

# Evaluation of Some Natural Antioxidants Efficiency on Healthy Peanut Butter Quality

Marwa, T. Hussein & \*Aziza, T. Gamal

Department of Horticulture Crops Research, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

## Original Article

### Article information

Received 20/01/2025

Revised 22/02/2025

Accepted 24/02/2025

Published 28/02/2025

### Available online

01/03/2025

### Keywords

Peanut butter, anise, cinnamon, clove buds, essential oils, beeswax, antioxidant, antimicrobial activity, storage

## ABSTRACT

This study aimed to improve the shelf life and quality of peanut butter by addressing challenges like rancidity and oil separation. Essential oils (EOs) from anise, cinnamon, and clove buds were tested as natural antioxidants and antimicrobials, alongside beeswax to prevent oil separation. The primary bioactive compounds in these EOs were identified as trans-anethole (anise), cinnamaldehyde (cinnamon), and eugenol (clove). Clove EO (CLEO) demonstrated the highest antioxidant activity, scavenging 95.78% of DPPH radicals, outperforming cinnamon EO (CNEO; 83.05%) and anise EO (ANEEO; 64.18%). Antimicrobial efficacy, measured by inhibition zones, increased with EO concentration, with cinnamon showing the broadest inhibition. Beeswax in the control sample (T1) effectively prevented 97.9% of oil separation, yielded the lowest calorie content (591.02 kcal), and had the lowest ether extract (45.6%). Water activity was also lower in T1 (0.46) compared to stabilizer-free T2 (0.58), indicating better microbial stability. During six-month storage, T7 (0.1% CLEO) and T9 (CLEO + ANEEO) maintained superior quality, with peroxide values below 10 meq/kg, outperforming other samples. CLEO and ANEEO+CNEO blends minimized free fatty acid increases and showed no detectable microbial counts. Texture analysis favored T1 over commercial samples, but sensory panels preferred T5 (0.3% ANEEO) for its sweetness, attributed to anethole. Post-storage, T5, T7, and T9 were top-rated, though preferences varied over time. T8 and T9 maintained consistent acceptability, while T2 (no stabilizer) scored lowest due to rancidity and stickiness. The study highlights beeswax as a critical stabilizer and clove and cinnamon EOs as potent natural preservatives. Blending EOs synergistically improved oxidative and microbial stability, offering a viable strategy to enhance peanut butter's shelf life while meeting consumer preferences for natural ingredients.

## 1. Introduction

The groundnut (*Arachis hypogaea*), a member of the Leguminosae family, holds significant importance due to its wide range of uses and nutritional value. Peanut seeds consist of 44–56% oil, 22–30% protein, and 9.5–19% carbohydrates on a dry-weight basis (Gulluoglu et al., 2016). In addition, they are rich in essential minerals such as phosphorous, calcium, magnesium, and potassium, along with vitamins E, K, and B-group vitamins. The composition of fatty acids in peanuts greatly influences their nutritional and storage properties (Shasidhar et al., 2017). Peanuts also offer

protective health benefits, aiding in the prevention of cardiovascular diseases, cancer, diabetes, osteoporosis and other degenerative conditions (Isanga and Zhang, 2007). They are widely used to produce nutritious, affordable foods such as peanut butter, peanut bars, peanut milk, and nimko, which help address protein-calorie malnutrition and iron deficiency, particularly in women and children (Ali and Nigam, 1993). Consumers and vendors prefer peanut butter to be easily spreadable and to have a long shelf life.

Peanut butter is also popular among candy, snack, and cookie manufacturers.

In candy production, peanut butter coatings cost about one-third as much as chocolate coatings and can be used to enrobe cake portions, cookies, candy centers, and other snack foods, as reported by Salunkhe et al. (1992) and Sanders (2003). However, one of the main limitations of peanut butter and other peanut-derived products is their susceptibility to rancidity, which results from the oxidation of unsaturated fatty acids. This oxidation process, accelerated by high temperatures, oxygen exposure, and enzymatic activity, reduces the quality of the oil (Nepote et al., 2006). The peroxide value is commonly used to gauge the extent of lipid oxidation, a value that increases with storage time and temperature (Abayomi et al., 2002). Peanut butter is made by grinding dry-roasted peanuts, often with the addition of seasonings and stabilizers to meet standards such as the U.S. FDA's requirement of at least 90% peanuts and no more than 55% oil (FDA, 2015). One challenge in natural peanut butter is oil separation, in which the oil rises to the top during storage. Commercial peanut butters address this issue by using stabilizers such as partially hydrogenated vegetable oils (Chang et al., 2013). Peanut butter is also prone to microbial, chemical, and physical degradation, which affects its final quality and shelf life. Quality deterioration can occur due to protein putrefaction by bacterial metabolism, darkening from sugar-protein interactions, and oxidative rancidity of the unsaturated oil portion when exposed to air. Efforts to improve peanut butter have focused on various aspects such as preventing oil separation, improving smoothness, spreadability, and consistency, and enhancing flavor and stability during storage (Woodroof 1983). Several methods have been explored, including special grinding techniques, heat treatment, and the incorporation of substances such as water, honey, and glycerin, with hydrogenated oils often suggested as stabilizers for their efficacy in homogenization and crystallization (Muego-Gnanasekharan, 1992). Essential oils (EOs) derived from aromatic plants are attracting growing attention due to their relative safety, broad consumer acceptance, and potential for multifunctional uses,

particularly as antioxidants and radical scavengers (Sacchetti et al., 2005 and Zengin et al., 2018). These oils contain bioactive compounds with potential applications in preventing or treating certain diseases (Taghipour et al., 2019). Anise (*Pimpinella anisum* L.), an annual spice and medicinal plant from the Apiaceae family, is native to the Mediterranean region. Medicinal plants are known for their therapeutic properties (Hussain et al., 2014 and Al-Juhaimi, 2014). Anise has gained popularity due to its antimicrobial, antifungal, insecticidal, and antioxidant effects (Tirapelli et al., 2007). Anise fruits contain 1.5–5.0% essential oil, with trans anethole as the major component, along with small amounts of estragole, anisaldehyde,  $\gamma$ -himachalene, and cis-anethole (Tabanca et al., 2005 and Omidbaingi et al., 2003). Cinnamon (*Cinnamomum verum*) is recognized for its antioxidant compounds that neutralize reactive oxygen species, including superoxide anions and hydroxyl radicals. Cinnamon oil and bark exhibit strong antimicrobial properties, effectively inhibiting the growth of bacteria and fungi.

Cinnamaldehyde, the active component, has been shown to reduce spoilage caused by rancidity and fat degradation. The U.S. Food and Drug Administration (FDA) approves cinnamaldehyde as a safe food additive due to its flavor enhancing and functional benefits (Matan et al., 2006). Clove essential oil has also demonstrated inhibitory activity against food spoilage microorganisms and is classified as “Generally Recognized as Safe” (GRAS) by the FDA, with an acceptable daily intake established by the World Health Organization (WHO) (Waterstrat, 1999 and Kildea et al., 2004). Clove oil, extracted from the flower buds, stems, and leaves of the clove tree, is primarily composed of eugenol (70–85%), eugenyl acetate (15%), and  $\beta$ -caryophyllene (5–12%). Besides its volatile compounds, clove oil contains non-volatile compounds such as tannins, sterols, triterpenes, and flavonoids, which contribute to its health benefits. It has antibacterial anticancer, and antioxidant properties, with its antioxidant activity surpassing that of synthetic antioxidants such as BHA (Butylated Hydroxyanisole) (Nagababu

and Lakshmaiah, 1992; Anderson et al., 1997). Although these oils are considered safe, their components are highly volatile, water-insoluble, and unstable to heat, oxygen, and light, making the development of topical preparations challenging. To address this, various strategies involving microparticulate systems have been developed to formulate oily bioactive substances (OBS) using polymeric and lipidic materials (LM) (Rojas et al., 2021). Beeswax (E901), a natural wax secreted by bees, is widely used in the cosmetics, the cosmetics and skincare industries as a thickening agent, emulsifier, and surfactant. However, its utilization in the food industry is more limited. The European Union permits beeswax as a glazing agent in certain food products such as confectionery (including chocolate), small bakery goods coated with chocolate, snacks, nuts, and dietary supplements. It is also used for surface treatment of specific fresh fruits at levels deemed necessary (*quantum satis*) (EU 1995). In addition to these uses, beeswax is increasingly being studied as a potential emulsifying agent, particularly in water-in-oil (W/O) emulsions. This has led to its application in the production of oleogels, which are used in functional foods, such as replacing animal fat in beef patties or creating oil-loaded beeswax microparticles (Gao et al., 2021). This wax can form an isotropic lipidic matrix (LM) with oils, cosolvents, and surfactants due to the low polarity, long-chain length, and high melting point of its compounds (Chubierre et al., 2019). Additionally, waxes create a crystalline network within the LM, providing a structured framework with specific hardness to prevent breakage during application (Petry et al., 2017). The goal of this study is to enhance the quality of spreadable peanut butter by incorporating essential oils as natural antioxidants and antimicrobial agents. Beeswax is also used to prevent oil separation, thereby prolonging the product's shelf life.

## 2. Materials and Methods

### Plant materials

Peanut (*Arachis hypogaea* L.) was purchased from a local market in Giza, Egypt.

Beeswax and honey were obtained from the apiary of the Faculty of Agriculture, Cairo University, Giza, Egypt. Anise fruits (*Pimpinella anisum*), cinnamon barks (*Cinnamomum cassia*), and clove buds (*Syzygium aromaticum*) were sourced from local suppliers. Wheat flour, quinoa seeds, carrageenan, flaxseeds, and commercial peanut butter (two different brands) were purchased from local markets in Giza, Egypt.

### Chemicals

All chemicals were analytical-grade and obtained from El-Nasr Pharmaceutical Chemicals Co., Egypt, and Sigma-Aldrich Company (USA). Plate Count Agar and Sabouraud Dextrose Agar were purchased from Sigma-Aldrich Company, USA.

### Methods

#### Extraction of essential oils and calculation of oil yield

Each crushed aromatic plant (100 g) was subjected to hydro-steam distillation in a Clevenger apparatus for 3 hours to extract essential oils, following the method of Mohamed et al. (2020). The obtained essential oils (EOs) were dried over anhydrous sodium sulfate and stored in dark, sealed glass vials at 4°C until analysis.

#### Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The essential oils (EOs) were analyzed using a gas chromatograph (Hewlett–Packard model 5890) paired with a mass spectrometer (Hewlett–Packard-MS model 5970) and fitted with a DB5 fused silica capillary column (60m, 0.32mm i.d., 0.25mm film thickness). The temperature program began at 50°C for 5 minutes, then increased to 250°C at a rate of 4°C/min. Helium was utilized as the carrier gas at a flow rate of 1.1mL/min. The essential oil was diluted in diethyl ether (30µL of essential oil per mL of diethyl ether), and 2µL of this mixture were injected into the GC with a split ratio of 1:10, at an injection temperature of 220°C. Mass spectra were collected in electron impact mode (EI) at 70eV, scanning from 39 to 400m/z. The retention indices (Kovats index) for the volatile compounds were calculated using retention times of

n-alkanes (C8–C22) as an external standard. The identification of isolated peaks was achieved by matching with the National Institute of Standards and Technology (NIST) mass spectra library, along with comparisons to known compounds and existing literature (Adams, 2001). The percentage composition of each essential oil was calculated using the normalization method based on the average GC peak area from three injections.

### Antimicrobial Activity of Essential Oils

Essential oils were diluted to 0.1%, 0.3%, and 0.5% (using 95% ethanol). Antimicrobial activity was assessed via disc diffusion assay (Hood et al., 2003). 20mL of Mueller Hinton agar (for bacteria) or Sabouraud agar (for fungi), containing 1% Tween 80 as an emulsifier, was poured into sterilized plates (90mm diameter) (Difco Manual, 1977). Ten  $\mu$ L of each diluted essential oil was loaded onto 6 mm blank antimicrobial discs, which were then placed on agar plates. Plates were incubated overnight at 30°C (fungi) or 37°C (bacteria). Inhibition zones (mm) were measured and recorded.

### Preliminary test for oil separation (stabilizer selection test)

This test was conducted to identify the optimal stabilizer for producing high-quality peanut butter (after roasting and grinding kernels, as described below). The materials evaluated included beeswax (1%, 2%), flaxseed powder (6%) + bees wax (1%), quinoa powder (6%) + beeswax (1%), carrageenan (0.5%), and wheat flour (2%). Peanut butter for each treatment was placed into centrifuge tubes (Hermle Z 206 A, Germany) and centrifuged at 6000 rpm for 15 minutes at 25°C. Surface oil was removed post-centrifugation, and the residual peanut butter was weighed. Oil loss was calculated as the ratio of surface oil weight to the initial peanut butter weight (Huang et al., 2020).

### Processing of peanut butter incorporated with essential oils

Peanut kernels with skins were roasted in trays using a laboratory oven (Fisher Scientific 750 G, USA) at 150°C for 20 minutes, then cooled until manageable. Skins were manually removed with

an electric fan, and defective seeds (yellowish with potential aflatoxin contamination) were discarded. De-skinned peanuts were re-roasted at 150°C for 15 minutes, then ground in an electric mixer (Moulinex, France) for 5 minutes to achieve a smooth, creamy texture. Ingredients were added as a percentage of total weight (Palomar et al., 2006). Per Egyptian Standardization guidelines, additives in peanut butter must not exceed 10%. The formulations were as follows:

- Beeswax (BW, melting range 53–67°C) was added at 2% for stabilization (all samples except T2, which contained only peanut paste).
- Honey was added at 7%.
- Salt was added at 0.5%.

Essential oils (EOs) of anise, cinnamon, or clove were added at varying concentrations.

Formulations:

- T1: Peanut butter with all ingredients except EOs.
- T2: Peanut butter without any additives.
- T3: Peanut butter (commercial brand 1).
- T4: Peanut butter (commercial brand 2).
- T5, T6, and T7: Peanut butter with EO of anise at 0.3%, 0.2%, and 0.1%, respectively.
- T8 and T9: Peanut butter with EO of cinnamon at 0.3% and 0.2%, respectively.
- T10 and T11: Peanut butter with EO of clove at 0.2% and 0.1%, respectively.
- T12: Peanut butter with essential oils mixture of anise 0.1% + cinnamon 0.2%.
- T13: Peanut butter with essential oils mixture of anise 0.15% + cinnamon 0.15%.
- T14: Peanut butter with essential oils mixture of anise 0.25% + clove 0.05%.
- T15: Peanut butter with essential oils mixture of anise 0.15% + clove 0.15%.

Samples were subjected to a preliminary panel test to select preferred formulations for further testing.

### Preparation of selected peanut butter samples

Approximately 300g of peanut butter was weighed into a clean jar for each treatment.



Salt (0.5%), melted beeswax (2%), honey (7%), and EO concentrations (specific to each treatment) were added to the ground peanuts. The mixture was blended thoroughly using a hand blender in a water bath (60°C for 2 minutes) to maintain the beeswax in a melted state. After mixing, each treatment (300g) was divided into three jars for replication. Following the selection of the most acceptable samples in the preliminary panel test, the formulations were renamed as follows:

- T1: Peanut butter with all ingredients except EOs.
- T2: Peanut butter without additives (pure peanut paste).
- T3: Commercial brand 1.
- T4: Commercial brand 2.
- T5: Peanut butter with anise EO (0.3%).
- T6: Peanut butter with cinnamon EO (0.2%).
- T7: Peanut butter with clove EO (0.1%).
- T8: Peanut butter with anise EO (0.15%) + cinnamon EO (0.15%).
- T9: Peanut butter with anise EO (0.25%) + clove EO (0.05%).

### Proximate chemical composition of peanut butter (T1, T3 and T4)

The chemical composition of peanut butter samples (T1, T3, and T4) was analyzed for moisture, ash, crude protein, crude fiber, and ether extract contents, following methods outlined in AOAC (2012). Each analysis was performed in triplicate. Readily available carbohydrates were calculated using the method described by Ceirwyn (1995), and energy content was determined via Atwater's equation (AOAC, 1990):

$$\text{Energy (kcal)} = (\text{Protein} \times 4) + (\text{Carbohydrate} \times 4) + (\text{Fat} \times 9).$$

### Long-term oil separation

Three tubes from each treatment and commercial sample, prepared as described in the preliminary oil separation test, were stored at room temperature. After 6 months, the tubes were reweighed and drained to determine the percentage of separated oil (Winkler-Moser et al., 2019).

### Water activity analysis

Peanut butter samples (2g) were spread into

sample cups and analyzed using a Decagon Aqua-Lab hygrometer (LabStart-aw, Novasina) to measure water activity  $a_w$  (Mohd Rozalli et al., 2016).

### Radical scavenging activity (RSA) of EOs and oil separated from peanut butter treatments

The radical scavenging activity (RSA) of essential oils (anise, cinnamon, and clove) and peanut butter-derived oil was evaluated using DPPH radicals in toluene, following the method by Ramadan et al. (2010) with modifications. A fresh DPPH solution  $10^{-4}\text{M}$  in toluene) was prepared. For each treatment, 100  $\mu\text{L}$  of essential oil or 10 mg of peanut butter oil (dissolved in 100  $\mu\text{L}$  toluene) was mixed with 390  $\mu\text{L}$  DPPH solution. The mixture was vortexed for 20 seconds, and after 30 minutes, absorbance at 515nm was measured using a spectrophotometer (Jenway Model 6705, UK). RSA was calculated by comparing absorbance differences between test samples and controls, with % inhibition determined using the formula:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample}) / \text{absorbance of control}] \times 100.$$

### Peroxide Value Assay

When lipids oxidize, unstable hydroperoxides are formed as primary compounds in the early stages of rancidity. The peroxide value (PV) assay is a widely used method for quantifying hydroperoxides, providing a measure of oxidized fat expressed in milliequivalents of peroxide per kilogram of fat.

The PV assay for peanut butter was conducted according to AOAC (1996) method 965.33. For the analysis, 5g of peanut butter was homogenized in a mixture of glacial acetic acid and chloroform, followed by the addition of potassium iodide. The resulting peroxides reacted with iodine, which was then titrated against sodium thiosulfate using starch as an indicator. The PV was calculated using the formula:

$$\text{PV} = (\text{S} - \text{B}) * \text{N} * 1000 / \text{sample weight}$$

where S is the titration value of the sample (mL), B is the titration value of the blank (mL), and N is the normality of the sodium thiosulfate solution (0.1N).

## Determination of % Free Fatty Acids

Free fatty acids were determined during the storage periods according to the method described by Kaur et al. (2011), with slight modifications. Five grams of butter fat were dissolved in 50 mL of neutralized ethanol. Then, 0.5 mL of phenolphthalein was added, and the mixture was titrated with 0.1 N NaOH until the pink color disappeared. The results were expressed as a percentage of free fatty acids (FFAs) and calculated as oleic acid.

## Microbiological Examination of Processed Peanut Butter Samples

Total plate count and fungal count (mold and yeast) were determined during storage at initial time, 3 months, and 6 months, following ISO 4833:2003 and ISO 21527-2:2008, respectively. For both analyses, 25g of peanut butter samples were homogenized with 225mL of sterile buffered peptone water (0.1% w/v) to prepare  $10^{-1}$  dilution. For total plate count, 0.1mL of the dilution was spread on Nutrient Agar and incubated inverted at 30°C for 48 hours. For fungal count, the same volume was spread on Sabouraud Dextrose Agar and incubated at 25°C for 48 hours. Visible colonies were counted post-incubation, and the log of total bacterial count per gram was calculated.

## Texture analysis of control and commercial peanut butter

Texture profile analysis (TPA) was performed using a universal testing machine (Cometech, B type, Taiwan) equipped with software for selected samples (T1, T2, T3, and T4). A 40mm diameter aluminum cylindrical probe was used in a double compression test, penetrating the sample to 50% depth at a speed of 1mm/s. Firmness (N), adhesiveness (N·s), and cohesiveness (ratio) were calculated from the TPA graph. Texture analysis was conducted following the method described by Bourne (2002).

## Sensory Evaluation of Tested Peanut Butter Samples

The selected peanut butter samples were evaluated organoleptically at zero time and after 2, 4, and 6 months of storage. The evaluation was conducted

by ten panelists from the Horticultural Crop Processing Lab, Food Technology Research Institute, Agricultural Research Center. Panelists assessed color, odor, taste, texture, and palatability using a 10-point scale to grade sample quality (Matsiko et al., 2014).

## Statistical analysis

According to (Steel et al., 1997), the statistical analysis was performed using a one-way analysis of variance (ANOVA) under a significant threshold of 0.05 for the entire set of findings using the statistical application CoStat (Ver. 6.400). The LSD test was used to determine the significance between different samples and during storage period.

## 3. Results and Discussion

### Essential oil (EO) yield of aromatic plants (Anise, Cinnamon, and Clove)

The highest essential oil (EO) yield was obtained from clove buds, followed by cinnamon bark and anise fruits, with yields of 3.53, 0.95, and 0.80mL/100g dry weight (d.w.), respectively. Our results closely align with the findings of Fadel et al. (2020). The observed variations may be attributed to environmental and climatic factors, as well as differences in drying methods used for the aromatic plants (Martos et al., 2007).

### Compounds Identified in Anise, Cinnamon, and Clove essential oils (ANEQ, CNEQ, and CLEQ)

The essential oils of anise (ANEQ), cinnamon (CNEQ), and clove (CLEQ) were fractionated and identified using GC/MS, as shown in Table 1. ANEQ consisted of 13 components, accounting for 98.43% of the total essential oil. The major component was trans-anethole, a phenolic ether, which constituted 82.31%, followed by methoxyphenyl-Z-methylbutanol (5.89%) and butanoic acid (3.82%). Additionally, minor components (<1%) were detected, including estragole (methyl chavicol). These results are consistent with those reported by Ullah et al. (2014), who found that trans-anethole was the predominant compound in ANEQ (82%). CNEQ contained 16 components, representing 73.71% of the total oil. The primary constituents were

cinnamaldehyde (48.83%) and caryophyllene (7.48%). These findings align with those of Paranagama et al. (2001), who reported cinnamaldehyde and caryophyllene at concentrations of 50.5% and 8%, respectively. Furthermore, cinnamaldehyde, caryophyllene, and eugenol have been identified as predominant compounds in other studies on the chemical composition of cinnamon bark essential oil (Kim et al., 2015). CLEO consisted of five major components, accounting for 98.79% of the total

oil. The dominant compounds were eugenol (79.66%), aceto eugenol (10.34%) and caryophyllene (6.94%). These results agree with those of Lee and Shibamoto (2001), who identified eugenol acetate as the main constituents in clove bud essential oil. Additionally, eugenol,  $\beta$ -caryophyllene,  $\alpha$ -caryophyllene,  $\alpha$ -caryophyllene, and carvacrol have been reported as major components in clove essential oil from Indonesia and India (Hossain et al., 2012).

**Table 1. Fractionation and identification of essential oil components by Gas Chromatography/ Mass Spectrum (GC/MS).**

Number of Peak	Components	ANEO	CNEO	CLEO
		% Area	% Area	%Area
*1	$\alpha$ -(Z,E)-Farnesene	0.26	-	-
2	Estragole	0.45	-	-
3	Hydroxymethoxyquinoline	3.72	-	-
4	Zingiberene	0.15	-	-
5	$\beta$ - Myrcene	0.19	-	-
6	Curcumene	0.20	-	-
7	Trans-anethole	82.31	-	-
8	L- Methoxy benzaldehyde	0.4	-	-
9	Anisyl acetone	0.20	-	-
10	Eugenol	0.50	1.63	79.66
11	Methoxy phenyl-Z- methyl butanole	5.89	-	-
12	Benzo-dioxol	0.34	-	-
13	Butanoic acid	3.82	-	-
14	Limonene	-	1.24	-
15	1-Methoxymethyl 4-methylenecyclohexanol	-	0.38	-
16	Benzene	-	0.61	-
17	Cyclohexene	-	0.80	-
18	Linalylformate	-	0.54	-
19	Caryophyllene	-	7.48	6.94
20	$\alpha$ -Ocimene	-	1.38	0.78
21	Carvone	-	0.61	-
22	$\gamma$ -Cadinene	-	0.83	-
23	Caryophyllene oxide	-	4.77	1.07
24	Cinnamaldehyde	-	48.83	-
25	Levomenthol	-	0.79	-
26	cinnamyl ester	-	1.07	-
27	Aceto eugenol	-	0.65	10.34
28	Apiol	-	2.10	-
Total identified compounds (%)		98.43	73.71	98.79
Total unidentified compounds (%)		1.57	26.29	1.21

\*Sesquiterpene Hydrocarbons (1,4,6,19,22) , Monoterpene Hydrocarbons (5,14,20), Oxygenated Sesquiterpenes (23) , Phenylpropenes (2, 28) , Phenols (Phenolic Ethers) (7, 10, 27) , Alcohols (11, 15, 25) , Aromatic Aldehydes (8, 24), Ketones (9, 21) , Esters (18, 26), Carboxylic Acids (13), Oxygenated Compounds (3,12), Aromatic Hydrocarbons (16), Cycloalkene Hydrocarbons (17).

## Radical scavenging activity (RSA) essential oils

A widely used method is the DPPH radical scavenging assay. In the present study, the essential oils of anise, cinnamon, and clove demonstrated the ability to donate hydrogen to the DPPH radical. As shown in Table 2, clove essential oil (CLEO) exhibited the highest DPPH radical scavenging activity (95.78%), followed by cinnamon essential oil (CNEO) with 83.05%, and anise essential oil (ANEO) with 64.18%. The differences among the essential oils were statistically significant. The lowest scavenging activity was observed for BHT. Jirovetz et al. (2006) reported that the antioxidant activity of 0.005mg of clove essential oil (CLEO) was comparable to that of standard butylated hydroxytoluene (BHT) at a concentration of 0.01%. The primary constituent of CLEO is eugenol, which is responsible for its strong antioxidant activity (Ogata et al., 2000). Similarly, Tomaino et al. (2005) attributed the high antioxidant activity of clove and cinnamon essential oils to the presence

of eugenol. This finding aligns with the concept that a phenolic group containing an electron-repelling group in the ortho position enhances radical scavenging activity. Furthermore, as shown in Table 2, the radical scavenging effect of anise essential oil (ANEO) significantly increased when mixed with either clove or cinnamon essential oil, reaching 91.12% and 73.12%, respectively. This enhancement is primarily due to the synergistic effect between phenolic compounds, even at low concentrations (Radünz et al., 2019). The high phenolic content in clove bud essential oil (Table 2) supports this observation. Trans-anethole, the major identified compound in ANEO, may also contribute to its antioxidant activity (Burits and Bucar, 2003). Similarly, El-Amrania et al. (2019) found that the DPPH radical inhibition percentage of cinnamon and clove essential oils exhibited a dose-dependent effect. Other studies have reported that the scavenging activity of anise essential oil was stronger than that of BHA and BHT, increasing with higher concentrations (Singh et al., 2008).

**Table 2. The radical scavenging activity of anise, cinnamon and clove essential oils (ANEO, CNEO and CLEO)**

Essential oils & their mixtures	RSA%
*ANEO (0.3%)	64.18 <sup>e</sup> ±0.11
CNEO (0.2%)	83.05 <sup>c</sup> ±0.19
CLEO (0.1%)	95.78 <sup>a</sup> ±0.21
ANEO (0.2) +CNEO (0.1)	73.12 <sup>d</sup> ±0.28
ANEO (0.25) + CLEO (0.05)	91.12 <sup>b</sup> ±0.11
**BHT (200 ppm)(0.02%)	54.43 <sup>f</sup> ±1.06

\*ANEO is anise essential oil, CNEO is cinnamon essential oil and CLEO is clove essential oil

\*\*BHT is butylated hydroxytoluene (artificial antioxidant). Values are means ± standard deviations of triplicate determinations. Means in the same column with different superscript (a,b,c,....) are significantly different (p≤0.05)

## Antimicrobial activity of tested essential oils (ANEO, CNEO, and CLEO)

The antimicrobial activity of essential oils (EOs) extracted from clove buds, anise seeds, and cinnamon bark, measured as the inhibition zone (mm), is presented in Table 3. Amphotericin and Gentamicin were used as standard positive controls for antimicrobial activity. The results in Table 3 indicate that both Gram-positive and Gram-negative bacteria exhibited sensitivity to clove essential oil (CLEO), anise essential oil (ANEO) and cinnamon essential oil (CNEO). Addi-

tionally, all tested EOs demonstrated antimicrobial activity. The results showed that the antimicrobial activity, measured by the inhibition zone (mm), increased gradually with higher concentrations of all tested essential oils (EOs). Among the studied EOs, cinnamon essential oil exhibited the highest inhibition rate, as observed in Table 3. Cinnamaldehyde ( $C_6H_5CH=CHCHO$ ) is the primary compound identified in cinnamon essential oil and is one of its most abundant components (Xing et al., 2010). Studies have demonstrated that cinnamaldehyde is more potent than other antimicrobial compounds containing an aldehyde group.



Its chemical structure, characterized by an outer CH chain and conjugated double bonds, plays a crucial role in its strong antimicrobial properties (Shams et al., 2003). Similarly, clove essential oil contains approximately 79.66% eugenol, which contributes to its antimicrobial activity. The antibacterial, antifun-

gal, and anti-yeast effects of essential oils are primarily attributed to the presence of secondary plant metabolites such as terpenoids, steroids, flavonoids, esters and acids, which are commonly found in EOs (Upadhyay, 2015).

**Table 3. Antimicrobial activity of tested essential oils by using agar disc diffusion method (mm)\***

Microbial Strains	Tested essential oils and reference antibiotics									Am-photeri- cin B*	Gen- tamic in*
	CLEO (%)			ANEQ (%)			CNEQ (%)				
	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3			
Gram positive bacteria											
<i>Bacillus subtilis</i> ATCC 33221	7.4 <sup>bD</sup> ± 0.13	12.3 <sup>aC</sup> ± 0.25	19.4 <sup>bB</sup> ± 0.07	0.0 <sup>bE</sup> ± 0.00	7.1 <sup>bD</sup> ± 0.03	8.8 <sup>abD</sup> ± 0.14	22.0 <sup>aB</sup> ± 0.3	>90 <sup>aA</sup>	12.2 <sup>aC</sup> ± 0.33	9.0 <sup>abD</sup> ± 0.1	
<i>Staphylococcus aure-</i> <i>us</i> ATCC 20231	0.0 <sup>cE**</sup> ± 0.00	7.1 <sup>cD</sup> ± 0.03	14.0 <sup>cC</sup> ± 0.21	0.0 <sup>bE</sup> ± 0.00	0.0 <sup>cE</sup> ± 0.00	9.2 <sup>bD</sup> ± 0.11	19.8 <sup>aB</sup> ± 0.3	>90 <sup>aA</sup>	12.3 <sup>aC</sup> ± 0.13	0.0 <sup>cE</sup> ± 0.00	
Gram negative bacteria											
<i>Escherichia coli</i> ATCC 6933	7.2 <sup>bB</sup> ± 0.05	10.0 <sup>bD</sup> ± 0.14	13.7 <sup>cC</sup> ± 0.14	0.0 <sup>bF</sup> ± 0.00	7.1 <sup>aE</sup> ± 0.07	7.3 <sup>bE</sup> ± 0.22	21.0 <sup>aB</sup> ± 0.2	>90 <sup>aA</sup>	8.5 <sup>bE</sup> ± 0.2	8.5 <sup>bE</sup> ± 0.17	
<i>Pseudomonasaer-</i> <i>oginosa</i> ATCC 9027	6.4 <sup>bD</sup> ± 0.00	7.4 <sup>cD</sup> ± 0.18	11.0 <sup>bcC</sup> ± 0.00	0.0 <sup>bE</sup> ± 0.00	6.8 <sup>bD</sup> ± 0.1	8.3 <sup>bC</sup> ± 0.10	22.8 <sup>aB</sup> ± 0.1	>90 <sup>aA</sup>	10.2 <sup>abC</sup> ± 0.2	9.0 <sup>bC</sup> ± 0.3	
Molds											
<i>Aspergillus niger</i> NRRL 2322	10.1 <sup>aCD</sup> ± 0.11	15.1 <sup>aC</sup> ± 0.15	22.0 <sup>bB</sup> ± 0.33	6.8 <sup>aD</sup> ± 0.11	8.5 <sup>aD</sup> ± 0.11	12.7 <sup>aCD</sup> ± 0.34	>90 <sup>aA</sup>	>90 <sup>aA</sup>	9.3 <sup>cD</sup> ± 0.21	16.0 <sup>aC</sup> ± 0.3	
<i>Aspergillus flavus</i> EMCC 100	9.8 <sup>aE</sup> ± 0.71	15.9 <sup>aC</sup> ± 0.22	19.8 <sup>bC</sup> ± 0.13	7.1 <sup>aF</sup> ± 0.16	9.3 <sup>aE</sup> ± 0.33	11.3 <sup>aD</sup> ± 0.33	33.6 <sup>aB</sup> ± 0.1	>90 <sup>aA</sup>	11.0 <sup>cD</sup> ± 0.01	16.3 <sup>aC</sup> ± 0.1	
Yeasts											
<i>Saccharomyces cere-</i> <i>visiae</i> NRRLY 2034	8.0 <sup>bD</sup> ± 0.22	13.0 <sup>bC</sup> ± 0.1	33.3 <sup>aB</sup> ± 0.11	0.0 <sup>bE</sup> ± 0.00	0.0 <sup>cE</sup> ± 0.00	0.0 <sup>cE</sup> ± 0.00	>90 <sup>aA</sup>	>90 <sup>aA</sup>	8.2 <sup>cD</sup> ± 0.19	9.0 <sup>abD</sup> ± 0.1	
<i>Candida lypolitica</i> NRRLY 1095	7.2 <sup>bCD</sup> ± 0.11	11.0 <sup>cC</sup> ± 0.1	13.7 <sup>dC</sup> ± 0.12	0.0 <sup>bD</sup> ± 0.00	8.1 <sup>aCD</sup> ± 0.1	10.3 <sup>bB</sup> ± 0.11	23.6 <sup>aB</sup> ± 0.1	44.6 <sup>bA</sup> ± 0.1	10.5 <sup>bC</sup> ± 0.2	8.9 <sup>bCD</sup> ± 0.11	

Values are mean inhibition zones of three replicates ± standard deviation. Means followed by different capital letters in the same row represents significant difference ( $p \leq 0.05$ ) between tested materials or concentrations. Means followed by different small letters in the same column represents significant difference ( $p \leq 0.05$ ), for each microorganism. \* Reference antibiotics at concentration (10 µg). \*\* No observed inhibition zone.

## Selection of Stabilizer for Reducing Oil Separation from Peanut Butter

Based on Table 4, the preliminary test indicates that beeswax at 2% was the most effective stabilizer for peanut butter, outperforming other tested materials, which were less efficient in stabilizing the product. After centrifugation, oil separation was observed in all peanut butter samples except for the sample containing 2% beeswax, which exhibited the lowest percentage of separated oil. Tekiki et al. (2022) demonstrated that beeswax functions as an effective emulsifying agent, facilitating the formation of a stable three-dimensional network structure in olive oil and honey, thereby maintaining an emulsion state.

**Table 4. Oil separation of peanut butter according to type of stabilizer**

Stabilizer type	Oil separation (%)
Without stabilizer	3.701 <sup>b</sup> ± 0.0005
Beeswax 1%	2.912 <sup>e</sup> ± 0.0015
Beeswax 2%	2.001 <sup>f</sup> ± 0.0022
Flaxseed 6% +1% beeswax	4.611 <sup>a</sup> ± 0.0104
Quinoa 6% +1% beeswax	2.918 <sup>e</sup> ± 0.0058
Carrageenan 0.5%	3.131 <sup>d</sup> ± 0.0100
Wheat flour 2%	3.621 <sup>c</sup> ± 0.0105

Values are means ± standard deviations of triplicate determinations.

Means in the same column with different superscript (a,b,c,...) are significantly different ( $p \leq 0.05$ )

### Selection of preferable samples.

Based on the results shown in Figure 1, certain treatments specifically T5, T9, T11, T13, T14 (peanut butter samples incorporating different essential oil concentrations) were found to be more

acceptable in terms of palatability and taste. Additionally, commercial samples (T3 and T4), as well as the control samples (T1 with beeswax and T2 without beeswax), were also among the preferred options.

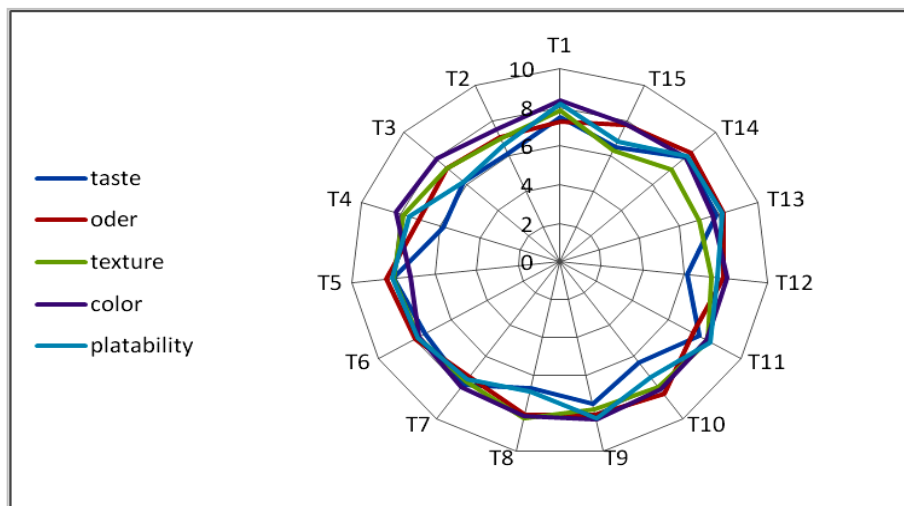


Figure 1. The preliminary panel test for choosing preferable treatments

\*T1: Peanut butter with all ingredients except Eos; T2: Peanut butter without other ingredients; T3: commercial peanut butter 1; T4: commercial peanut butter 2; T5, T6 and T7: Peanut butter with Eo of anise 0.3%, 0.2 and 0.1, respectively; T8 and T9: Peanut butter with Eo of cinnamon 0.3 and 0.2%, respectively; T10 and T11: peanut butter with EO of clove 0.2 and 0.1 %, respectively; T12: peanut butter with essential oils mixer of anise 0.1% + cinnamon 0.2 %; T13: Peanut butter with essential oils mixer of anise 0.15% + cinnamon 0.15 %; T14: Peanut butter with essential oils mixer of anise 0.25% + clove 0.05 %; T15: Peanut butter with essential oils mixer of anise 0.15% + clove 0.15 %

### Chemical composition of peanut butter samples

The proximate chemical composition of peanut butter samples (T1, T3, and T4) is presented in Table 5. The data indicate that the moisture and ash content in the control sample (T1) were significantly higher than in the commercial brands (T3 and T4). This increase may be attributed to the presence of honey in the control sample, which contrib-

utes to higher moisture and ash levels. The results align with those of Shrestha (2017), who reported that peanut spreads typically have a moisture content of 2.8%. Additionally, Dhamsaniya et al. (2011) stated that the moisture content of peanut butter should be less than 1%, as lower moisture levels contribute to an extended shelf life and enhanced microbial stability.

Table 5. Chemical composition of peanut butter samples (T1, T3 and T4)

Peanut butter	Moisture	Protein	Ether extract	Fiber	Ash	Available carbohydrates	Calories
*T1	2.61 <sup>a</sup> ±0.06	25.357 <sup>a</sup> ±0.12	45.6 <sup>c</sup> ±0.46	2.753 <sup>a</sup> ±0.42	3.88 <sup>a</sup> ±0.07	19.80 <sup>a</sup> ±0.19	591.02 <sup>b</sup> ±3.98
T3	1.55 <sup>b</sup> ±0.04	25.75 <sup>a</sup> ±1.22	47.6 <sup>b</sup> ±0.20	2.247 <sup>b</sup> ±0.03	3.63 <sup>b</sup> ±0.09	19.22 <sup>a</sup> ±1.32	608.28 <sup>a</sup> ±1.35
T4	1.61 <sup>b</sup> ±0.07	26.18 <sup>a</sup> ±0.80	48.67 <sup>a</sup> ±0.21	2.867 <sup>a</sup> ±0.06	3.65 <sup>b</sup> ±0.15	17.02 <sup>b</sup> ±0.42	610.81 <sup>a</sup> ±0.39

\*T1: Peanut butter with all ingredients except EOs.; T3: Peanut butter (commercial brand 1); T4: Peanut butter (commercial brand 2), Means in the same column with different superscript (a,b,c,...) are significantly different ( $p \leq 0.05$ ).

Following the recommendations of Yang et al. (2013) regarding the maximum shelf life of roasted almonds, the optimal moisture content range is 1.5–2.5%. Similarly, Mohd et al. (2016) recommended

that natural peanut butter can be stored for more than 16 weeks at 10°C and approximately 10 weeks at 25°C and 35°C, with a moisture content ranging from 1.6% to 2.5%.

There were no significant differences in protein content among all tested samples. Our results align with those of Shibli et al. (2019), who reported that protein content in different peanut cultivars ranged from 20.5% to 23%, and Mangels (2001), who found that peanut butter contained 26.7% protein. On the other hand, the control sample (T1) had the lowest fat content, which may be attributed to the presence of hydrogenated fatty acids in commercial brands, as indicated on their labels. Additionally, as shown in Table 6, the total fiber content exceeded 2%. Mangels (2001) reported that fiber content in peanut butter varies between 2.11% and 4.46%. The results also indicate that available carbohydrate content ranged between 17.02% and 19.80% for the studied samples. Moreover, the control sample had the lowest caloric value, consistent with the findings of Shrestha (2017).

The effect of beeswax as a stabilizer for peanut butter after six months

As shown in Table 6, oil visibly separated and accumulated on the surface during storage in the unstabilized peanut butter sample (T2). In contrast, no oil separation was observed over the six-month storage period in the commercial brands (T3 and T4). The use of beeswax as a stabilizer in T1 had a significant positive effect, preventing oil separation by approximately 97.9%. These findings are consistent with those of Winkler-Moser et al. (2019). Tekiki et al. (2022) demonstrated that even a minimum beeswax concentration of 1% was sufficient to bind the liquid and oily phases, effectively stabilizing the peanut butter. In this study, beeswax performed similarly to other hydrogenated oils and commercial peanut butter stabilizers. Additionally, beeswax has been recognized as an excellent oleogelator (Da Silva et al., 2018).

Table 6. Oil separation after six months from peanut butter (T1,T2,T3 and T4)

Samples	% Oil separation
*T1(beeswax 2% as stabilizer)	0.170 <sup>b</sup> ±0.023
T2 (without stabilizer)	7.979 <sup>a</sup> ±0.09
T3 (commercial brand from India)	0.049 <sup>c</sup> ±0.017
T4 (commercial brand from Egypt)	0.052 <sup>c</sup> ±0.004

\*T1 : Peanut butter with all ingredients except EOs.; T2: Peanut butter without other ingredients; T3: Peanut butter (commercial brand 1);T4: Peanut butter (commercial brand 2). Values are means ± standard deviations of triplicate determinations. Means in the same column with different superscript (a,b,c.....) are significantly different (p≤0.05)

Water activity (a<sub>w</sub>) of peanut butter of control and commercial samples

Peanut butter is known for its very low water activity (a<sub>w</sub>), typically below 0.7, which prevents the production of toxins (Behling et al., 2010). The measured a<sub>w</sub> values for commercial peanut butter samples were 0.37 and 0.41 (Table 7). The addition of stabilizers and other additives contributes to this lower water activity, creating an environment that inhibits microbial growth during storage. However, the unstabilized sample (T2) recorded a higher a<sub>w</sub> than the other samples due to the absence of a stabilizer. Moisture levels beyond a water activity of 0.40 led to an increase in oxidation rate, likely due to enhanced mobility of pro-oxidant factors and possible hydrolytic degradation (Evranuz, 1993).

Table 7. Water activity (a<sub>w</sub>) of peanut butter (T1, T2, T3, T4)

Samples	Water activity (a <sub>w</sub> )
*T1	0.46
T2	0.58
T3	0.37
T4	0.41

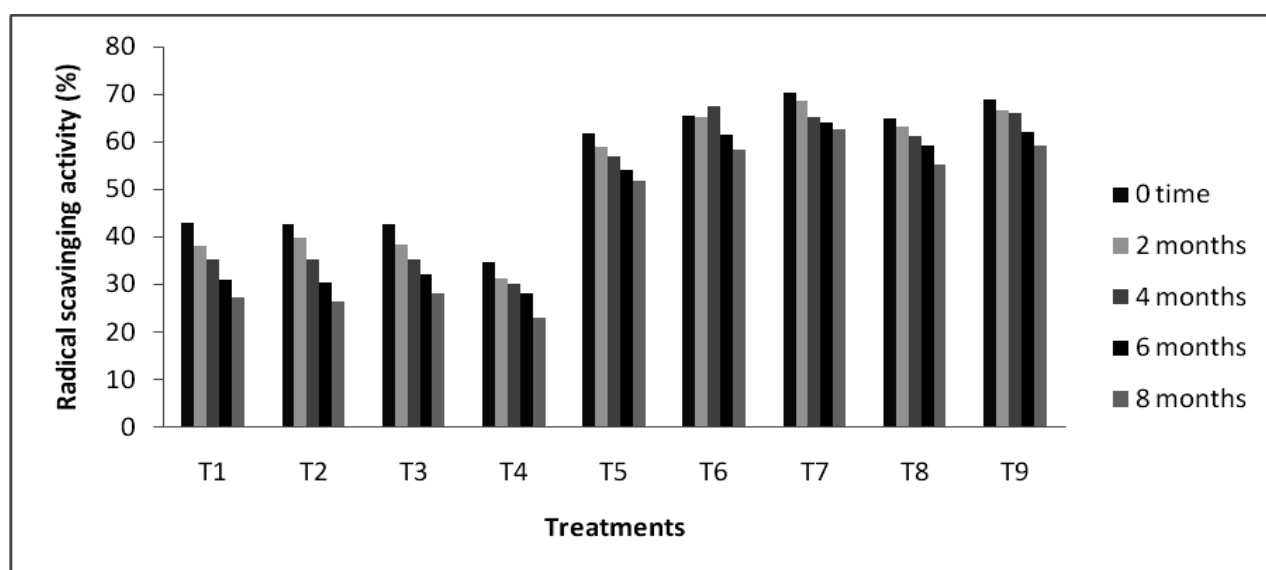
\*T1 : Peanut butter with all ingredients except EOs.; T2: Peanut butter without other ingredients; T3: Peanut butter (commercial brand 1);T4: Peanut butter (commercial brand 2)

Radical scavenging activity of peanut butter incorporated with essential oils

Results presented in Figure 2 illustrate the radical scavenging activity of peanut butter samples with essential oils compared to control samples (T1 and T2) and commercial samples (T3 and T4).

No significant differences were observed among T1, T2 and T3 across all storage months. However, T4 (the second commercial sample) exhibited the lowest radical scavenging activity, with a significant reduction compared to the other treatments. The highest radical scavenging activity was recorded in peanut butter samples incorporated with clove essential oil (CLEO) and a combination of anise and clove essential oils (ANEQ + CLEO), specifically in treatments T7 and T9. Their antioxidant activity ranged from 70.23% to 62.61% and 68.99% to 59.08%, respectively, from the beginning of storage up to six months. This superior activity is attributed to the potent antioxidant properties of clove essential oil. Following T7 and T9, the next highest radical scavenging activity was observed in treatments T6 and T8, with no significant differences between them. However, the lowest activity among EO-treated samples was recorded for peanut

butter treated with ANEO alone (T5). Additionally, a significant decline in radical scavenging activity was observed during storage, from zero time up to eight months, with the highest values recorded at the start of storage. These findings align with D'Avila-Farias et al. (2014), who demonstrated that the antioxidant potential of CLEO is primarily due to the presence of polar polyphenols such as eugenol and eugenol acetate. These compounds exhibit synergistic activity, providing broad spectrum antioxidant effects by donating hydrogen atoms and stabilizing free radicals. Furthermore, Pagthinathan (2020) reported a higher antioxidant activity in butter fortified with clove oil then it decreased over time, likely due to interactions with lipid radicals, leading to more stable oxidation products. This suggests that clove oil acts as an effective antioxidant, enhancing the shelf life of peanut butter during storage.



**Figure 2. Radical scavenging activity of peanut butter treatments**

\*T1 : Peanut butter with all ingredients except EOs.; T2: Peanut butter without other ingredients; T3: Peanut butter (commercial brand 1); T4: Peanut butter (commercial brand 2); T5 : Peanut butter with EO of anise 0.3%; T6 : Peanut butter with EO of cinnamon 0.2%; T7 : peanut butter with EO of clove 0.1 %; T8 : Peanut butter with essential oils mixer of anise 0.15% + cinnamon 0.15 %; T9: Peanut butter with essential oils mixer of anise 0.25% + clove 0.05 .

Eugenol, the primary component of clove essential oil (CLEO), is a phenolic compound containing an aromatic ring. This structure enables it to stabilize free radicals by donating hydrogen atoms. The stabilization mechanism is facilitated by its resonant structure, as reported by Gülçin (2010). Similarly,

the antioxidant capacity of cinnamon essential oil (CNEO) is attributed to its phenolic content. These phenolics inhibit fat oxidation by donating electrons and reacting with free radicals to form more stable products, thereby interrupting free radical chain reactions (Schmedes and Holmer, 1989; Han and



Rhee, 2005). Studies such as those by Mathew and Abraham (2006) and Hammad et al. (2013) have associated cinnamon oil’s antioxidant activity with the presence of cinnamaldehyde and eugenol. In the case of anise essential oil (ANEO), Singh et al. (2008) observed that at a concentration of 200 ppm, the volatile oil and oleoresins of anise demonstrated strong DPPH radical scavenging activity. This activity increased with concentration and even exceeded that of synthetic antioxidants like BHA and BHT. The scavenging power of anise oil is primarily due to trans-anethole, as noted by Burits and Bucar (2003). However, other minor compounds may also contribute to its overall antioxidant effect.

Peroxide Value in Peanut Butter During Storage

Oxidation is an undesirable reaction involving oxygen, which leads to the degradation of oil quality, resulting in rancidity, off-flavors, and unpleasant odors. The rate of oxidation depends on several factors, including, oil type, temperature, exposure to light and oxygen and presence of moisture and metals (e.g., iron) (Matsiko et al., 2014). The primary oxidation process in oils involves the formation of

hydroperoxides, which are quantified by measuring the peroxide value. Figure 3 illustrates the trends in peroxide values in peanut butter samples, showing the progression of oxidation over time. From Figure 3, it is evident that significant differences in peroxide values were observed across all treatments during storage. The values gradually increased from zero time up to 8 months, indicating ongoing lipid oxidation. Based on the results, peanut butter can be stored at ambient temperature (20–42°C) for up to 6 months while maintaining an acceptable peroxide value. Specifically: T5 and T8 reached a peroxide value of 10.69meq/kg at 6 months, T6, T7, and T9 maintained peroxide values below 10meq/kg (9.80, 9.41, and 9.77meq/kg, respectively), with no significant differences among them. Meanwhile, the control sample (T2) had the highest peroxide value, likely due to the absence of preservatives or stabilizers. In contrast, the commercial samples (T3 and T4) contained hydrogenated fatty acids, which prevent oil separation and contribute to oxidative stability. The other control (T1), stabilized with beeswax, functioned similarly to hydrogenated oils in peanut butter stabilization.

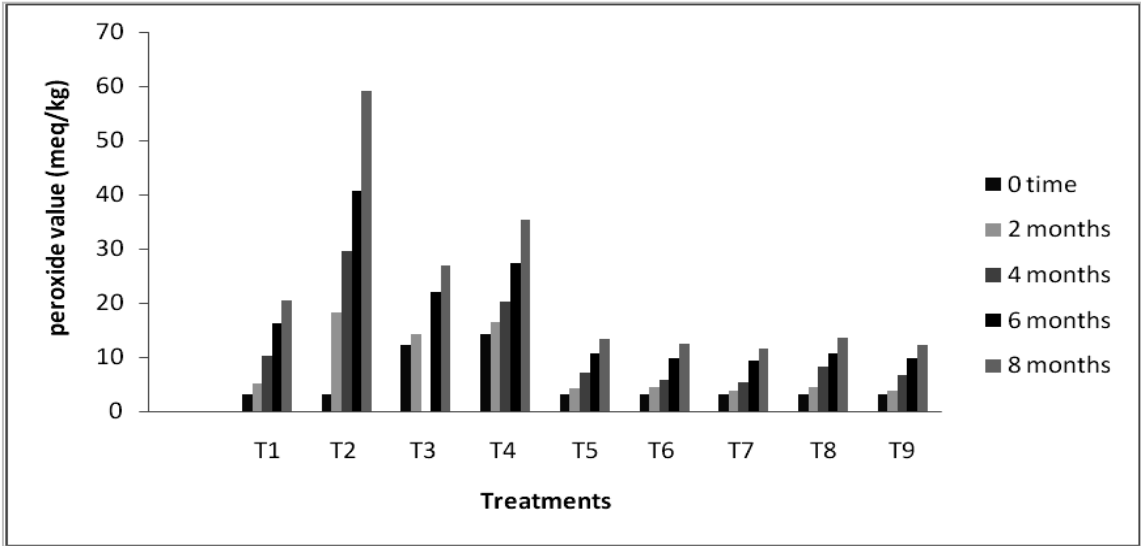


Figure 3. Peroxide value in peanut butter treatments during storage

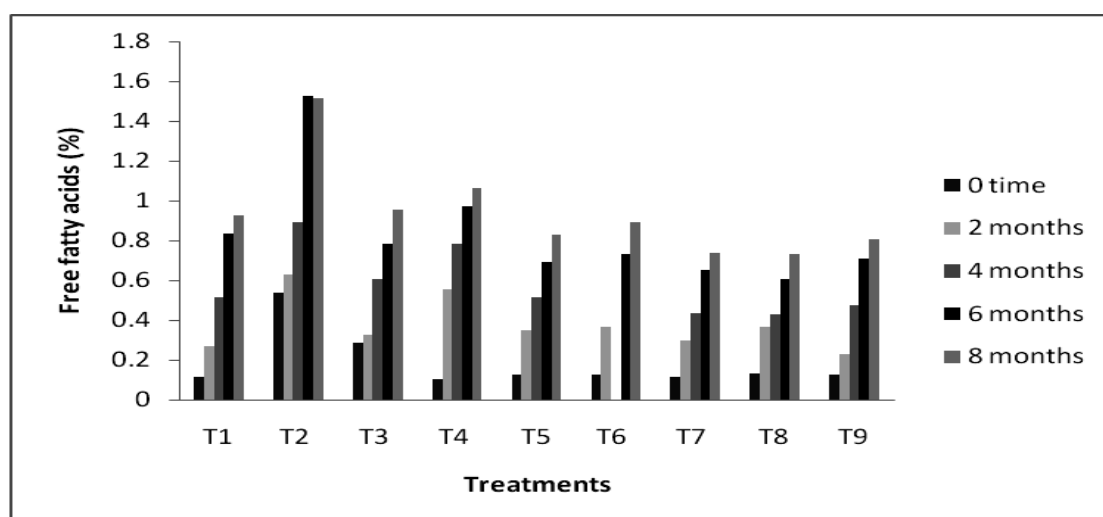
\*T1 : Peanut butter with all ingredients except EOs.; T2: Peanut butter without other ingredients; T3: Peanut butter (commercial brand 1); T4: Peanut butter (commercial brand 2); T5 : Peanut butter with EO of anise 0.3%; T6 : Peanut butter with EO of cinnamon 0.2%; T7 : peanut butter with EO of clove 0.1 %; T8 : Peanut butter with essential oils mixer of anise 0.15% + cinnamon 0.15 %; T9: Peanut butter with essential oils mixer of anise 0.25% + clove 0.05

## Free fatty acids (FFA) (%) in peanut butter

The data in Figure 4 show an increasing trend in the percentage of free fatty acids (% FFA) for samples T1, T2, T3, and T4. Notably, T2 (which contained neither beeswax nor essential oil) exhibited significant increments in %FFA during storage. However, the addition of clove essential oil (CLEO) in T7 or a combination of anise essential oil (ANEO) and cinnamon essential oil (CIEO) in T8 resulted in lower %FFA values. Moreover, the rate of increase in %FFA for these samples was slower during storage. There were no significant differences between T7 and T8 in the 6th and 8th months.

A similar trend was observed in other samples treated with essential oils, though the rate of increase in %FFA was slower compared to the control and commercial samples without essential oils. The continuous rise in %FFA over the storage period may be attributed to the hydrolytic decomposition of fats into free fatty acids (Vidanagamage et al., 2016).

Our findings align with those of Pagthinathan (2020), who reported that clove essential oil effectively reduced the rate of increase in free fatty acid values in butter. Additionally, Sindhu et al. (2000) found that free fatty acid levels increased due to both hydrolysis and fat oxidation.



**Figure 4. % Free fatty acids in peanut butter treatments during storage**

\*T1 : Peanut butter with all ingredients except EOs.; T2: Peanut butter without other ingredients; T3: Peanut butter (commercial brand 1); T4: Peanut butter (commercial brand 2); T5 : Peanut butter with EO of anise 0.3%; T6 : Peanut butter with EO of cinnamon 0.2%; T7 : peanut butter with EO of clove 0.1 %; T8 : Peanut butter with essential oils mixer of anise 0.15% + cinnamon 0.15 %; T9: Peanut butter with essential oils mixer of anise 0.25% + clove 0.05 .

## Microbial load of peanut butter samples during storage for 6 months

According to Table 8, there were no significant differences in microbial growth during storage, except for T2 and T4, which showed a slight increase in mold and yeast counts over time. Moreover, microbial load (bacteria, yeast, and mold) was not detected (-ve) in: T6 (cinnamon EO treatment), T8 and T9 (EO mixtures). This finding is supported by Table 3, which confirmed the strong antimicrobial properties of cinnamon oil. Also there is a synergistic antimicrobial effect among the essential oil compounds, which enhanced microbial inhibition. The antifungal activity of essential oils varies based on

their composition and concentration. Omidbeygi et al. (2007) highlighted that different fungal species exhibit varying levels of sensitivity to essential oils, with their effectiveness largely dependent on their chemical composition. These results confirm that incorporating essential oils, especially cinnamon, can significantly improve the microbial stability of peanut butter, reducing the risk of contamination and extending shelf life. Any compound may exhibit antifungal activity alone or in combination with other compounds (Shams Ghahfarokhi et al., 2003). Cinnamon essential oil has a strong antimicrobial effect and is widely used as an aromatic condiment and flavoring additive.

Referring to Table 1, the main component of cinnamon essential oil is cinnamaldehyde, which exhibits antibacterial activity (Wei et al., 2011 and Khare et al., 2014). Cinnamaldehyde acts on microorganisms by binding to the carbonyl group of microbial cell proteins and inhibiting the decarboxylation of amino acids (Moghadam et al., 2019). Nevertheless, natural peanut butters remain safe for consumption,

as aerobic plate counts are below the regulatory limit of 10,000CFU/g for peanut butter (USDA, 2010). Anise and clove essential oils also demonstrate significant inhibitory activity against fungi. The most bioactive component in anise is anethole (Shukla and Tripathi, 1987 and Chon et al., 2020), while eugenol is the primary antimicrobial agent in clove oil.

Table 8. Microbial load of peanut butter samples during storage for 6 months

Treatments	Total count (log CFU/g)			Mold & Yeast (log CFU/g)		
	0 time	3 months	6 months	0 time	3 months	6 months
T1	2.084cA	2.221cA	2.36cA	-ve	-ve	-ve
T2	3.063aA	3.24aA	3.563aA	4.004aB	4.222aAB	4.306aA
T3	2.655bA	2.655bA	2.747bA	3.377bA	3.323bA	3.403aA
T4	1.486dA	1.485dA	1.458dA	3.015cB	3.122bB	3.392bA
T5	2.055cA	2.041cA	2.047cA	-ve	-ve	-ve
T6	-ve	-ve	-ve	-ve	-ve	-ve
T7	1.028eA	1.140eA	1.158dA	-ve	-ve	-ve
T8	-ve	-ve	-ve	-ve	-ve	-ve
T9	-ve	-ve	-ve	-ve	-ve	-ve

\*T1 : Peanut butter with all ingredients except EOs.; T2: Peanut butter without other ingredients; T3: Peanut butter (commercial brand 1); T4: Peanut butter (commercial brand 2); T5 : Peanut butter with EO of anise 0.3%; T6 : Peanut butter with EO of cinnamon 0.2%; T7 : peanut butter with EO of clove 0.1 %; T8 : Peanut butter with essential oils mixer of anise 0.15% + cinnamon 0.15 %; T9: Peanut butter with essential oils mixer of anise 0.25% + clove 0.05. \*\*A& B: The means with the different capital (A& B) superscript letters within the same raw indicate significant (LSD ≤0.05) differences during 6 months of storage. \*\*\* a, b,c,...: The means with the different small (a, b,...) superscript letters within the same column indicate significant (LSD ≤0.05) differences among treatments. The data are means of 3 replicates ± SD. -Ve means no microbial load was detected

Effect of beeswax on the texture of processed peanut butter

Peanut butter is a spread possessing both elastic and viscous properties. Hardness (firmness) is an important indicator that determines its spreadability and texture (Swenson et al., 2000). Hardness influences the force required to spread the peanut butter, as hardness increases, spreading peanut butter on food becomes more difficult. Conversely, if hardness is too low, peanut butter becomes soft and collapses or flows easily. Another desired textural characteristic is stickiness, which is influenced by adhesiveness. Stickiness allows peanut butter to adhere to food surfaces better. However, excessive stickiness causes peanut butter to cling to utensils and resist spreading in the mouth, making it difficult to chew and swallow (Yadav, 2019). As shown in Figure 5, T2 (without beeswax) had the lowest firmness and cohesiveness but the highest adhesion, resulting in a sticky, viscous texture. In contrast, commercial samples T3 and T4 had the highest firmness values. T1 exhibited lower firm-

ness than commercial samples, making its texture preferable.

Sensory evaluation of selected samples during storage period

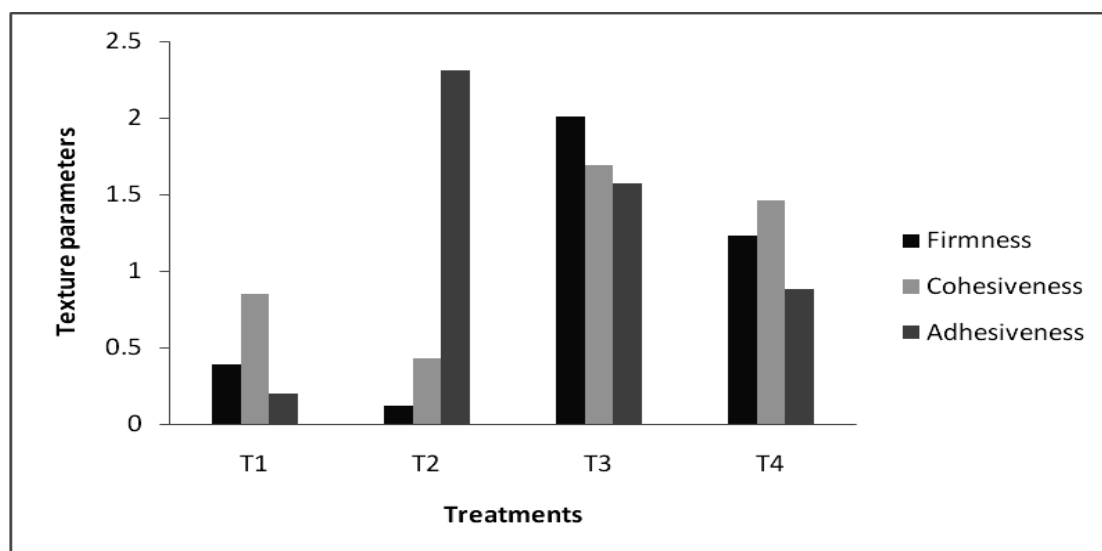
As shown in Figure 6, significant differences (p ≤ 0.05) were observed in taste parameters across all tested samples during storage, particularly in T2, where rancidity developed as peroxide values increased. T2 exhibited the worst taste, characterized by a fatty flavor and sticky mouthfeel. In contrast, commercial samples (T3 and T4) showed no significant differences during storage, though they had a moderately rancid taste from the outset. At zero time and the second month, T5 (peanut butter with 0.3% anise essential oil) showed no significant differences compared to other EO-treated samples. Panelists preferred T5, likely due to the sweet, aromatic flavor imparted by anethole, the primary compound in anise (Sun et al., 2019). By the sixth month, T5, T7, and T9 were rated highest by panelists. However, significant differences emerged among all EO-treated and untreated samples over

time, except for T8 and T9, which maintained consistent acceptability throughout storage.

**Texture:** T2 (without beeswax) scored lowest in texture, exhibiting viscoelasticity and stickiness. T3 had a slightly darker color, possibly due to extended roasting, though color remained stable in other samples during storage. T5 was consistently preferred

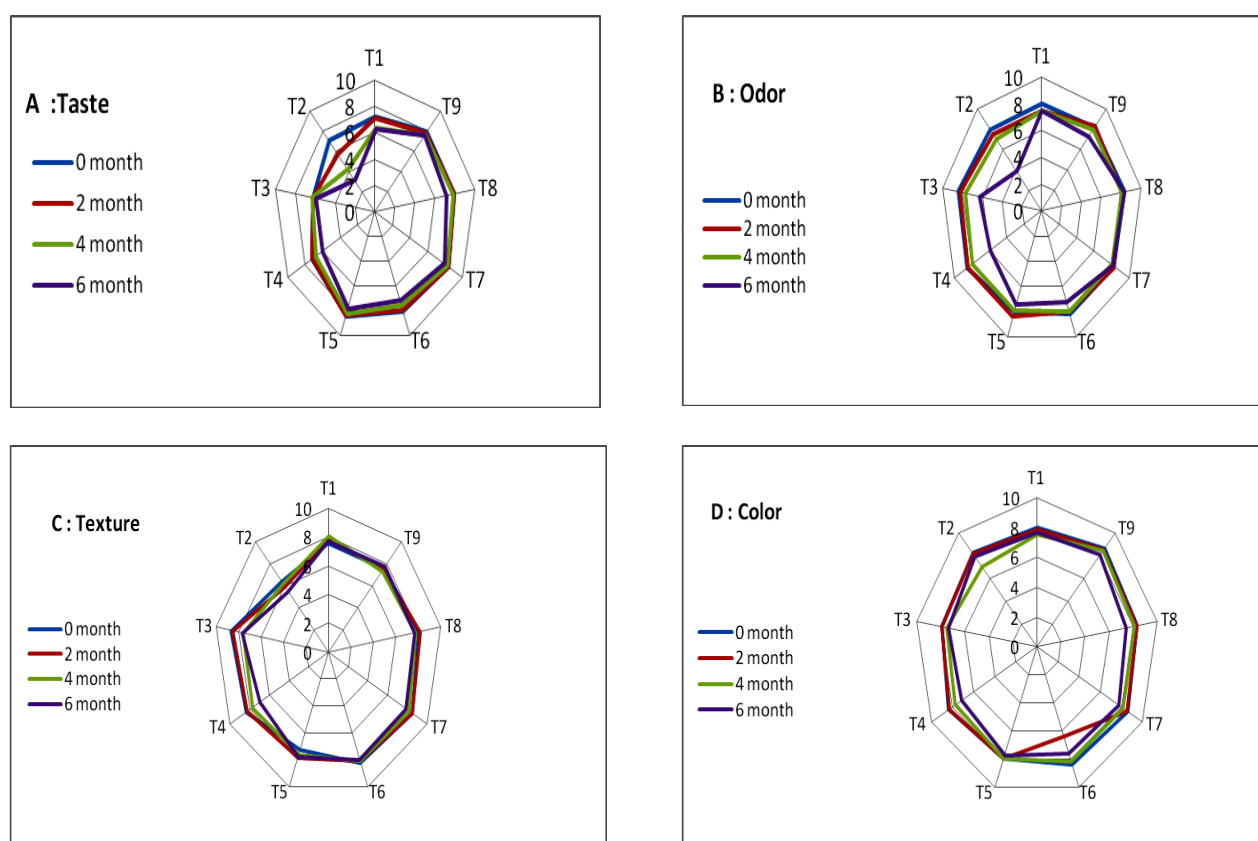
for palatability (Figure 6).

**Odor:** Odor trends mirrored taste, with T2 declining in approval due to rancidity. Samples with essential oils retained desirable odors. Texture differences were insignificant except in T2, which lacked cohesiveness and beeswax, resulting in a viscous texture.

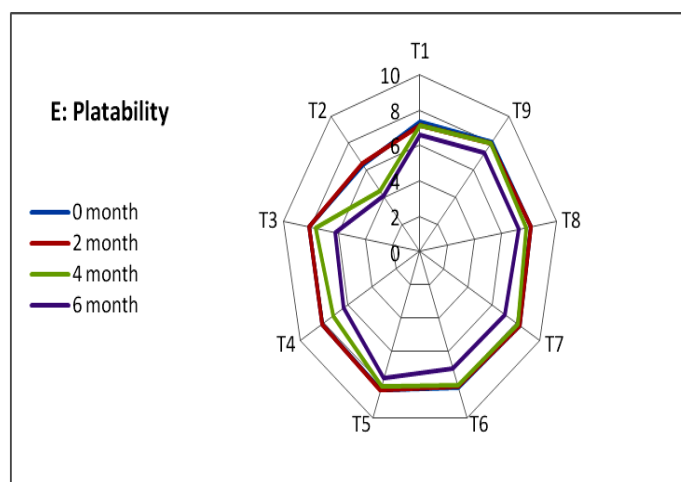


**Figure 5. The textural characteristics of peanut butter control and commercial samples**

\*T1 : Peanut butter with all ingredients except EOs.; T2: Peanut butter without other ingredients; T3: Peanut butter (commercial brand 1); T4: Peanut butter (commercial brand 2)







**Figure 6. Sensory characteristics of peanut butter samples during storage period (up to 6 months)**

\*T1: Peanut butter with all ingredients except Eos; T2: Peanut butter without other ingredients; T3: commercial peanut butter 1; T4: commercial peanut butter 2; T5, T6 and T7: Peanut butter with Eo of anise 0.3%, 0.2 and 0.1, respectively; T8 and T9 : Peanut butter with Eo of cinnamon 0.3 and 0.2%, respectively; T10 and T11 : peanut butter with EO of clove 0.2 and 0.1 % , respectively; T12 : peanut butter with essential oils mixer of anise 0.1% + cinnamon 0.2 %; T13 :Peanut butter with essential oils mixer of anise 0.15% + cinnamon 0.15 %; T14: Peanut butter with essential oils mixer of anise 0.25% + clove 0.05 %; T15: Peanut butter with essential oils mixer of anise 0.15% + clove 0.15 %

#### 4. Conclusion

This study demonstrates that incorporating essential oils (EOs) and beeswax can significantly enhance the quality and shelf life of peanut butter. Clove essential oil (CLEO) exhibited the highest antioxidant activity, followed by cinnamon (CNEO) and anise (ANEO). The synergistic effects of EO blends further improved oxidative stability and antimicrobial properties. The use of beeswax as a stabilizer effectively minimized oil separation, maintaining product consistency. Among the tested formulations, T7 (0.1% CLEO) and T9 (ANEO + CLEO) showed the best overall performance, with low peroxide values and microbial stability during six-month storage. Sensory evaluation highlighted T5 (0.3% ANEO) as the most preferred due to its sweetness, while T8 and T9 maintained consistent acceptability. These findings support the application of natural antioxidants and stabilizers as viable alternatives to synthetic additives in peanut butter production, aligning with consumer preferences for clean-label, preservative-free products. Future research should explore the long-term stability of EO-treated peanut butter under different storage conditions and optimize formulations for commercial scalability.

#### References

- AOAC (1996). Official methods of analysis. Washington, D.C. Association of Official Analytical Chemists.
- AOAC. (1990). Official Methods of Analysis, 15th edn. Virginia, USA: Association of Official Analytical Chemists.
- AOAC. (2012). Official Methods of Analysis, 19th edn. Gaithersburg, Maryland: Association of Official Analytical Chemists.
- Abayomi, E.O., Adetunji, A.E., and Odebunmi, O.O. (2002). Peroxide value as an indicator of lipid oxidation in peanut-based products during storage, *International Journal of Food Science and Technology*, 37(9):903–908.
- Adams, R.P. (2001). Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Publishing Corporation, Carol Stream Illinois, Allured.
- Al-Juhaimi, Y.F. (2014). Citrus fruits by-products as source of bioactive compounds with antioxidant potential, *Pakistan Journal of Botany*, 46 (4): 1459–1462.
- Ali, M. and Nigam, S. (1993). Peanut-based products and their role in addressing iron deficiency in developing countries, *Food Science and*

- Journal, 34(3):129–135.
- Anderson, W.G., McKinley, R.S. and Colavecchia, M., (1997). The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. *North American Journal of Fisheries Management*, 17, 301–307.
- Behling, R.G., Eifert, J., Erickson, M.C., Gurtler, J.B. and Kornacki, J.L. (2010). Selected pathogens of concern to industrial food processors: infectious, toxigenic, toxico-infectious, selected emerging pathogenic bacteria. In: Kornacki JL (Ed), *Principles of microbiological troubleshooting in the industrial food processing environment*, Food microbiology and food Safety. Behling food safety associates, Springer Science + Business Media publishers, Madison, Wisconsin, USA.
- Bourne, M.C. (2002). *Food texture and viscosity: Concept and measurement*, 2<sup>nd</sup> ed. London: Academic Press.
- Burits, M., and Bucar, F. (2003). Antioxidant activity of *Nigella sativa* essential oil, *Phytotherapy Research*, 14, pp. 323–328.
- Ceirwyn, S.J. (1995). *Analytical Chemistry of Food*, Part 1, p. 135.
- Chang, A.S., Sreedharan, A., and Schneider, A.R. (2013). Peanut and peanut products: A food safety perspective, *Food Control*, 32(1), pp. 296–303.
- Chon, J.W., Seo, K.H., Bae, D., Kim, B., Jeong, D. and Song, K.-Y. (2020). Antibacterial activity of clove oil against foodborne pathogenic bacteria and sensory attributes in clove oil-enriched dairy products: A preliminary study. *Journal of Dairy Science and Biotechnology*, 38(4), pp.197–206.
- Chuberre, B., Araviiskaia, E., Bieber, T. and Barbaud, A. (2019). Mineral oils and waxes in cosmetics: An overview mainly based on the current European regulations and the safety profile of these compounds. *Journal of the European Academy of Dermatology and Venereology.*, 33, 5–14.
- D'Avila-Farias, M., Souto-Oliveira, P., Pereira-Dutra, F., Jacobsen-Fernandes, T., de Pereira, C., Quintana-de Oliveira, S., Moro-Stefanello, F., Leonetti-Lencina, C. and Gatto-Barschak, A. (2014). Eugenol derivatives as potential antioxidants: Is phenolic hydroxyl necessary to obtain an effect?, *Journal of Pharmacy and Pharmacology*, 66, pp. 733–746.
- Da Silva, S.L., Amaral, J.T., Ribeiro, M., Sebastiao, E.E., Vargas, C., de Lima Franzena, F., Schneider, G., Lorenzo, J.M., Fries, L.L.M., Cichoski, A.J. and Campagnol, P.C.B. (2018). Fat replacement by oleogel rich in oleic acid and its impact on the technological, nutritional, oxidative, and sensory properties of bologna-type sausages, *Meat Science*, 149, pp. 141–148.
- Dhamsaniya, N.K., Patel, N.C., and Dabhi, M.N. (2011). Selection of groundnut variety for making a good quality peanut butter, *Journal of Food Science and Technology*, 49(1):115–118.
- Difco- Manual (1977). *Dehydrated Culture Media and Reagent Microbiological and Clinical Laboratory Procedures*. 9th Ed., Pub. Difco- Lab., Detroit, Michigan, USA., pp.582-585.
- EL-Amrania, S.E., Lalamib, A.E.O., Zoubic, Y.E., Moukhafia, K., Bouslamtia, R., Lairinia, S. (2019). Evaluation of antibacterial and antioxidant effects of cinnamon and clove essential oils from Madagascar, *Material Today Proceeding*, 13:762–770.
- EU (1995). European Commission Directive 95/2/EC on food additives other than colours and sweeteners. Brussels.
- Evranzuz, E.O. (1993). 'The effects of temperature and moisture content on lipid peroxidation during storage of unblanched salted roasted peanuts: Shelf life studies for unblanched salted roasted peanuts', *International Journal of Food Science and Technology*, 28(2):193–199.
- FDA (2015). *Food standards: Peanut butter and peanut products*, U.S. Food and Drug Administration.
- Fadel, H.H.M., El-Ghorab, A.H., Ahmed, M.S., Hussein, M., El-Massry, K.F., Lotfy, S.N., Ahmed, M.Y.S. and Soliman, T.N. (2020). 'Correlation between chemical composition and radical scavenging activity of commercial essential oils: Impact of microencapsulation on

- functional properties of essential oils', Arabian Journal of Chemistry, 13, pp. 6815–6827.
- Gao, Y., Lei, Y., Wu, Y., Hongshan, L., Li, J., Pei, Y., Li, Y., Li, B., Luo, X. and Liu, S. (2021). Beeswax: A Potential Self-Emulsifying Agent for the Construction of Thermal- Sensitive Food W/O Emulsion. Food Chemistry, 349, Article ID: 129203.  
https://doi.org/10.1016/j.foodchem.2021.129203.
- Gulluoglu, L., Bakal, H., Onat, B., El Sabagh, A., and Arioglu, H. (2016). Characterization of peanut (*Arachis hypogaea* L.) seed oil and fatty acids composition under different growing seasons under Mediterranean environment', Journal of Experimental Biology and Agricultural Sciences, 4(5S), pp. 564–571.
- Gülçin, I. (2010). Antioxidant properties of resveratrol: A structure-activity insight, Innovative Food Science & Emerging Technologies, 11(1): 210–218.
- Hammad, H.M., Albu, C., Matar, S.A., Litescu, S.C. and Al Jaber, H.I. (2013). Biological activities of the hydro-alcoholic and aqueous extracts of *Achillea biebersteinii* (Forssk) grown in Jordan, African Journal of Pharmacy and Pharmacology, 7(25):1686–1694.  
DOI: 10.4236/ns.2014.61005.
- Han, J. and Rhee, K.S. (2005). Antioxidant properties of selected oriental non-culinary/ nutraceutical herb extracts as evaluated in raw and cooked meat, Meat Science, 70(1):25–33.  
DOI: 10.1016/j.meatsci.2004.11.017.
- Hood, J.R., Wilkinson, J.M., and Cavanagh, H.M.A. (2003). Evaluation of common antibacterial screening methods utilized in essential oil research, Journal of Essential Oil Research, 15(6): 428–433.  
DOI: 10.1080/10412905.2003.9698631.
- Hossain, M.A., Al-Hashmi, R.A., Weli, A.M., Al-Riyami, Q. , Al- Sabahi, J. (2012). Constituents of the essential oil from different brands of *Syzigium caryophyllatum* L. by gas chromatography-mass spectrometry. Asian Pacific Journal of Tropical Biomedicine, 2(3), S1446-S1449 .  
DOI:10.1016/S2221-1691(12)60435-3
- Huang, Z., Guo, B., Deng, C., Luo, S., Liu, C., and Hu, X. (2020). Stabilization of peanut butter by rice bran wax, Journal of Food Science, 85(6), pp. 1793–1801.  
DOI: 10.1111/1750-3841.15176.
- Hussain, J., Rehman, N., Shinwari, Z.K., Khan, A.L., Al-Harrasi, A., Ali, L. and Mabood, F. (2014). Preliminary comparative analysis of four botanicals used in the traditional medicines of Pakistan, Pakistan Journal of Botany, 46(4), pp. 1403–1407.
- ISO 21527-2: (2008). Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of yeasts and moulds. International Organization for Standardization, Geneve, Switzerland. pp. 1-17.
- ISO 4833: (2003). Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of microorganisms. Colony-count technique at 30°C. International Organization for Standardization, Geneve, Switzerland. pp.1-19.
- Isanga, J., and Zhang, M. (2007). Peanut seeds play a significant role in protecting against cardiovascular diseases, cancer, diabetes, osteoporosis, and other degenerative conditions, Journal of Food Science and Technology, 44(5):1247–1251.
- Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A. and Schmidt, E. (2006). Chemical composition and antioxidant properties of clove leaf essential oil', Journal of Agricultural and Food Chemistry, 54, pp. 6303–6307.
- Kaur, D., Wani, A.A., Singh, D.P. and Sogi, D.S. (2011). Shelf life enhancement of butter, ice-cream, and mayonnaise by addition of lycopene, International Journal of Food Properties, 14(6), pp. 1217–1231.
- Khare, A.K., Biswas, A.K. and Sahoo, J. (2014). Comparison Study of Chitosan, EDTA, Eugenol and Peppermint Oil for Antioxidant and Antimicrobial Potentials in Chicken Noodles and Their Effect on Colour and Oxidative Stability at Ambient Temperature Storage. LWT– Food Science

- and Technology, 55(1):286–293.
- Kildea, M.A., Allanb, G.L. and Kearney, R.E., (2004). Accumulation and clearance of the anaesthetics clove oil and AQUIS<sub>2</sub> from the edible tissue of silver perch (*Bidyanus bidyanus*). Aquaculture, 232, 265–277.
- Kim, J., Lee, J.H., Kim, D.S. (2015). Insecticidal and acetylcholine esterase inhibition activity of Asteraceae plant essential oils and their constituents against adults of the German cockroach (*Blattella germanica*), Journal of Agricultural and Food Chemistry, 63, pp. 1187–1194.
- Lee, K.G., Shibamoto, T. (2001). Antioxidant property of aroma extract isolated from clove buds (*Syzygium aromaticum* L.) Merr. et Perry, Food Chemistry, 74, pp. 443–448. DOI: 10.1016/S0308-8146(01)00161-3.
- Mangels, R. (2001). Guide to nuts and nut butters, Vegetarian Journal, 21, pp. 20–23.
- Martos, M.V., Mohamady, M.A., Lopez, F.J., Abdelrazik, K.A. (2007). In vitro antioxidant and antibacterial activities of essential oils obtained from Egyptian aromatic plants, Food Control, 22, pp. 1715–1722. DOI: 10.1016/j.foodcont.2011.04.003.
- Matan, N., Rimkeeree, H., Mawson, A.J., Chompreeda, P., Haruthaithanasan, V., Parker, M., (2006). Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. Int. J. Food Microbiol. 107, 180–185.
- Mathew, S., and Abraham, T.E. (2006). Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models, Food Chemistry, 94(4), pp. 520–528. DOI: 10.1016/j.foodchem.2004.11.043.
- Matsiko, F., Murindwa, E., Niyigena, C., Hitimana, B. and Vasanthakalam, H. (2014). Comparison and evaluation of the quality and storage stability of soy and peanut butter, Pakistan Journal of Food Science, 24(1), pp. 50–56.
- Moghadam, Z.A., Hosseini, H., Hadian, Z., Asgari, B., Mirmoghtadaie, L., Mohammadi, A., Shamloo, E., and Javadi, N.H.S., (2019). Evaluation of the antifungal activity of cinnamon, clove, thymes, *Zataria multiflora*, cumin, and caraway essential oils against ochratoxigenic *Aspergillus ochraceus*. Journal of Pharmaceutical Research International, 26(1), pp.1–16.
- Mohamed, S.A.M., Tawfik, M.S.H. and Ibrahim, A.E. (2020). Influence of two extraction methods on essential oils of some Apiaceae family plants. Egyptian Pharmaceutical Journal, 18:160–164
- Mohd, R.N.H., Chin, N.L., Yusof, Y.A. and Mahyudin, N. (2016). Quality changes of stabilizer-free natural peanut butter during storage, Journal of Food Science and Technology, 53 (1):694–702. DOI: 10.1007/s13197-015-2006-x.
- Muego-Gnanasekharan, K.F. (1992). Physicochemical and sensory characteristics of peanut paste stored at different temperatures, Journal of Food Science, 57:1385–1389.
- Nagababu, E., and Lakshmaiah, N., (1992). Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. Biochem. Pharmacol. 43, 2393–2400.
- Nepote, V., Zygadlo, J., and Valle, D. (2006). Rancidity in peanut butter and peanut-derived products: The role of unsaturated fatty acids oxidation, Food Chemistry, 97(2), pp. 273–279.
- Ogata, M., Hoshi, M., Urano, S and Endo, T. (2000). Antioxidant activity of eugenol and related monomeric and dimeric compounds. Chemical and Pharmaceutical Bulletin, 48:1467–1469..
- Omidbaigi, R., Hadjiakhoondi, A., and Saharkhiz, M. (2003). Changes in content and chemical composition of *Pimpinella anisum* L. oil at various harvest times, Journal of Essential Oil Bearing Plants, 6, pp. 46–50.
- Omidbeygi, M., Barzegar, M., Hamidi, Z., and Naghdibadi, H. (2007). Antifungal activity of thyme, summer savory, and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste, Food Control, 18(12):1518–1523.
- Pagthinathan, M. (2020). Impact of physicochemical and sensory acceptability of clove oil added butter, International Journal of Scientific and Research Publications, 10(5).



- against *Aspergillus flavus* in liquid medium and tomato paste, *Food Control*, 18(12):1518–1523.
- Pagthinathan, M. (2020). Impact of physicochemical and sensory acceptability of clove oil added butter, *International Journal of Scientific and Research Publications*, 10(5).
- Palomar, L.S., Galvez, L.A., Dotollo, M.O., Lustre, A.O. and Resurreccion, A.V.A. (2006). Standardization of Stabilized Peanut Spread with Roasted Cassava Flour. In: *Peanut Butter and Spreads*. USA and Philippines: Peanut Collaborative Research Support Program (Peanut-CRSP), pp. 108-126.
- Paranagama, P.A., Wimalasena, S., Jayatilake, G.S., Jayawardena, A.L., Senanayake, U.M. and Mubarak, A.M. (2001). A comparison of essential oil constituents of bark, leaf, root, and fruit of cinnamon (*Cinnamomum zeylanicum* Blume) grown in Sri Lanka, *Journal of the National Science Foundation of Sri Lanka*, 29(3&4), pp. 147–153.
- Petry, T. Bury, D. Fautz, R. Hauser, M. Huber, B. Markowetz, A. Mishra, S. Rettinger, K. Schuh, W. and Teichert, T. (2017). Review of data on the dermal penetration of mineral oils and waxes used in cosmetic applications. *Toxicology Letter*, 280, 70–78.
- Pidatala, P.K., Bellmer, D., and McGly, W. (2021). Oxidative stability of a new peanut butter bite product, *International Journal of Food Science*, 2021, 1-9. DOI: 10.1155/2021/5528315.
- Radünz, M., Da Trindade, M.L.M., Camargo, T.M., Radünz, A.L., Borges, C.D., Gandra, E.A. and Helbig, E. (2019). Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (*Syzygium aromaticum*, L.) essential oil, *Food Chemistry*, 15, pp. 180–186. DOI: 10.1016/j.foodchem.2018.09.173.
- Ramadan, M.F., Kinni, S.G., Seshagiri, M. and Mörsel, J. (2010). Fat-soluble bioactives, fatty acid profile and radical scavenging activity of *Semecarpus anacardium* seed oil', *Journal of the American Oil Chemists' Society*, 87, pp. 885–894. DOI: 10.1007/s11746-010-1567-0.
- Rojas, J., Cabrera, S., Benavides, J., Lopera, Y. and Yarce, G. K. (2021). Lipidic matrixes containing clove essential oil: biological activity, microstructural and textural studies. *Molecules*, 26(9):2425. DOI: 10.3390/molecules26092425.
- Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. and Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods', *Food Chemistry*, 91, pp. 621–632.
- Salunkhe, D.K., Chavan, J.K., Adsule, R.N. and Kadam, S.S. (1992). *World oilseeds: chemistry, technology and utilization*. Van Nostrand, Reinhold, New York, pp 140–196.
- Sanders, T.H. (2003) Peanut. In: *Encyclopedia of food sciences and nutrition*, Academic press, Amsterdam, pp 4420–4427
- Schmedes, A. and Holmer, G. (1989). A new thio-barbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation', *Journal of the American Oil Chemists' Society*, 66(6), pp. 813–817. DOI: 10.1007/BF02653674.
- Shams Ghahfarokhi, M., Razafsha, M., Allameh, A., Razzaghi, M. and Abyaneh, M. (2003). Inhibitory effects of aqueous onion and garlic extracts on growth and keratinase activity in *Trichophyton mentagrophytes*', *Iranian Biomedical Journal*, 7(3), pp. 113–118.
- Shasidhar, Y., Vishwakarma, M.K., Pandey, M.K., (2017). Molecular mapping of oil content and fatty acids using dense genetic maps in groundnut (*Arachis hypogaea* L.), *Frontiers in Plant Science*, 8, p. 794. DOI: 10.3389/fpls.2017.00794
- Shibli, S., Siddique, F., Raza, S., Ahsan, Z. and Raza, I. (2019). Chemical composition and sensory analysis of peanut butter from indigenous peanut cultivars of Pakistan', *Pakistan Journal of Agricultural Research*, 32(1):159.
- Shrestha, A. (2017) Roasting time-temperature optimization for preparation of peanut butter and study on its shelf life. B.Tech. thesis. Tribhuvan

- University, Nepal.
- Shukla, H.S and Tripathi, S.C. (1987). Antifungal substance in the essential oil of anise (*Pimpinella anisum* L.). Biol. Chem. Agric. , 51, 1991–1993. [https://www.jstage.jst.go.jp/article/bbb1961/51/7/51\\_7\\_1991/\\_pdf](https://www.jstage.jst.go.jp/article/bbb1961/51/7/51_7_1991/_pdf)
- Sindhu, J.S., Sumit, A. and Nayak, S.K. (2000) ‘Physico-chemical aspects of indigenous dairy products, Indian Dairyman, 52(10):51–64.
- Singh, G., Kapoor, I.P.S., Singh, P., de Heluani, C.S. and Catalan, C.A.N. (2008). Chemical composition and antioxidant potential of essential oil and oleoresins from anise seeds (*Pimpinella anisum* L.), International Journal of Essential Oil Therapeutics, 2, pp. 122–130.
- Steel, R., Torrie, J. and Dickey, D. (1997) Principles and Procedures of Statistics: A Biometrical Approach. New York: McGraw-Hill.
- Sun, W., Shahrajabian, M.H. and Cheng, Qi. (2019) ‘Anise (*Pimpinella anisum* L.), a dominant spice and traditional medicinal herb for both food and medicinal purposes’, Cogent Biology, 5, pp. 1673688.
- Swenson, B.J., Wendorff, W.L. and Lindsay, R.C. (2000) ‘Effects of ingredients on the functionality of fat-free processed cheese spreads’, Journal of Food Science, 65, pp. 822–825.
- Tabanca, N., Demirci, B., Kirimer, N., Baser, C.H.K., Bedir, E., Khan, A.I. and Wedge, E.D. (2005) ‘Gas chromatographic-mass spectrometric analysis of essential oil from *Pimpinella aurea*, *Pimpinella corymbosa*, *Pimpinella peregrina* and *Pimpinella puberula* gathered from Eastern and Southern Turkey’, Journal of Chromatography A, 1097, pp. 192–198.
- Taghipour, Y.D., Hajialyani, M., Naseri, R., Hesari, M., Mohammadi, P., Stefanucci, A., Mollica, A., Farzaei, M.H., and Abdollahi, M. (2019) ‘Nanoformulations of natural products for management of metabolic syndrome’, International Journal of Nanomedicine, 14:5303–5321. DOI: 10.2147/IJN.S213831
- Tekiki, A., Charfi, I., Helal, R., and Bornaz, S. (2022). Formulation and optimization of new spreads based on olive oil and honey: A response surface methodology Box-Benken design’, Food and Nutrition Sciences, 13, pp. 358–372.
- Tirapelli, C.R., Andrade, R.C., de Cassano, O.A., de Souza, A.F., Ambrosio, R.S., Costa, B.F. and da Oliveira, M.A. (2007) ‘Antispasmodic and relaxant effects of the hydroalcoholic extract of *Pimpinella anisum* (Apiaceae) on rat anococcygeous smooth muscle’, Journal of Ethnopharmacology, 110, pp. 23–29.
- Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A. and Saija, A. (2005) ‘Influence of heating on antioxidant activity and the chemical composition of some spice essential oils’, Food Chemistry, 89, pp. 549–554.
- Ullah, H., Mahmood, A. and Honermeier, B. (2014) ‘Essential oil and composition of anise (*Pimpinella anisum* L.) with varying seed rates and row spacing, Pakistan Journal of Botany, 46 (5):1859–1864.
- United States Department of Agriculture (2010) Commodity requirements PP12 peanut products for use in domestic programs, Farm Service Agency (FSA), Washington, DC.
- Upadhyay, R.K. (2015). GC-MS analysis and in vitro antimicrobial susceptibility of *Foeniculum vulgare* seed essential oil’, American Journal of Plant Sciences, 6(7), pp. 1058–1067.
- Vidanagamage, S.A., Pathiraje, P.M.H.D. and Perala, O.D.A.N. (2016). Effects of cinnamon (*Cinnamomum verum*) extract on functional properties of butter, Proceedings of the International Conference of Sabaragamuwa University of Sri Lanka, 6, pp. 136–142.
- Waterstrat, P.R., (1999). Induction and recovery from anesthesia in channel catfish *Ictalurus punctatus* fingerlings exposed to clove oil. J. World Aquaculture Society, 30, 250–255.
- Wei, Q.Y., Xiong, J.J., Jiang, H., Zhang, C. and Ye, W. (2011). The Antimicrobial Activities of the Cinnamaldehyde Adducts with Amino Acids. International Journal of Food Microbiology, 150.

- Winkler-Moser, J.K., Anderson, J.E., Byars, J.A., Singh, M. and Hwang, H.S. (2019) 'Evaluation of beeswax, candelilla wax, rice bran wax, and sunflower wax as alternative stabilizers for peanut butter', *Journal of the American Oil Chemists' Society*, 96, pp. 1235–1248.
- Woodroof, J.G. (1983). Peanut Butter. In: *Peanuts: Production, Processing, Products* (3rd ed.). (J.G. Woodroof, Ed.). pp. 181-227. Westport, Connecticut. AVI Publishing Co., Inc.
- Xing, Y., Li, X., Xu, Q., Yun, J. and Lu, Y. (2010) 'Antifungal activities of cinnamon oil against *Rhizopus nigricans*, *Aspergillus flavus* and *Penicillium expansum* in vitro and in vivo fruit test', *International Journal of Food Science and Technology*, 45(9), pp. 1837–1842.
- Yadav, A. (2019). Effect of flaxseed addition on rheological, texture and color characteristics of peanut butter, *International Journal of Chemical Studies*, 7(2):1989–1993.
- Yang, J., Pan, Z., Takeoka, G., Mackey, B., Bingol, G., Brandl, M.T., Garcin, K., McHugh, T.H. and Wang, H. (2013). Shelf-life of infrared dry-roasted almonds, *Food Chemistry*, 138(1):671–678.
- Zengin, M., Genc, H., Taslimi, P., Kestane, A., Gulclu, E., Ogutlu, A., Karabay, O. and Gulçin, I. (2018). Novel thymol bearing oxypropanolamine derivatives as potent some metabolic enzyme inhibitors – Their antidiabetic, anticholinergic and antibacterial potentials, *Bioorganic Chemistry*, pp. 119–126.  
DOI: 10.1016/j.bioorg.2018.08