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Evaluation of Some Natural Antioxidants Efficiency on Healthy Peanut Butter Quality

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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 (ANEO; 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 99.83% 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 + ANEO) maintained superior quality, with peroxide values below 10 meq/kg, outperforming other samples. CLEO and ANEO+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% ANEO) 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.

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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 (Woodroof, 1983). 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; 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, γ-himachalene, and cis-anethole (Tabanca et al., 2005 and Omidbaigi 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 β-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) (European Union, 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 (Chuberre 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.

Preliminary test for accelerated oil release: 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 300 g 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 (300 g) 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)

Chemical Analysis

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):

Energy (kcal) = (Protein × 4) + (Carbohydrate × 4) + (Fat × 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 (2 g) were spread into sample cups and analyzed using a Decagon AquaLab hygrometer (LabStart-aw, Novasina) to measure water activity (a_w) (Mohd Rozalli et al., 2016).

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). Twenty mL of Mueller Hinton agar (for bacteria) or Sabouraud agar (for fungi), containing 1% Tween 80 as an emulsifier, was poured into sterilized plates (90 mm diameter) (Difco Manual, 1977). Ten µL 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.

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, 25 g of peanut butter samples were homogenized with 225 mL of sterile buffered peptone water (0.1% w/v) to prepare a 10^{-1} dilution. For total plate count, 0.1 mL 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 $\log_{10}$ of total bacterial count per gram was calculated.

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} M in toluene) was prepared. For each treatment, 100 µL of essential oil or 10 mg of peanut butter oil (dissolved in 100µL toluene) was mixed with 390 µL 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:

% inhibition = [(absorbance of control – absorbance of test sample) / absorbance of control] × 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:

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

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 Acid Determination

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 40 mm diameter aluminum cylindrical probe was used in a double compression test to penetrate 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 according to Bourne (2002).

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 (ANEO, CNEO, and CLEO)

The essential oils of anise (ANEO), cinnamon (CNEO), and clove (CLEO) were fractionated and identified using GC/MS, as shown in Table 1. ANEO 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%).

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^{e}±0.11
CNEO (0.2%)	83.05^{c}±0.19
CLEO (0.1%)	95.78^{a} ±0.21
ANEO (0.2) +CNEO (0.1)	73.12^{d}±0.28
ANEO (0.25) + CLEO (0.05)	91.12^{b}±0.11
**BHT (200 ppm)(0.02%)	54.43^{f}±1.06

*ANEO is anis 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 ($p \leq 0.05$)

Antioxidant activity of essential oils

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).

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

The antimicrobial activity of essential oils (EOOs) 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). Additionally, 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, antifungal, 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). 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 ANEO (82%). CNEO 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%), acetoeugenol (10.83%), 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, β-caryophyllene, α-caryophyllene, and carvacrol have been reported as major components in clove essential oil from Indonesia and India

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	α-(Z,E)-Farnesere	0.26	-	-
2	Estragole	0.45	-	-
3	Hydroxymethoxyquinoline	3.72	-	-
4	Zingiberene	0.15	-	-
5	β- 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	α-Ocimene	-	1.38	0.78
21	Carvone	-	0.61	-
22	γ-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).

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

Microbial Strains	Tested essential oils and reference antibiotics									
	CLEO (%)			ANEO (%)			CNEO (%)		Amphotericin B*	Gentamicin*
	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3		
Gram positive bacteria										
Bacillus subtilis ATCC 33221	7.4^{bD}± 0.13	12.3^{aC} ± 0.25	19.4^{bB} ± 0.07	0.0^{bE} ± 0.00	7.1^{bD} ±0.03	8.8^{abD} ± 0.14	22.0^{aB} ± 0.3	$>90^{aA}$	12.2^{aC} ±0.33	9.0^{abD} ±0.1
Staphylococcus aureus ATCC 20231	0.0^{cE**} ± 0.00	7.1^{cD} ± 0.03	14.0^{cC} ± 0.21	0.0^{bE} ± 0.00	0.0^{cE} ±0.00	9.2^{bD} ± 0.11	19.8^{aB} ±0.3	$>90^{aA}$	12.3^{aC} ±0.13	0.0^{cE} ±0.00
Gram negative bacteria										
Escherichia coli ATCC 6933	7.2^{bB} ±0.05	10.0^{bD} ±0.14	13.7^{cC} ±0.14	0.0^{bF} ±0.00	7.1^{aE} ±0.07	7.3^{bE} ±0.22	21.0^{aB} ±0.2	$>90^{aA}$	8.5^{bE} ±0.2	8.5^{bE} ±0.17
PesudomonasaeroginosaATCC 9027	6.4^{bD} ± 0.00	7.4^{cD} ± 0.18	11.0^{bcC} ± 0.00	0.0^{bE} ± 0.00	6.8^{bD} ± 0.1	8.3^{bC} ± 0.10	22.8^{aB} ± 0.1	$>90^{aA}$	10.2^{abC} ± 0.2	9.0^{bC} ± 0.3
Molds										
Aspergillus nigerNRRL 2322	10.1^{aCD} ± 0.11	15.1^{aC} ± 0.15	22.0^{bB} ± 0.33	6.8^{aD} ± 0.11	8.5^{aD} ± 0.11	12.7^{aCD} ± 0.34	$>90^{aA}$	$>90^{aA}$	9.3^{cD} ± 0.21	16.0^{aC} ± 0.3
Aspergillus flavusEMCC 100	9.8^{aE} ± 0.71	15.9^{aC} ± 0.22	19.8^{bC} ± 0.13	7.1^{aF} ± 0.16	9.3^{aE} ± 0.33	11.3^{aD} ± 0.33	33.6^{aB}± 0.1	$>90^{aA}$	11.0^{cD} ± 0.01	16.3^{aC} ± 0.1
Yeasts										
Saccharomyces cerevisiae NRRLY 2034	8.0^{bD} ± 0.22	13.0^{bC} ± 0.1	33.3^{aB} ± 0.11	0.0^{bE} ± 0.00	0.0^{cE} ± 0.00	0.0^{cE} ± 0.00	$>90^{aA}$	$>90^{aA}$	8.2^{cD} ± 0.19	9.0^{abD} ± 0.1
Candida lypolitica NRRLY 1095	7.2^{bCD} ±0.11	11.0^{cC} ±0.1	13.7^{dC} ±0.12	0.0^{bD} ±0.00	8.1^{aCD} ± 0.1	10.3^{bB} ± 0.11	23.6^{aB} ±0.1	44.6^{bA} ±0.1	10.5^{bC} ±0.2	8.9^{bCD} ±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.

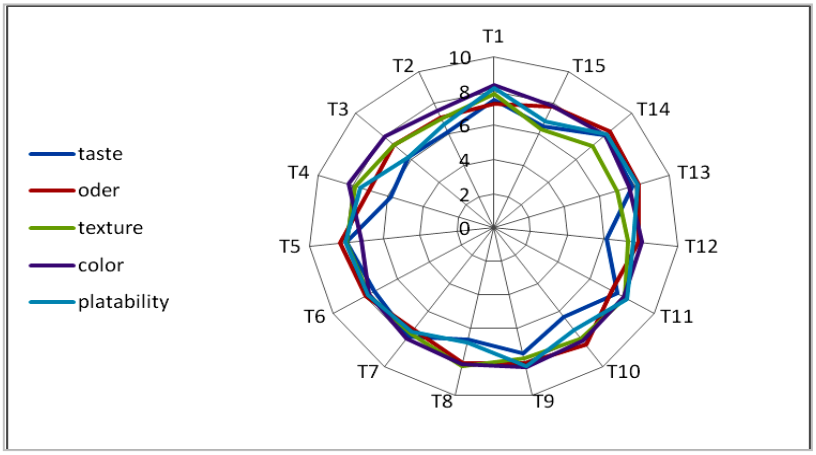
Selection of Stabilizer for Reducing Oil Separation from Peanut Butter. Based on Table 4, the preliminary test indicates that beeswax at 2% was the most effective stabilizer for peanut butter, outperforming other tested materials, which were less efficient in stabilizing the product. After centrifugation, 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 beewax 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^{b}±0.0005
Beeswax 1%	2.912^{e}±0.0015
Beeswax 2%	2.001^{f}±0.0022
Flaxseed 6% +1% beeswax	4.611^{a}±0.0104
Quinoa 6% +1% beeswax	2.918^{e}±0.0058
Carrageenan 0.5%	3.131^{d}±0.0100
Wheat flour 2%	3.621^{c}±0.0105

Values are means ± standard deviations of triplicate determinations.
Means in the same column with different superscript (a,b,c…..) are significantly different ($p \leq 0.05$)

Preliminary panel test for selection preferable samples. Based on the results shown in Fig. 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.



*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 %

Figure 1. The preliminary panel test for choosing preferable treatments

Chemical composition of peanut butter samples

The proximate chemical composition of peanut butter samples (T1, T3, and T4) is 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 contributes 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 samples	Moisture	Protein	Ether extract	Fiber	Ash	Available carbohydrates	Calories
*T1	$2.61^{a}\pm0.06$	$25.357^{a}\pm0.12$	$45.6^{c}\pm0.46$	$2.753^{a}\pm0.42$	$3.88^{a}\pm0.07$	$19.80^{a}\pm0.19$	$591.02^{b}\pm3.98$
T3	$1.55^{b}\pm0.04$	$25.75^{a}\pm1.22$	$47.6^{b}\pm0.20$	$2.247^{b}\pm0.03$	$3.63^{b}\pm0.09$	$19.22^{a}\pm1.32$	$608.28^{a}\pm1.35$
T4	$1.61^{b}\pm0.07$	$26.18^{a}\pm0.80$	$48.67^{a}\pm0.21$	$2.867^{a}\pm0.06$	$3.65^{b}\pm0.15$	$17.02^{b}\pm0.42$	$610.81^{a}\pm0.39$

*T1 : Peanut butter with all ingredients except EOs.; T3: Peanut butter (commercial brand 1);T4: Peanut butter (commercial brand 2)

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 Rozalli 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 7, 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 99.83%. 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 7. Oil separation after six months from peanut butter (T1,T2,T3 and T4)

Samples	% Oil separation
*T1(beeswax 2% as stabilizer)	0.170^{b}±0.023
T2 (without stabilizer)	7.979^{a}±0.09
T3 (commercial brand from India)	0.049^{c}±0.017
T4 (commercial brand from Egypt)	0.052^{c}±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 ($p \leq 0.05$)

Water activity (a_w) of peanut butter of control and commercial samples

Peanut butter is known for its very low water activity (aw), typically below 0.7, which prevents the production of toxins (Behling et al., 2010). The measured aw values for commercial peanut butter samples were 0.37 and 0.41 (Table 8). 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 aw than the other samples due to the absence of a stabilizer. An increase in moisture content can reduce lipid oxidation rates up to an a_w of 0.40. Beyond this threshold, further increases in a_w accelerate oxidation, as expected. Lipid oxidation occurs even at low temperatures, but the reaction rate significantly increases with rising temperatures (Evranuz, 1993).

Table 8. Water activity (a_w) of peanut butter (T1, T2, T3, T4)

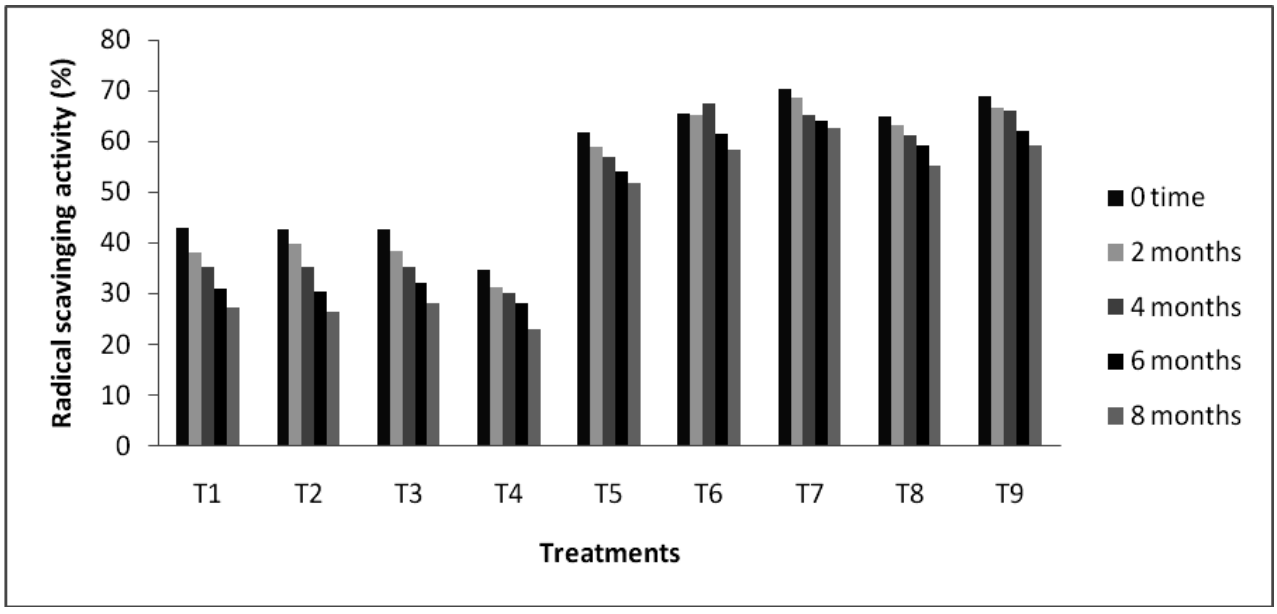
Samples	Water activity (a_w)
*T1	0.46
T2	0.58
T3	0.37
T4	0.41

*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 (ANEO + 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 decline in antioxidant activity in butter fortified with clove oil 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.

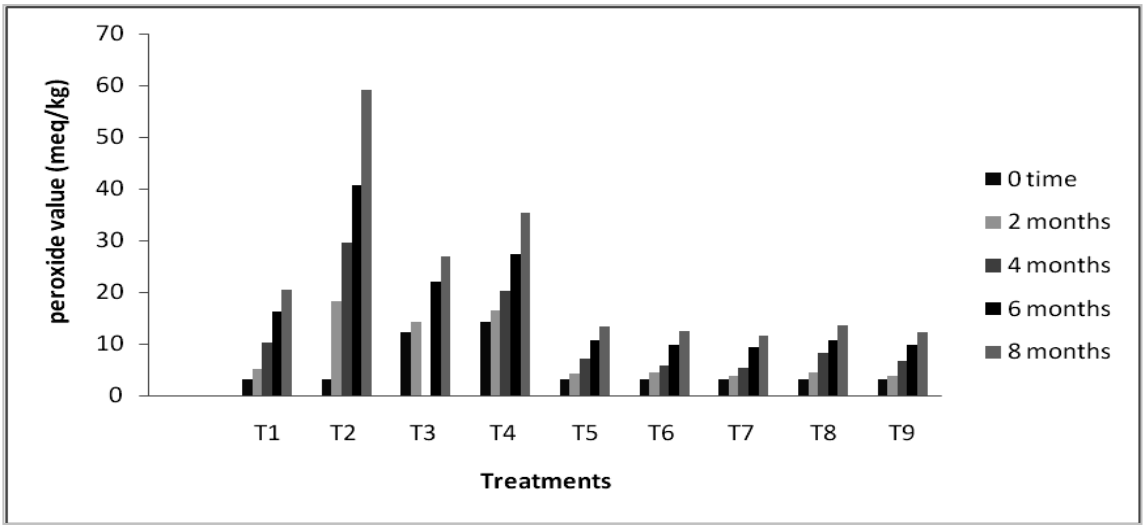


*T1 : Peanut butter with all ingredients except EOs.; T2: Peanut butter without other ingredients; T3: Peanut butter (commercial brand 1); T4: Peanut butter (commercial brand 2); T5 : Peanut butter with EO of anise 0.3%.; T6 : Peanut butter with EO of cinnamon 0.2%; T7 : peanut butter with EO of clove 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 .

Figure 2. Radical scavenging activity of peanut butter treatments

Antioxidant Activity of Essential Oils in Peanut Butter

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. 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.



*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

Figure 3. Peroxide value in peanut butter treatments during storage

Peroxide Value Trends in Peanut Butter During Storage

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

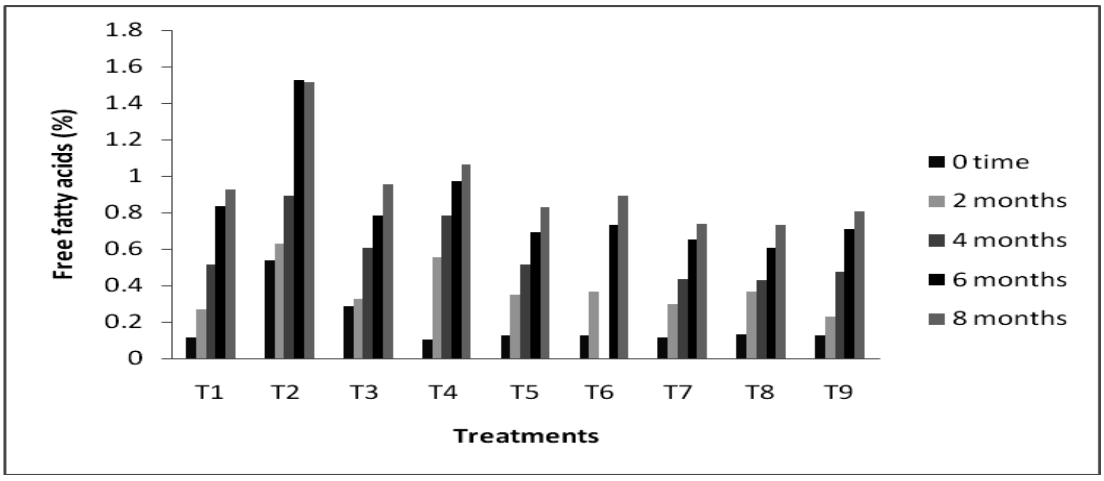
values below 10 meq/kg (9.80, 9.41, and 9.77meq/kg, respectively), with no significant differences among them. Meanwhile, the control sample (T2) had the highest peroxide value, likely due to the absence of preservatives or stabilizers. In contrast, the commercial samples (T3 and T4) contained hydrogenated 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.

These findings align with Pidatala et al. (2021), who reported peroxide values ranging from 2.6 to 31.51 meq/kg over six months. Additionally, Rojas et al. (2021) demonstrated that a lipidic matrix (LM), similar to beeswax, reduces oil mobility and leakage, leading to greater stability than raw clove essential oil (CEO). Their study found that LM exhibited no signs of oxidation, whereas raw CEO had a peroxide value of 13meqO/kg. Furthermore, Vidanagamage et al. (2016) confirmed that cinnamon extract reduced peroxide values in butter due to its antioxidant activity, ensuring stability for approximately two months. These findings emphasize the importance of using stabilizers like beeswax and essential oils to enhance peanut butter's oxidative stability, extending its shelf life under ambient conditions.

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

The data in Fig. 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.



*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 .

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

Microbial load of peanut butter samples during storage for 6 months

According to Table 9, 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. Among the tested samples, T6 exhibited the lowest microbial count due to the antimicrobial activity of cinnamon essential oil. This finding is supported by Table 3, which confirmed the strong antimicrobial properties of cinnamon oil. Moreover, microbial load (bacteria, yeast, and mold) was not detected (-ve) in: T6 (cinnamon EO treatment), T8 and T9 (EO mixtures) This suggests 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.

Table 9. 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.

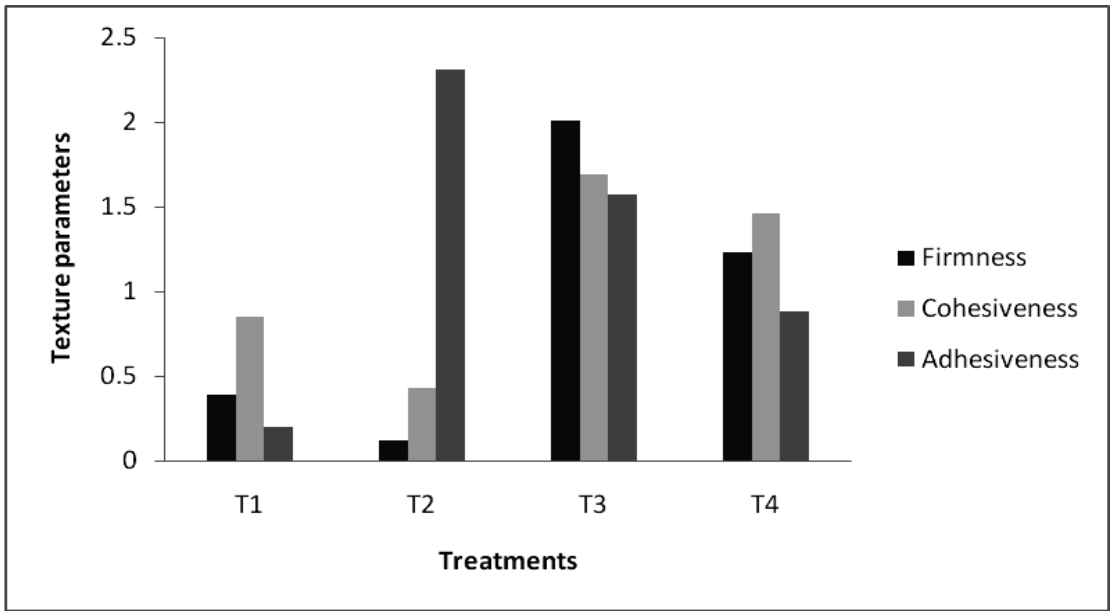
-Ve means no microbial load was detected

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.

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 firmness than commercial samples, making its texture preferable.



*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)

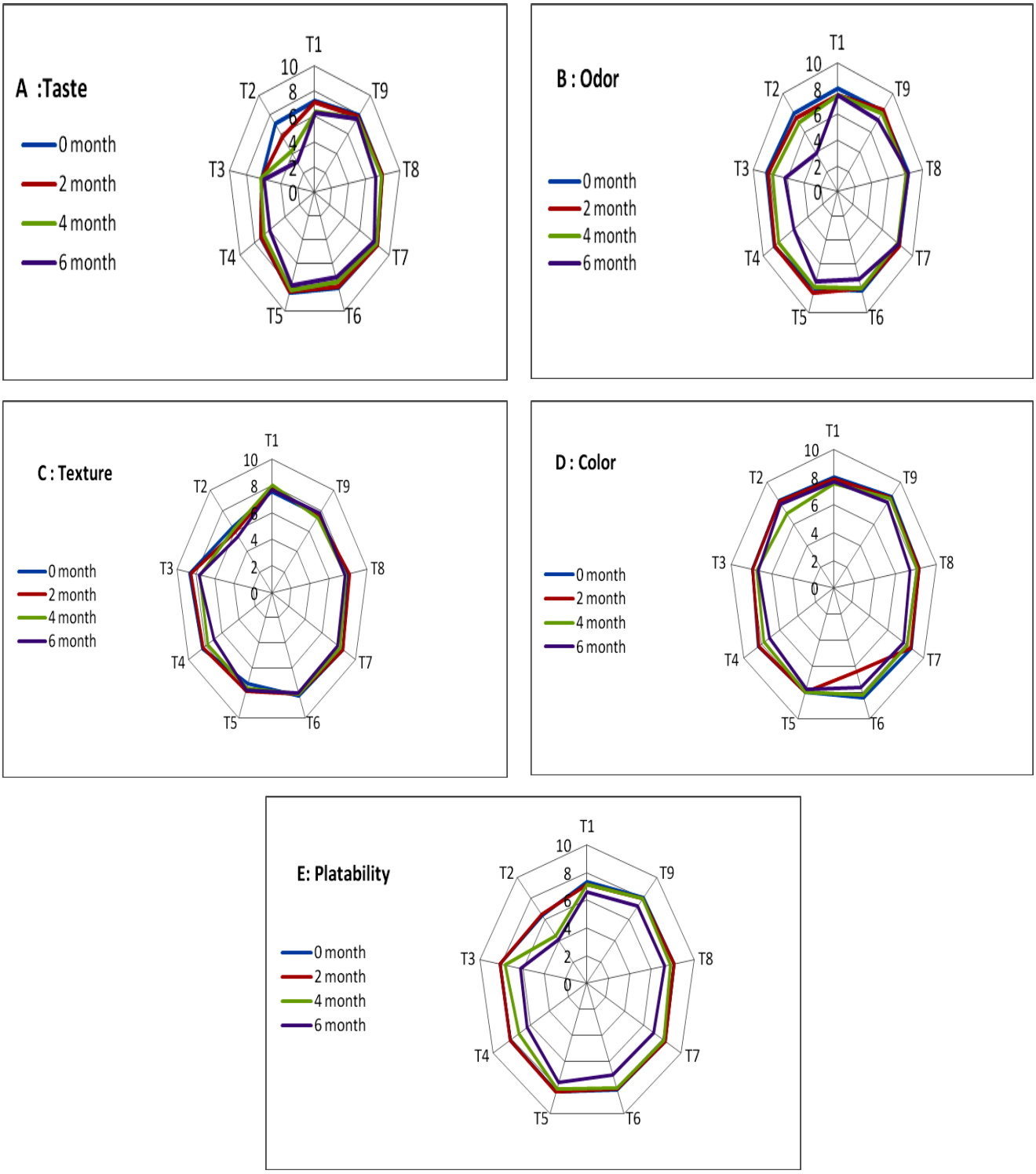
Figure 5. The textural characteristics of peanut butter control and commercial samples

Sensory evaluation of selected samples during storage period

As shown in Figure 6, significant differences ($p \leq 0.05$) 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.



*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 %

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

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.

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