leaves and avocado seeds were cleaned, dried, and ground into a fine powder.^{[7][8]} The powders were subjected to solvent extraction to obtain the antioxidant-rich extracts. Castor oil and Vitamin E were used in their standard pure forms.

➤ Sample Preparation

Samples of sunflower oil were treated separately with each antioxidant at a predetermined concentration. A control sample without any antioxidant was also maintained. All samples were stored in identical conditions to monitor the oxidative stability over time.^{[9][10]}

➤ Assessment of Lipid Peroxidation

Lipid peroxidation was evaluated using the Thiobarbituric Acid Reactive Substances (TBARS) assay.^[11] The degree of peroxidation was measured by quantifying malondialdehyde (MDA) formation, which is a key indicator of lipid oxidation. Absorbance was read spectrophotometrically^[12], and results were recorded in terms of MDA equivalents.

➤ New oil formulation

100 mL total oil blend → Use 3 proportions are taken

- (a) 25 mL castor oil + 75 mL sunflower oil,
- (b) 10 mL castor oil + 90 mL sunflower oil,
- (c) 50 mL castor oil + 50 mL sunflower oil.

Simply mix the oils thoroughly by stirring in a sterile container, Mildly Heated for better homogenization, Stored in a dark, airtight bottle to prevent oxidation.^{[13][14]}

➤ Statistical Analysis

All experiments were conducted in triplicate. Results were analyzed using standard statistical methods, and the significance of antioxidant efficacy was assessed using ANOVA.

RESULTS

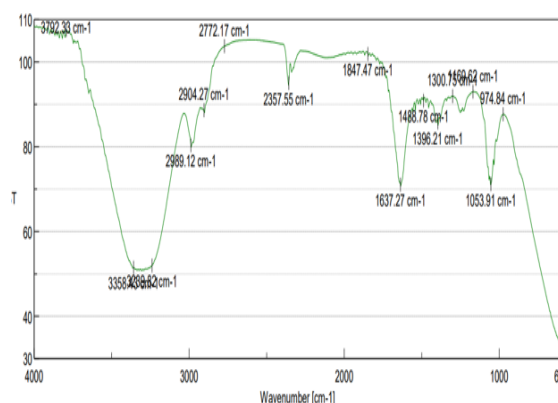
The antioxidant activity of rosemary leaves, avocado seed, Vitamin E, and castor oil was assessed using TBARS assay, total phenolic content (TPC), and DPPH radical scavenging activity.

The TBARS results showed that castor oil exhibited the greatest reduction in malondialdehyde (MDA) levels, followed by rosemary, avocado seed, and Vitamin E. The antioxidant properties were further supported by TPC and DPPH assay results, as shown in the tables below.

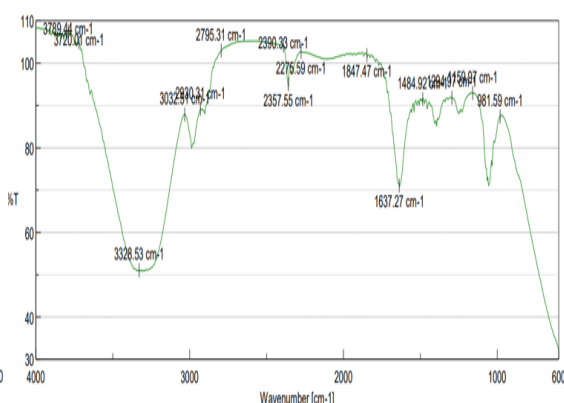
Table 1: Antioxidant Activity Analysis.

Sample	Total phenolic content (mgGAE/g)	DDPH Scavenging activity (%)
Castor oil	48.2	81.6
Rosemary extract	52.4	78.3
Avacado seed	36.4	65.9
Vitamin. E	28.5	60.2

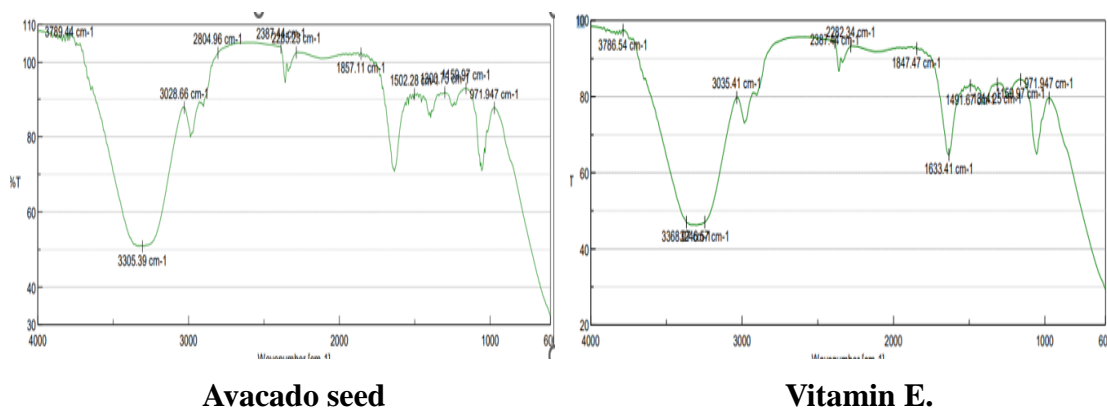
Fourier Transform Infrared Spectroscopy (FTIR)



Castor oil



Rosemary leaves



Fourier Transform Infrared Spectroscopy (FTIR) was conducted to identify the functional groups present in the antioxidant-treated oil samples, confirming the presence of bioactive compounds that contribute to antioxidant activity.^[15]

The FTIR spectra of treated sunflower oil showed characteristic peaks associated with antioxidant compounds.

Castor Oil-Treated Sample showed broad absorption around 3400 cm^{-1} , indicating --OH stretching from hydroxyl groups of ricinoleic acid. Peaks near 2924 cm^{-1} and 2854 cm^{-1} corresponded to C--H stretching of aliphatic chains. A sharp peak at 1743 cm^{-1} indicated the ester carbonyl (C=O) stretching, confirming the presence of fatty acid esters.

Rosemary Extract-Treated Sample revealed peaks at 1605 cm^{-1} and 1510 cm^{-1} corresponding to aromatic C=C stretching, indicating the presence of rosmarinic acid and other polyphenols. The O--H bending and stretching vibrations were also observed around 3400 cm^{-1} .

Avocado Seed-Treated Sample exhibited bands near 3300 cm^{-1} (O--H stretching), 2920 cm^{-1} (C--H stretching), and 1735 cm^{-1} (C=O stretching), suggesting the presence of flavonoids and phenolic acids.

Vitamin E-Treated Sample displayed characteristic --OH and C--H peaks, with notable bands near 3450 cm^{-1} (O--H stretching) and 2950 cm^{-1} (C--H stretching), as well as 1650 cm^{-1} (C=C aromatic ring), consistent with tocopherol structure.

These spectra confirm the incorporation of antioxidant compounds into the oil matrix. The presence of hydroxyl, ester, and aromatic functional groups supports the observed antioxidant behavior in the treated oils.

Table 2: Lipid Peroxidation Assay (TBARS Method).

Sample	MDA value	Antioxidant effectiveness
Control (no antioxidant)	3.42	-
Castor oil	1.08	Highest
Rosemary leaves	1.26	High
Avacadoseed extract	1.65	Moderate
Vitamin E	1.89	Least

The results demonstrate that all tested natural antioxidants possess significant antioxidant potential. Castor oil and rosemary extract showed the highest antioxidant capacity, aligning with their stronger inhibition of lipid peroxidation in sunflower oil.

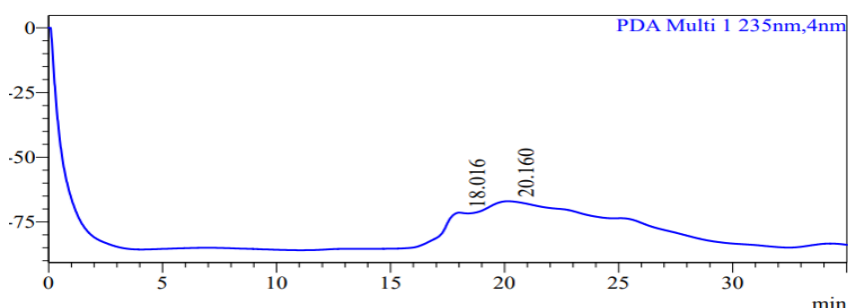
The results demonstrate that all tested natural antioxidants possess significant antioxidant potential. Castor oil and rosemary extract showed the highest antioxidant capacity, aligning with their stronger inhibition of lipid peroxidation in sunflower oil in the oil formulation the 50 ml castor oil and 50 ml sunflower oil propotion shows the best results compared to other propotions, as shown in the tables below.

Table 3: Antioxidant activity of new oil.

50 mL castor oil + 50 mL sunflower oil	25 ml castor oil + 75ml sunflower oil,	10 ml castor oil + 90 ml sunflower oil.
peroxide value befor cooking		
0.2	0.2	0.4
Peroxide value after cooking		
0.7	0.9	1.5

Castor oil exhibiting a strong volatile taste in 50 ml castor oil+ 50 ml sunflower oil case, it effecting tne sensory properties and 10 ml castor oil + 90 ml sunflower oil contain less properties with compared to other, so here 25ml castor oil + 75ml sunflower oil is more better

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



hplc result of 25ml castor oil + 75ml sunflower oil

Based on your chromatogram result at 235 nm, you have two distinct peaks at retention times of **18.016 min and 20.160 min**, with the second peak being significantly larger in area and concentration.

The presence of oxidative degradation compounds suggests a peroxide value analysis. fewer oxidation-related compounds are observed.^[16]

DISCUSSION

The results of this study highlight the significant role of natural antioxidants in reducing lipid peroxidation in sunflower oil. Among the tested substances, castor oil showed the highest antioxidant efficiency, followed closely by rosemary extract. These findings are supported by both TBARS results and antioxidant analysis, including total phenolic content (TPC) and DPPH radical scavenging activity.

The strong antioxidant performance of castor oil may be attributed to its high content of ricinoleic acid and other bioactive compounds, which enhance its free radical scavenging capacity. Similarly, rosemary extract is rich in phenolic diterpenes such as carnosic acid and rosmarinic acid, which are known to exhibit strong antioxidant properties.

Avocado seed extract, though moderately effective, still contributed to a notable reduction in lipid peroxidation. This can be linked to its polyphenol and flavonoid content. Vitamin E, while widely recognized for its antioxidant function, exhibited the least effectiveness in this comparative study. This may be due to the instability of α -tocopherol in the presence of heat or oxygen, or its lower synergistic interaction with the oil matrix compared to plant extracts.

The observed differences in antioxidant activity among the samples also align with their TPC and DPPH values. Higher phenolic content generally correlates with stronger radical scavenging potential, supporting the idea that antioxidant capacity is largely influenced by the phytochemical profile of each sample.

These findings suggest that natural antioxidants such as castor oil and rosemary extract can serve as effective alternatives to synthetic antioxidants in preserving the quality and extending the shelf life of sunflower oil.

CONCLUSION

This study demonstrates that natural antioxidants can significantly reduce lipid peroxidation in sunflower oil. Among the tested substances, castor oil exhibited the highest antioxidant

activity, followed by rosemary extract, avocado seed extract, and Vitamin E. The effectiveness of these antioxidants was supported by TBARS, total phenolic content, and DPPH assays. And a new edible oil was formulated using castor oil with specific proportion, it can reduce lipid oxidation.

The results confirm that natural plant-based antioxidants, especially castor oil and rosemary, offer a safer and more effective alternative to synthetic additives in preserving oil quality. Their incorporation into edible oils could enhance shelf life while meeting consumer demands for cleaner, more natural food products.

Further research may explore the synergistic effects of combining these antioxidants or their application in other lipid-based food systems.

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