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Evaluation of Essential Oils as Natural Antibacterial Agents for Eggshell Sanitization and Quality Preservation

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Abstract: Eggs are considered a nutritionally complete food and an excellent source of protein. However, they are prone to bacterial contamination, which can affect their internal quality and safety. This study aimed to evaluate the effectiveness of essential oils (EOs) as natural sanitizers for eggshells to reduce the bacterial load and improve shelf life. Ten EOs—cinnamon, clove, lavender, peppermint, thyme, rosemary, green tea, mustard, basil, and marjoram—were tested for antibacterial activity against three Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Listeria monocytogenes*) and three Gram-negative bacteria (*Salmonella* Enteritidis, *Pseudomonas aeruginosa*, and *Escherichia coli*) using the well diffusion method. The most effective EOs were assessed in vapor form using a disc volatilization technique. A total of 288 eggs from a local farm in South Lebanon were treated with selected EOs and stored at room temperature for three weeks to evaluate microbial load and quality. The eggs were grouped into six categories: dry control, ultraviolet (UV) treated, and eggs sprayed with EOs, based on the recorded minimum inhibitory concentration (MIC) values. At each time point, 12 eggs from each group were analyzed for microbiological testing and external and internal quality. Results indicated that nine of the ten EOs showed antibacterial activity, with the highest inhibition zones recorded for cinnamon against *S.* Enteritidis (14 mm) and *E. coli* (9 mm), peppermint against *P. aeruginosa* (10 mm), and rosemary against *L. monocytogenes* (22 mm), *S. aureus* (19 mm), and *E. faecalis* (12 mm). Vapor phase testing showed that rosemary, thyme, clove, and cinnamon EOs had average inhibition zones ranging from 21 to 33 mm against the tested bacteria, and peppermint EO achieved complete inhibition of *P. aeruginosa*. Clove EO consistently reduced the bacterial load as a sanitizer on eggs, initially achieving complete inhibition. While egg quality parameters such as weight, shell thickness, Haugh unit, and yolk color declined over time, EOs helped maintain better quality compared to dry controls. GC-MS analysis of the tested EOs revealed that monoterpene alcohols are the major constituents of most oils. This study concludes that EOs are effective natural sanitizers for eggs, enhancing safety and preserving quality during storage.

Keywords: eggs; eggshell; essential oils; antibacterial; sanitizers; food safety; food quality

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1. Introduction

Eggs are one of the most widely consumed food products around the world. This is due to their low price and high nutritional value, making them an important part of the human

diet [1]. Despite significant improvements in egg safety and quality research over the past decade, eggs have been implicated in serious foodborne illnesses [2]. Indeed, in recent years, the egg industry has faced several food safety issues that have raised consumer concerns, such as the *Salmonella* outbreak in the United States (US) in 2015 [3] and the fipronil pesticide contamination in Europe in 2017 [4]. Contaminated table eggs pose a significant public health hazard worldwide [5]. Over the past 18 years, 75% of foodborne outbreaks involving *S.* Enteritidis have been linked to eggs or egg-based products in the US. In the last three years, hundreds of thousands of eggs have been recalled due to the presence of *Salmonella* [6]. Both Gram-negative and Gram-positive bacteria, pathogenic fungi, and mycotoxins have been detected on the eggshell surface and in the egg white [2]. Most egg-associated illnesses have been attributed to the *Salmonella* serovar Enteritidis, although other serovars, notably *S.* Heidelberg and *S.* Typhimurium, are also occasionally implicated [7]. Additionally, several bacteria, including *Escherichia*, *Staphylococcus*, *Pseudomonas*, and *Bacillus*, can cause foodborne diseases in eggs [6,8].

There are numerous pathways through which eggs can become contaminated by pathogenic microorganisms that can penetrate the eggshell and persist throughout their shelf life [2]. Bacterial contamination can occur through endogenous contamination, where the egg is affected before oviposition due to infection in the reproductive organs of hens, or through exogenous contamination, which occurs due to bacterial shell contamination and penetration [9]. Some countries employ a method called eggshell washing, typically performed before packaging, to remove dirt or fecal material and reduce microbial contamination [10]. The US, Japan, and Australia routinely wash eggs, while countries like the United Kingdom and those in the European Union (EU) do not, following Regulation (EC) No 589/2008 [11].

Interestingly, eggs are the only food of animal origin that can be stored for several weeks in their natural condition without losing specific characteristics. However, environmental factors can affect both the internal and external qualities of eggs, leading to their deterioration over time. In recent years, consumers have increasingly preferred eggs with firm albumen, dense colored yolk, large size, and good quality [12]. Consequently, preserving the shelf life and internal quality of eggs has become a top priority for the food industry. Key egg quality parameters include the Haugh unit (HU), pH, egg weight, yolk color, shell thickness, and specific gravity [9].

New food processing technologies have evolved in the last 30 years, focusing on non-thermal processing alternatives to inactivate microorganisms in specific food products, minimize quality changes, and reduce processing times [6]. These technologies can be classified into three major categories: physical, chemical, and biological [13]. Examples include pressure, electricity, light, sound waves, chemical compounds, natural antimicrobials, and free radicals. The conventional disinfection technology for cleaning and reducing microbial activity on egg surfaces relies on chlorine, which has several disadvantages, such as the formation of carcinogenic compounds and loss of effectiveness in the presence of organic matter. Therefore, the search for natural antimicrobials to disinfect food products has become a priority. Some natural antimicrobials tested to reduce eggshell microbial loads include probiotics, prebiotics, and essential oils [14].

Since ancient times, essential oils (EOs) have been studied for their potential as natural antimicrobial agents and have demonstrated antibacterial activity [15]. EOs are defined as volatile secondary metabolites in plants that impart distinctive smells and tastes. Moreover, humans have used EOs for thousands of years in various ways, including in folk medicine, due to their antimicrobial properties. Chemical analysis of EOs has shown that they contain over 20 bioactive compounds [16]. Depending on the essential oil, the dominant compounds may include monoterpenes, phenols, aldehydes, ketones, alcohols, hydrocarbons, and

other substances. The chemical composition of EOs is believed to play a key role in their antimicrobial properties, including their effectiveness against bacterial strains, whether isolated from poultry or not. This is due to the interaction of these compounds with microbial cell walls and membranes, which increases the permeability of these structures, leading to leakage or disruption of microbial homeostasis [17,18]. EOs have potential applications in the poultry industry, as eggs treated with EOs have shown a significant reduction in the number of total aerobic mesophilic bacteria (up to 80.77%) in eggshells, as demonstrated by Oliveira et al. [19]. Additionally, Vale et al. [20] reported that essential oils, when used at specific concentrations, exhibited antibacterial effects in vitro, significantly reducing the load of mesophilic bacteria and enterobacteria on eggshells by at least 3 and 2 $\log_{10}$ CFU/mL, respectively.

In this context, this study aims to evaluate the effectiveness of essential oils in the vapor state as a novel, cost-effective, and eco-friendly eggshell sanitizer to reduce shell-associated bacterial load while maintaining the internal quality and shelf life of eggs during storage. The primary objective is to assess the use of natural antimicrobial agents to inactivate microorganisms while preserving egg quality.

2. Materials and Methods

2.1. Essential Oils and Egg Collection

All EOs were obtained from a local store in North Lebanon. The EOs were selected based on their antimicrobial activity in previous studies. Ten EOs were included in this study, namely, Clove (*Syzygium aromaticum*), Mustard (*Brassica nigra*), Marjoram (*Origanum majorana*), Cinnamon (*Cinnamomum verum*), Thyme (*Thymus vulgaris*), Basil (*Ocimumbasilicum*), Peppermint (*Mentha piperita*), Lavender (*Lavandula* spp.), Rosemary (*Salvia rosmarinus*) and Green Tea (*Camellia sinensis*).

A total of 288 eggs of the same age were collected from a local farm in South Lebanon. They were transported to the laboratory in cardboard trays, with each tray containing 30 eggs. Throughout the experiment, the eggs were stored at room temperature in accordance with EU regulations [21].

2.2. Chemicals, Media, and Microorganisms

Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (Steinem, Germany). Plate Count Agar (PCA), Violet Red Bile Agar (VRBA), Tryptone Soya Broth (TSB), Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB), and peptone water were obtained from HIMEDIA (Mumbai, India). Six clinical isolates, including three Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Listeria monocytogenes*) and three Gram-negative bacteria (*Salmonella* Enteritidis, *Escherichia coli,* and *Pseudomonas aeruginosa*), provided from Beirut Arab University laboratory, were used to evaluate the antibacterial activity of EOs. The bacterial isolates were inoculated into TSB and incubated at 37 °C for 48 h to remove bacteria from the stock cultures [22].

2.3. Essential Oils Preparation and Microbiological Analysis

2.3.1. Essential Oils Preparation

Before assessing their antimicrobial activities, the selected EOs were dissolved in 1:1 (v/v) ratio in 1% DMSO to enhance EOs diffusion in the agar medium (preliminary analyses with 1% (v/v) DMSO did not affect the growth of the test organisms). Then, 200 mg/mL EOs stock solutions were prepared and sterilized using 0.45 µm disposable syringe filters. Stock solutions of the EOs were stored in dark glass bottles at room temperature for subsequent use [23].

2.3.2. Essential Oils Microbiological Analysis

For the total count, 100 µL of each EO was added to PCA and gently rotated to ensure thorough mixing. The plates were then incubated at 30 °C for 48 h [24]. For total coliform detection, 100 µL of the selected EOs was added and spread on VRBA plates; then, the plates were incubated at 37 °C for 24 h [25].

2.4. Antibacterial Activity of EOs

2.4.1. Inoculum Standardization

For antibacterial activity assays, bacterial suspensions were first prepared according to the 0.5 McFarland Standard by taking 3 to 5 bacterial pure colonies and placing them in 0.9% saline solution to obtain a bacterial concentration of 1.5×10^8 CFU/mL to test the antibacterial effects of EOs against the tested Gram-positive and Gram-negative bacterial strains. On the other hand, from the fresh bacterial suspensions (10^8 CFU/mL), decimal dilutions were made in 0.9% sterile saline to reach a bacterial suspension of 10^6 CFU/mL to perform the disc volatilization technique and determine the minimum inhibitory concentration (MIC) of the tested EOs [23].

2.4.2. Antibacterial Activity of EOs in Liquid State

The agar well diffusion method was used to evaluate the antibacterial activity of tested EOs against *S. aureus*, *E. faecalis*, *L. monocytogenes*, *S.* Enteritidis, *E. coli*, and *P. aeruginosa*. First, plates of MHA were inoculated with the prepared bacterial suspensions (1.5×10^8 CFU/mL). Seven-millimeter wells were created using a sterile cork borer, and 50 µL of each EO stock was added. The plates were left for 30 min at room temperature to allow the oils to diffuse and then incubated at 37 °C for 24 h. Negative controls (distilled water with 1% DMSO) and positive controls (Gentamicin disc, 10 µg/disc) were also included. After overnight incubation, the average inhibition zones were measured in millimeters (mm) [26].

2.4.3. Antibacterial Activity of EOs in Vapor State

The disc volatilization method was used to determine the antibacterial effect of the selected EOs in vapor form against the six foodborne pathogens mentioned in Section 2.4.2. Only EOs that showed promising results against the tested foodborne pathogens in liquid form were selected for this method to study their effects in the vapor phase.

In the first step, 5 mL of MHA was poured into the upper lid of each plate to prevent adsorption of EOs onto the plastic material of the lid. An ample amount of MHA (30 mL) was added to the base of the Petri dish to ensure a small space (2 mm) between the lid and base. A standardized inoculum of bacterial suspension (10^6 CFU/mL) was inoculated and swabbed onto the surface of the MHA plate using sterile cotton swabs. Sterile paper discs were then placed on the agar in the upper lid, and 35 µL of each EO was added. The plates were sealed with MHA in the space between the lid and the base and covered with parafilm to prevent any vapor loss. After sealing, each plate was placed directly in an incubator at 37 °C for 24 h. Negative controls (distilled water with 1% DMSO) and positive controls (Gentamicin disc, 10 µg/disc) were also included. After overnight incubation, the antibacterial effect of the EOs in vapor form against the tested bacteria was assessed by measuring the average diameter of the inhibition zones in mm [27].

2.4.4. Determination of the MIC

The MIC of the EOs that demonstrated promising inhibition of the respective bacterial strains in the vapor phase was determined using the microdilution broth method [28]. In a 96-well microtiter plate, 100 µL of MHB was added to all wells (1–12). In the first well,

100 µL of the EO was added, followed by two-fold serial dilutions in the next ten wells, where 20 µL of the bacterial suspension (10^6 CFU/mL) was added. For well 11, 10 µL of a 0.9% saline solution was added as a positive control, while well 12 received the inoculum, serving as a negative control. Finally, the plates were covered and incubated for 24 h at 37 °C. After incubation, the MIC was defined as the lowest concentration of the tested substance that prevented visible bacterial growth.

2.5. Egg Disinfection by EOs Spraying Technique

The eggs were divided into six treatment groups: dry control, eggs sanitized with ultraviolet (UV) light, and four groups of eggs sprayed with EOs that previously demonstrated the highest inhibition against the tested bacterial strains in the vapor phase.

The dry control group consisted of eggs that had not undergone any treatment and were left as received from the farm. In the second group, the eggs were sanitized using UV light. The eggshells were exposed to UV-C light (253.7 nm) at an intensity of 15 mW/cm^2/s for 10 min on each side. The third, fourth, fifth, and sixth groups were sprayed with clove, cinnamon, rosemary, and peppermint EOs at concentrations of 0.39 mg/mL, 1.56 mg/mL, 1.56 mg/mL, and 12.5 mg/mL, respectively. These concentrations were determined based on the recorded MIC results [29].

Microbiological and quality analyses were conducted on the eggs from each group at the beginning of the experiment (t_0) and after one (t_1), two (t_2), and three (t_3) weeks. A total of 288 eggs were allocated to the six treatment groups, with 48 eggs per group. At each time interval, six eggs were tested from each group. Since the experiment was conducted in duplicate, the total number of eggs tested was 288 (6 eggs × 6 groups × 4-time intervals × 2 repetitions).

2.6. Microbiological Analysis of Eggs

At each time interval from t_0 to t_3, six eggs were selected from each group for microbiological analysis. These eggs were placed together in a sterile container containing 200 mL of 0.1% peptone water and agitated on an orbital shaker for 5 min on each side at a speed of 60 rpm.

To determine the aerobic plate count, the pour plate technique was employed according to ISO 4833-1:2013 [30]. One milliliter of peptone water from the pooled sample was serially diluted ten-fold with 9 mL sterile 0.1% peptone water. Then, 1 mL of each dilution was transferred to a sterile Petri dish, and 25 mL of PCA was poured over and mixed gently with the inoculum. The plates were then incubated at 37 °C for 24 h. The number of colonies was reported as $\log_{10}$ CFU/mL [9].

2.7. External and Internal Quality Analysis of Eggs

After microbiological analysis at each time interval for all groups, the same eggs were used for quality analysis, focusing on four main criteria: egg weight, shell thickness, Haugh unit, and yolk color.

2.7.1. Egg Weight

The eggs were taken from each group at each time interval, individually weighed on a digital weighing scale, and then recorded.

2.7.2. Shell Thickness

After the eggs were broken, the contents (yolk and albumen) were separated from the shells. The eggshells were then cleaned and their thicknesses were measured using a Thickness Gauge (Mitutoyo Corp., Kawasaki, Japan) [31].

2.7.3. Haugh Unit

The Haugh unit (HU), introduced by Haugh in 1937, is widely regarded as the primary measure of albumen quality [32]. The height of the albumen was measured using a standard tripod micrometer (Ames Inc., Melrose, MA, USA) [33].

2.7.4. Yolk Color

Yolk color was determined using a Roche Yolk Color Fan (RYCF). After cracking the egg, the yolk was separated and compared to the fan, which features a scale from 1 to 16, with 1 being the lightest and 16 the darkest. A number was then selected from the fan that best represented the yolk color [34].

2.8. Gas Chromatography of EOs

The GC-MS analysis was performed by utilizing gas chromatography (GC) coupled with a quadrupolar mass-spectrometer selective detector (MSD) with an Agilent-6890 N Network for GC-MS (Agilent Technologies, Santa Clara, CA, USA) and an Agilent-5975 Network detector for MSD (Agilent Technologies, Santa Clara, CA, USA) [35]. The GC-MS system was equipped with NIST (NIST 11.0, National Institute of Standards and Technology, Gaithersburg, MD, USA) and Wiley (Wiley, Chichester, West-Sussex, UK) library search databases. The oven temperature was elevated from 40 to 200 °C at a rate of 5.5 °C/min. Then, 200–290 °C at a rate of 29.5 °C/min. Then, post-run for 10 min at 290 °C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were set at 260 °C. GC-MS was performed by injecting 1 µL of the sample (0.1% in absolute hexane) in scan and splitless modes. The major essential oil peaks were compared to external standards, and the reference retention times were correlated with the NIST and Wiley mass spectra libraries.

2.9. Statistical Analysis

Data were analyzed using IBM-SPSS Statistics for Windows, Version 25.0 (Released 2017. IBM Corp., New York, NY, USA). All experiments were conducted in duplicate. Means and standard deviations were calculated for the inhibition zones and bacterial survival rates ($\log_{10}$ CFU/mL). Pearson's correlation was used to assess the relationship between variables, with statistical significance determined using a two-tailed test ($p < 0.05$) and a 95% confidence interval.

3. Results and Discussion

3.1. Microbiological Analysis of Essential Oils

When the EOs were tested for total count and total coliforms, no bacterial growth was detected in any of the PCA or VRBA plates incubated overnight.

3.2. Evaluation of the Antibacterial Effect of Essential Oils in Liquid Phase

The antibacterial activity of ten EOs against *S. aureus*, *E. faecalis*, *L. monocytogenes*, *S.* Enteritidis, *E. coli*, and *P. aeruginosa* was evaluated using the agar well diffusion method, with results summarized in Table 1. The antibacterial efficacy was expressed as the average inhibition zone diameter in mm. All EOs in the liquid phase showed an inhibitory effect against foodborne pathogens, except for lavender. The most effective EOs for *S. aureus* was rosemary, which had the largest inhibition zone of 19 mm, followed by thyme (17 mm), clove (11 mm), mustard (9 mm), cinnamon (9 mm), and green tea (8 mm). *E. faecalis* was only inhibited by rosemary, with a zone of 12 mm. In the case of *L. monocytogenes*, rosemary again showed the greatest inhibition at 22 mm, followed by thyme (15 mm), clove (11 mm), and cinnamon (9 mm). Cinnamon was the most effective against *S.* Enteritidis, achieving a

14 mm zone, with clove, basil, and marjoram at 9 mm, 9 mm, and 8 mm, respectively. For *P. aeruginosa*, only peppermint EO demonstrated inhibition, with an average zone diameter of 10 mm. Lastly, cinnamon and clove were effective against *E. coli*, with inhibition zones of 9 mm and 8 mm, respectively. No significant relationship was observed between the bacterial strains and the inhibition zones for most essential oils ($p > 0.05$), indicating that the inhibition zones did not vary significantly across the bacterial strains tested. Additionally, no significant relationship was found between the essential oils and the inhibition zone ($p > 0.05$), except for *E. coli*, where a significant relationship was noted ($p < 0.05$).

Table 1. Antibacterial activity of the tested essential oils in the liquid phase against foodborne pathogens.

Bacterial Strain	Zone of Inhibition (mm)										*p*-Value
	Essential Oils (Liquid)										
	Cinnamon	Clove	Lavender	Peppermint	Thyme	Rosemary	Green Tea	Basil	Mustard	Marjoram	
S. aureus	9 ± 0.71	11 ± 1.41	NI *	NI	17 ± 1.71	19 ± 1.06	8 ± 0.71	NI	9 ± 0.71	NI	>0.05
E. faecalis	NI	NI	NI	NI	NI	12 ± 1.77	NI	NI	NI	NI	-
L. monocytogenes	9 ± 0.71	11 ± 0.00	NI	NI	15 ± 4.24	22 ± 2.83	NI	NI	NI	NI	>0.05
S. Enteriditis	14 ± 0.00	9 ± 0.35	NI	NI	NI	NI	NI	9 ± 0.00	NI	8 ± 0.71	>0.05
E. coli	9 ± 0.35	8 ± 0.71	NI	NI	NI	NI	NI	NI	NI	NI	<0.05
P. aeruginosa	NI	NI	NI	10 ± 1.06	NI	NI	NI	NI	NI	NI	-
p-value	>0.05	>0.05	-	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	

Values represent mean (mm) of two experiments ± SD. * NI: no inhibition.

A significant property of EOs is their hydrophobic nature, which determines their mode of action against bacteria. This characteristic allows them to disrupt the lipids in the bacterial cell membrane and mitochondria, thereby disturbing cell structures and increasing permeability. This extensive leakage from bacterial cells, along with the exit of critical molecules and ions, ultimately leads to cell death [36].

Rosemary EO exhibited the highest average inhibition zones against *S. aureus* (19 mm) and *L. monocytogenes* (22 mm). It was also the only oil that was effective against *E. faecalis*, with an average inhibition zone of 12 mm. According to Gouveia et al. (2016), rosemary EO demonstrated bactericidal activity against *L. monocytogenes* after 24 h of incubation [37]. Similar results were reported by Mohsen et al., who found that rosemary EO had an inhibition zone of 15 mm against *S. aureus* [38]. Additionally, Bansal et al. identified rosemary EO as the most effective against *E. faecalis*, with an inhibition zone of 28.83 mm [39].

Following rosemary, cinnamon EO showed the highest inhibition zone against S. Enteritidis, measuring 14 mm. These findings align with those of Prabuseenivasan et al. and Kon et al., who reported that cinnamon EO exhibited the strongest antimicrobial activity among 21 to 35 plant EOs tested against foodborne pathogens [36,40]. The antibacterial activity of cinnamon EO is attributed to its active constituent, cinnamaldehyde. Furthermore, a study by Olaimat et al. confirmed that cinnamon EO was the most effective against *S.* Enteritidis, with the highest inhibition zone recorded at 39 mm [41]. The highest inhibition zones were observed with EOs against Gram-positive bacteria compared to those against Gram-negative bacteria. This difference in susceptibility could be attributed to the presence of an outer membrane containing lipopolysaccharides in the cell walls of Gram-negative bacteria, which generally makes them more resistant to essential oils [42].

3.3. Evaluation of the Antibacterial Effect of Essential Oils in Vapor Phase

The EOs that demonstrated effectiveness against the six selected bacteria using the agar well diffusion method were further tested for their antibacterial effects in the vapor phase using the disc volatilization method. Table 2 shows the average inhibition zones of

these EOs against the tested bacteria in the vapor phase. The lavender EO did not show any antibacterial effect in the liquid phase; hence, it was excluded from this part.

Table 2. Antibacterial activity of the selected essential oils in the vapor state against foodborne pathogens.

Bacterial Strain	Zone of Inhibition (mm)									*p*-Value
	Essential Oils (Vapor)									
	Cinnamon	Clove	Peppermint	Thyme	Rosemary	Green Tea	Basil	Mustard	Marjoram	
S. aureus	12 ± 1.41	12 ± 0.35	*[A]	17 ± 3.18	26 ± 0.35	20 ± 0.00	*	NI [B]	*	>0.05
E. faecalis	*	*	*	*	NI	*	*	*	*	-
L. monocytogenes	31 ± 1.06	16 ± 1.77	*	29 ± 3.54	33 ± 2.12	*	*	*	*	>0.05
S. Enteriditis	18 ± 1.41	32 ± 1.77	*	*	*	*	NI	*	*	<0.05
E. coli	21 ± 1.41	NI	*	*	*	*	*	*	*	>0.05
P. aeruginosa	*	*	CI [C]	*	*	*	*	*	*	-
p-value	>0.05	>0.05	-	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	

[A] Not treated. [B] No inhibition. [C] Complete inhibition. * Not tested in the vapor state.

S. aureus was sensitive to several EOs in the vapor phase, with rosemary showing the largest inhibition zone (26 mm), followed by green tea (20 mm), thyme (17 mm), and both clove and cinnamon (12 mm each), while mustard EO was ineffective. For *E. faecalis*, only rosemary was effective in the liquid state but showed no inhibition in vapor. In the vapor phase, rosemary exhibited the largest inhibition zone (33 mm) against *L. monocytogenes*, followed by cinnamon (31 mm), thyme (29 mm), and clove (16 mm). For *S.* Enteritidis, only clove (32 mm) and cinnamon (18 mm) were effective in vapor. While both inhibited *E. coli* in the liquid phase, only cinnamon (21 mm) was effective in the vapor phase. Peppermint EO was the only oil that completely inhibited *P. aeruginosa* vapor. The results indicate that while most essential oils exhibited varying degrees of inhibition against different bacteria, no statistically significant relationship was found between bacterial type and inhibition zone size ($p > 0.05$), except for peppermint, which demonstrated complete inhibition against *P. aeruginosa*. A significant correlation between essential oils and the inhibition zone was observed for *S.* Enteritidis ($p < 0.05$), suggesting their inhibitory potential against this bacterium, whereas no significant effects were observed against *S. aureus*, *E. faecalis*, *L. monocytogenes*, *E. coli*, and *P. aeruginosa*.

In the vapor phase, the number of EOs that inhibited Gram-positive bacteria was generally higher than that for Gram-negative bacteria, except for *E. faecalis*. Notably, Gram-negative strains showed increased susceptibility to EO vapors, with peppermint EO achieving complete inhibition of *P. aeruginosa*. This aligns with the findings of Tyagi and Malik, who reported significant morphological changes in bacteria exposed to EO vapors, including complete deformation of *P. fluorescens* [43]. The antimicrobial activity of EOs is influenced by the volatility of their compounds, with high volatility contributing to their effectiveness as antimicrobial agents [44].

3.4. MIC of Essential Oils

The most promising essential oils (cinnamon, clove, peppermint, and rosemary), which demonstrated antibacterial activity in the vapor state against both Gram-positive and Gram-negative bacterial strains, were selected to evaluate their MIC values. Rosemary EO had the highest MIC value at 12.5 mg/mL against *L. monocytogenes*. Cinnamon and peppermint EOs exhibited an identical MIC of 1.56 mg/mL against *E. coli* and *P. aeruginosa*, respectively, while clove EO recorded the lowest MIC of 0.39 mg/mL against *S.* Enteritidis. No significant differences were observed in the recorded MIC values ($p > 0.05$).

Our results align with and expand upon the findings of previous studies. For instance, Medeiros Barbosa et al. reported the MIC of rosemary EO against *L. monocytogenes* as 5 mL/mL [45], while Saraiva et al. recorded a MIC of 0.40 µL/mL for the same EO against *L. monocytogenes* [46]. Zhang et al. found that the MIC of cinnamon EO was

1 mg/mL against *E. coli* [47], whereas Raeisi et al. reported a lower MIC of 780 µg/mL [48]. Peppermint EO showed varying MIC values against *P. aeruginosa*, with Mahboubi et al. reporting 16 µL/mL [49], and Muntean et al. reporting 40 mg/mL [50]. Similarly, Somrani et al. reported an MIC value of 0.1 mg/mL for clove EO against *S.* Enteritidis, closely reflecting our results [51]. These comparisons highlight the variability in MIC values across different studies, likely influenced by factors such as testing conditions, bacterial strains, and EO composition.

3.5. Microbiological Analysis of Sanitized Eggs with Essential Oils

The results of disinfecting eggs with EOs—cinnamon, clove, peppermint, and rosemary—as well as UV light, are presented in Figure 1. Measurements were taken immediately after treatment (t_0) and again after one week (t_1), two weeks (t_2), and three weeks (t_3), along with a dry control.

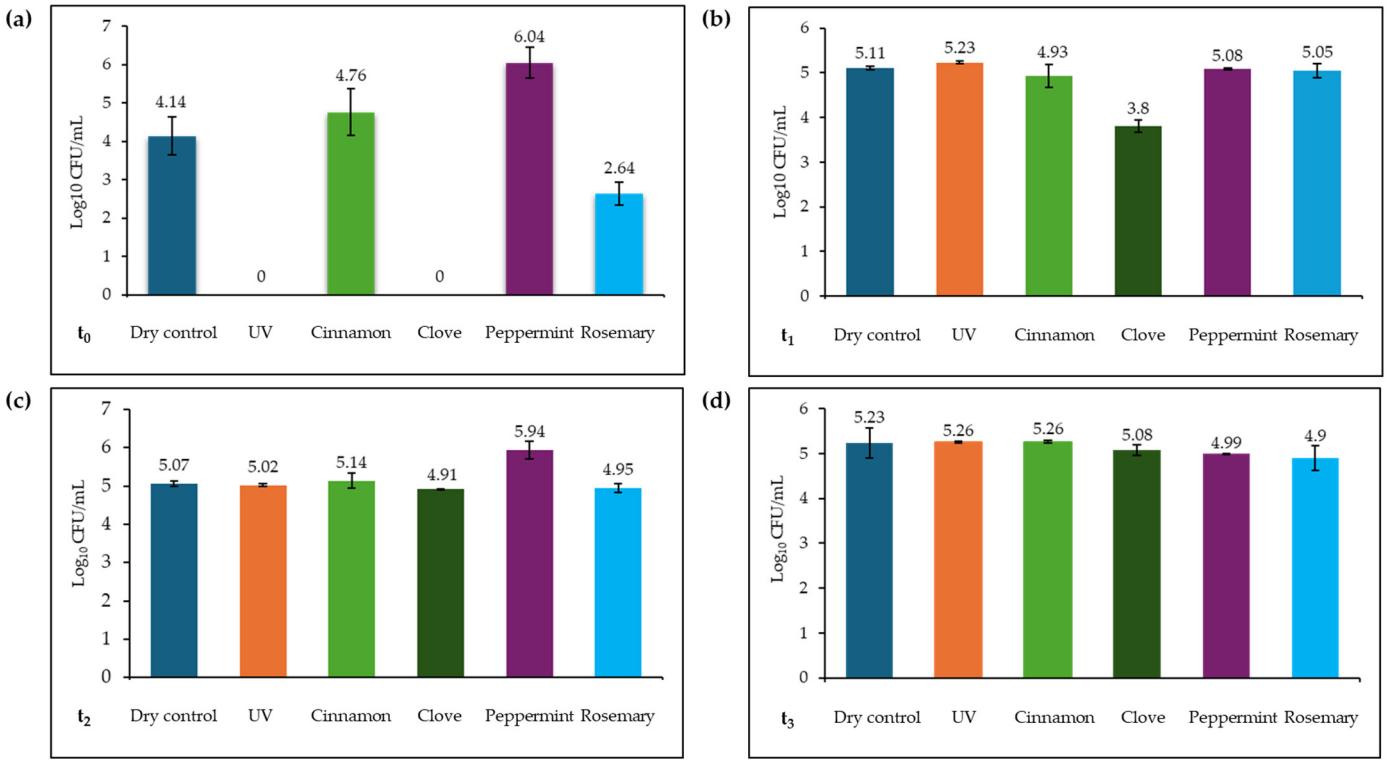


Figure 1. Enumeration of total aerobic bacteria on eggshell surfaces on dry control and according to disinfection processes with ultraviolet light (UV), cinnamon, clove, peppermint, and rosemary essential oils measured after (**a**) treatment directly (t_0), (**b**) one week (t_1), (**c**) two weeks (t_2), and (**d**) three weeks (t_3).

At t_0, UV disinfection of the eggshells resulted in complete bacterial inhibition (Figure 1a). Clove EO was the most effective among EOs, inhibiting bacteria growth on the treated eggshells. This was followed by rosemary EO, which had a bacterial count of 2.64 CFU/mL. In contrast, cinnamon-sprayed eggshells had a bacterial load of 4.76 CFU/mL, which was similar to that of the dry control (4.14 CFU/mL). Peppermint-sprayed eggs showed the highest bacterial load of 6.04 CFU/mL.

At t_1, the bacterial loads were reassessed (Figure 1b). The dry control had a mean count of 5.11 CFU/mL, while all other treatments showed lower counts ($p > 0.05$). Clove EO was the most effective, resulting in the lowest bacterial load of 3.80 CFU/mL. The counts of eggs sprayed with peppermint, rosemary, and cinnamon were lower than that

of the dry control, with values of 5.08, 5.05, and 4.93 CFU/mL, respectively. In contrast, UV-sanitized eggs had a higher count than the dry control, measuring 5.23 CFU/mL.

After two weeks of treatment (t_2), as shown in Figure 1c, the bacterial loads across groups were similar, with the dry control at 5.07 CFU/mL ($p > 0.05$). UV-sanitized eggs had 5.02 CFU/mL, cinnamon-sprayed eggs had 5.14 CFU/mL, and peppermint-sprayed eggs had 5.94 CFU/mL. Clove- and rosemary-treated eggs had the lowest counts, at 4.91 and 4.95 CFU/mL, respectively.

Finally, at t_3, the aerobic counts are illustrated in Figure 1d. There was a notable decrease in bacterial load on eggs treated with clove, peppermint, and rosemary, with counts of 5.08, 4.99, and 4.90 CFU/mL, all lower than the dry control at 5.23 CFU/mL ($p < 0.05$). Both UV-sanitized and cinnamon-sprayed eggs had similar counts of 5.26 CFU/mL, comparable to untreated eggs.

Eggs sanitized with clove EO exhibited the highest level of inhibition throughout the treatment period, except at t_3, where it was still effective. This finding aligns with that of Oliveira et al., who reported that clove EO effectively reduced microbial contamination of eggshells [52]. The strong antimicrobial properties of clove EO are attributed to its high phenolic compound content, which interacts with phospholipids in bacterial cell membranes, increasing permeability and ultimately leading to cell death by disrupting vital cellular functions [53].

In this study, EOs proved to be effective in reducing microbial contamination in eggshells. A study by Mustafa et al. [54] also demonstrated the effectiveness of essential oils in reducing bacterial counts on eggshells. As noted by Aouadhi et al. [55], the interaction between bacteria and essential oils can render bacteria nonviable.

3.6. Effect of Disinfecting by Spraying EOs on External Egg Quality Parameters

The results of the effect of disinfecting eggs by spraying EOs, UV treatment, and storage time on the egg weight and shell thickness are shown in Figure 2.

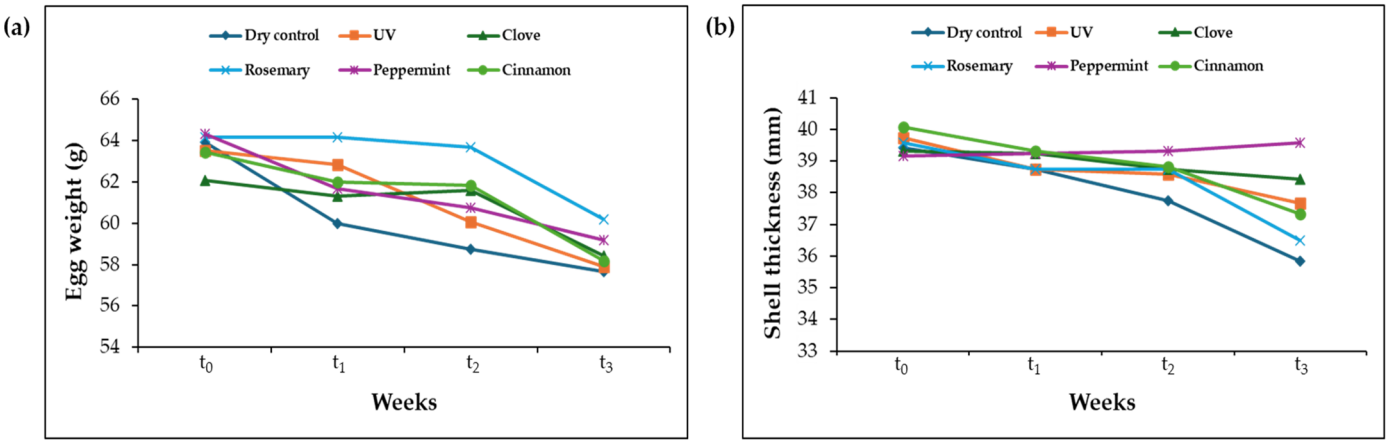


Figure 2. Effect of egg disinfection by spraying EOs, UV treatment, and storage time (weeks) on (**a**) weight and (**b**) shell thickness of the eggs.

Figure 2a illustrates the changes in egg weight across the six groups over the three-week storage period. Overall, the egg weights in all groups declined throughout the study, reaching their lowest points by the end of the study. The dry control group experienced a decline, with average egg weights dropping from 63.92 g at the start (t_0) to 57.67 g by the end of week three (t_3) ($p > 0.05$). In contrast, UV-sanitized eggs maintained a stable average weight of 63.50 g initially (t_0), which decreased slightly to 62.83 g after one week (t_1), followed by a significant decline to 57.92 g at t_3 ($p < 0.05$). Notably, the groups treated with rosemary and clove EOs exhibited relatively stable average weights until the second

week of storage (t_2), starting at 64.17 g and 62.08 g at t_0 and decreasing to 63.67 g and 61.58 g at t_2, respectively. However, by the third week (t_3), average weights had further declined to 60.17 g for rosemary-sprayed eggs and 58.42 g for clove-sprayed eggs, with no significant difference between these groups ($p > 0.05$). For peppermint-sprayed eggs, the weight decreased significantly from an initial 64.33 g to 59.17 g by week three (t_3) ($p < 0.05$). Eggs treated with cinnamon EO showed a gradual decline, starting at 63.42 g and reaching 58.17 g at t_3 ($p > 0.05$). The egg weight parameter showed a consistent decline over time across all groups. This finding aligns with the research by Sert et al., which indicated that storage time negatively affects egg weight; eggs stored for 10 days, regardless of temperature, weighed less than fresh eggs [56]. A subsequent study by Sert et al. observed an increase in weight loss over storage time, with eggs losing weight from day 1 to day 14 [57]. The weight reduction may be attributed to the drying and shrinking of the cuticle, which allows moisture, ammonia, carbon dioxide, and nitrogen to escape through the shell pores via evaporation [58,59]. Notably, eggs treated with clove and rosemary EOs exhibited a slower decline in weight, indicating a potential protective effect on the cuticle. Additionally, the EO-treated eggs maintained higher weights compared to the dry control and UV-treated groups, although no significant differences were observed between them ($p > 0.05$). Our findings indicate that none of the EO treatments caused damage to the cuticle.

Figure 2b presents the results of shell thickness analysis for all six groups over the three-week storage period. The dry control group displayed a gradual decline in shell thickness, with the most significant decrease occurring after two weeks of storage, dropping from 37.75 mm at t_2 to 35.83 mm at t_3 ($p < 0.05$). Eggs sprayed with peppermint EO maintained a relatively stable average shell thickness of approximately 39 mm throughout the study ($p < 0.05$). Similarly, clove EO-treated eggs exhibited a consistent average thickness of 39.33 mm at t_0 and 39.25 mm at t_1, with only a slight decrease noted in subsequent weeks—38.75 mm at t_2 and 38.42 mm at t_3 ($p < 0.05$). In contrast, cinnamon EO-treated eggs started with an average shell thickness of 40.08 mm at t_0, gradually decreasing by about 1 mm each week to reach 37.33 mm at t_3 ($p < 0.05$). Eggs sanitized with rosemary EO showed a consistent average thickness of 38.75 mm at t_1 and t_2 but dropped to 36.5 mm by t_3 ($p > 0.05$). Similarly, UV-sanitized eggs had an average thickness of 38.75 mm at t_1 and a comparable 38.58 mm at t_2, decreasing to 35.83 at t_3 ($p < 0.05$). Regarding shell thickness, all groups, except those sprayed with peppermint EO, demonstrated a decrease over the three-week storage period. This finding aligns with the research by Alsobayel et al., which indicated that prolonged storage negatively impacts shell thickness [60]. However, eggs treated with essential oils showed higher shell thickness compared to both the dry control and UV-treated groups, with no significant differences found among these treatments ($p > 0.05$). This aligns with other studies that similarly found no significant differences in the thickness of eggshells treated with grain alcohol, clove essential oil, or paraformaldehyde [52,61].

3.7. Effect of Disinfecting by Spraying EOs on Internal Egg Quality Parameters

The results of the effect of disinfecting eggs by spraying EOs, UV treatment, and storage time on the Haugh unit and yolk color are shown in Figure 3.

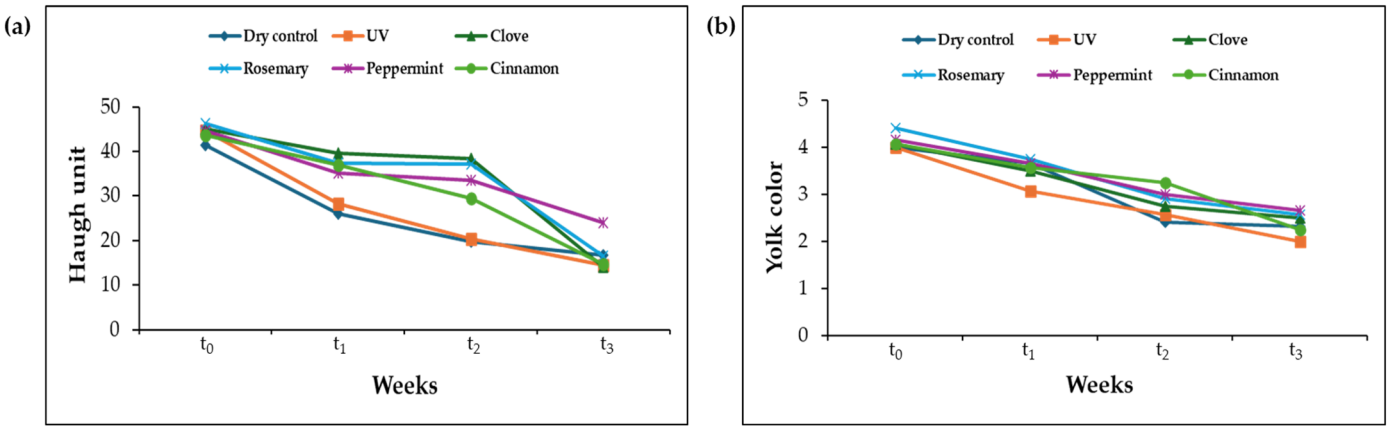


Figure 3. Effect of egg disinfection by spraying EOs, UV treatment, and storage time (weeks) on (**a**) Haugh unit and (**b**) yolk color of eggs.

The average Haugh unit (HU) measurements over the three-week storage period for the different egg groups are illustrated in Figure 3a. At t_0, all groups had similar HU averages ranging from 62.08 to 64.33 ($p > 0.05$). However, HU values gradually decreased for all eggs, reaching their lowest point at t_3. The dry control group exhibited HU values of 41.42 at t_0, 26.08 at t_1, 19.75 at t_2, and 16.75 at t_3, showing a consistent decline over time with no significant differences ($p > 0.05$). From the first to the second week of storage, eggs treated with clove EO maintained a relatively stable HU average, starting at 39.67 at t_1 and decreasing slightly to 38.42 at t_2. By t_3, the HU average had decreased to 15.75 ($p > 0.05$). A similar trend was observed in eggs sprayed with rosemary EO, with HU averages of 37.33 at t_1 and 37.08 at t_2, declining to 16.50 at t_3 ($p > 0.05$). In contrast, peppermint EO-treated eggs showed a gradual decline in HU but managed to maintain a significantly higher average at t_3, measuring around 24 ($p < 0.05$). Eggs sanitized by UV radiation and those treated with cinnamon EO also showed a gradual decline in HU over time ($p < 0.05$). This decline in HU may be attributed to the loss of carbon dioxide from the egg albumen, resulting in a watery structure, and consequently, a deterioration in HU. This is because HU is calculated based on the height of the albumen and the weight of the egg [58]. However, no significant differences were observed among these treatments when compared to the dry control at t_3 ($p > 0.05$).

The results of yolk color analysis, presented in Figure 3b, indicate the average values for all groups over the three-week storage period. At t_0, the average yolk color was similar across the groups, ranging from 4.00 to 4.42 ($p > 0.05$). However, these averages declined over time, with a significant decrease observed in all groups ($p < 0.05$), except for the dry control group, which showed no significant change ($p > 0.05$). At t_1, eggs treated with rosemary EO had the highest average yolk color of 3.75, and by t_2, eggs sanitized with cinnamon EO had the highest average yolk color of 3.25, with no significant differences among treatments ($p > 0.05$). By t_3, all groups exhibited similar yolk color averages, with the peppermint EO group showing the highest average at 2.67. However, this was not significantly different from the other treatments ($p < 0.05$). An increase in storage temperature and duration is linked to the breakdown of the albumen's protein structure and the vitelline membrane surrounding the yolk. This breakdown allows albumen components (protein and water) to seep into the yolk, diluting its pigment and subsequently reducing the yolk color [62]. However, there were no significant differences in yolk color at t_3 among the groups ($p < 0.05$), with all essential oils except for cinnamon showing higher values than the dry control and UV-treated eggs. This suggests that the essential oils effectively preserved the yolk color in this study.

3.8. Gas Chromatography of EOs

GC-MS analysis identified the major components of the essential oils, as shown in Table 3. The GC-MS chromatograms of the essential oils are shown in Figure S1. Cinnamon EO was dominated by cinnamic alcohol (70.82%), followed by α-himachalene (12.98%) and caryophyllene (8.03%). In clove EO, eugenol was the primary component (61.09%), followed by eugenyl acetate (22.57%) and caryophyllene (16.31%). Peppermint EO contained the highest proportion of *p*-mentha-6,8-dien-2-one (45.30%), followed by *p*-menthan-3-ol (25.12%), borneol (8.65%), and (R)-(-)-limonene (5.38%). Thyme EO was mainly composed of thymol (59.06%), *p*-cymene (15.28%), γ-terpinene (6.32%), and a smaller amount of linalool (4.18%). In rosemary EO, linalool (70.82%) was the most abundant, with trans-bornyl acetate (12.98%) and tricyclo[4.2.1.1(2,5)]decane (8.03%) also present in notable quantities. Green tea EO contained linalool (27.30%), jasmone (6.79%), geraniol (5.46%), and linalool oxide III (4.26%). The major compounds in the basil EO were linalool (42.23%), 1,8-cineol (13.36%), caryophyllene oxide (8.67%), eugenol (8.41%), and iso-caryophyllene (3.03%). The mustard EO contained benzoic acid, 4-ethoxy, ethyl ester (19.51%), butyl 9,12-octadecadienoate (14.29%), 9,12-octadecadienoic acid (Z,Z), 2-hydroxy-1-(hydroxymethyl)ethyl ester (10.38%), and Alpha-phenyl-alpha-tropylacetaldehyde tosylhydrazone (6.91%). Finally, Marjoram EO was characterized by trans-sabinene hydrate (37.95%), terpinen-4-ol (18.89%), spathulenol (8.67%), caryophyllene oxide (8.67%), and α-terpineol (6.48%).

Table 3. The chemical composition of Eos determined using GC-MS.

EO	Peak Number	Retention Time (min)	Compounds	Area (%)
Cinnamon	1	6.10	α-Pinene	0.45
	2	6.50	Camphene	<0.1
	3	7.35	Benzaldehyde	<0.1
	4	9.78	β-Pinene	<0.1
	5	11.86	6-Methyl-5-hepten-2-one	<0.1
	6	12.10	*p*-Cymene	0.13
	7	12.24	D-limonene	0.16
	8	21.86	Phenethyl alcohol	0.24
	9	22.51	Phenylpropyl aldehyde	0.30
	10	23.17	Borneol	<0.1
	11	23.49	Cinnamic alcohol	70.82
	12	25.78	α terpineol	0.38
	13	26.51	Cinnamic aldehyde	0.40
	14	26.74	*o*-Anisaldehyde	0.10
	15	30.69	Phenyl ethyl acetate	<0.1
	16	30.69	Trans-2-decenal	<0.1
	17	32.50	Cyclosativene	0.20
	18	41.97	α-Ylangene	0.23
	19	41.97	α-Copaene	<0.1
	20	43.52	Eugenol	0.17
	21	44.65	β-Elemene	<0.1
	22	45.36	Caryophyllene	8.03
	23	47.66	α-Himachalene	12.98
	24	47.98	Cinnamyl acetate	0.36
	25	48.56	2-Methoxycinnamaldehyde	0.18
	26	49.25	γ- Muurolene	0.16
	27	49.89	α-Muurolene	<0.1
	28	50.33	α-Curcumene	0.25
	29	50.95	β-Bisabolene	0.14
	30	51.36	δ-Cadinene	0.49
	31	53.67	α-Longipinene	0.58
	32	54.98	Nerolidol	0.35
	33	55.29	Spathulenol	0.12
	34	56.10	Globulol	0.45
	35	57.65	Benzyl benzoate	<0.1
			Total	97.67

Table 3. *Cont.*

EO	Peak Number	Retention Time (min)	Compounds	Area (%)
Clove	1	12.15	(E)-4,8-Dimethyl-1,3,7-nonatriene	tr *
	2	13.39	Acetic acid, phenylmethyl ester	<0.1
	3	14.09	Methyl salicylate	<0.1
	4	14.20	Bicyclobutylidiene	tr
	5	15.59	Chavicol	0.34
	6	17.71	α-Cubebene	<0.1
	7	17.80	Eugenol	61.09
	8	18.50	α-Copaene	0.89
	9	18.60	Cis-isoeugenol	<0.1
	10	18.70	β-Elemene	<0.1
	11	19.30	Caryophyllene	16.31
	12	20.12	α-Humulene	3.03
	13	21.01	Alloaromadendrene	<0.1
	14	21.50	δ-Cadinene	<0.1
	15	21.88	(E)-5-Acetyl-2,2-dimethyl-1-(3′-methyl-1′,3′-butadien-1′-yl)bicyclo[2.1.0]pentan	0.45
	16	22.01	γ-Muurolene	0.19
	17	22.04	α-Amorphene	0.21
	18	22.09	α-Muurolene	0.13
	19	22.20	β-Selinene	0.14
	20	22.40	α-Selinene	0.14
	21	22.54	β –Cadinene	<0.1
	22	22.68	α-Farnese	0.19
	23	23.10	Eugenyl acetate	22.57
	24	2321.00	Cadina-1,4-diene	0.54
	25	23.34	α-Cadinene	tr
	26	23.41	α-Calacorene	0.11
	27	24.50	1-Vinyl-2,6,6-Trimethylcyclohex-1-Ene	0.34
	28	24.60	1,Z-5,E-7-Dodecatriene	0.01
	29	24.69	(-)-Caryophyllene oxide	0.78
	30	24.81	Junipene	<0.1
	31	24.98	Ledol	<0.1
	32	25.30	Tau-Muurolol	tr
	33	25.83	Caryophylla-4(12),8(13)-dien-5.beta.-ol	<0.1
	34	26.94	Vulgarol B	<0.1
	35	27.89	2,3,4-Trimethoxyacetophenone	tr
	36	29.40	Benzyl benzoate	0.35
			Total	107.80
Peppermint	1	8.20	α-Phellandrene	0.16
	2	8.60	α-Pinene	0.24
	3	9.45	Camphene	<0.1
	4	11.88	B-Pinene	0.47
	5	13.96	o-Cymene	0.81
	6	14.20	D-Limonene	5.38
	7	14.34	Eucalyptol	1.95
	8	19.23	B-Linalool	0.28
	9	23.96	Borneol	8.65
	10	24.61	*p*-Menthan-3-ol	25.12
	11	25.27	*p*-Cymen-8-ol	0.21
	12	25.59	*p*-menth-1-en-8-ol	0.21
	13	27.88	2-Isopropenyl-5-methylhex-4-enal	0.19
	14	28.61	*p*-Mentha-6,8-dien-2-one	45.30
	15	28.84	7-Oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl)	1.99
	16	32.79	α-Santalol	0.32
	17	32.79	Santalol	0.32
	18	34.60	2-isopropylidenecyclohexanone	4.72
	19	44.07	1H-cycloprop[e]azulen-7-ol,decahydro--1,1,7-trimethyl-4-methylene	0.48
	20	44.07	Caryophyllene oxide	0.40
	21	44.97	Epiglobulol	0.48
			Total	97.68
Thyme	1	4.94	α-Pinene	0.62
	2	5.34	α-Thujene	0.49
	3	6.17	Camphene	0.60
	4	8.57	ß-Pinene	0.17
	5	10.63	Myrcene	1.16
	6	10.87	α-Terpinene	1.00

Table 3. *Cont.*

EO	Peak Number	Retention Time (min)	Compounds	Area (%)
Thyme	7	11.00	Limonene	0.35
	8	15.83	1,8-Cineole	0.24
	9	20.50	ß-Phellandrene	0.13
	10	21.14	ɣ-Terpinene	6.32
	11	21.80	*p*-Cymene	15.28
	12	22.11	Camphor	0.39
	13	24.38	Linalool	4.18
	14	25.09	ß-Caryophyllene	1.30
	15	25.32	Terpinen-4-ol	0.92
	16	29.22	Thymol methyl ether	0.51
	17	29.22	Boroneol methyl ether	1.74
	18	31.01	α-Terpineol	0.32
	19	40.36	Isothymol	0.12
	20	41.25	Thymol	59.56
	21	42.16	Carvacrol	2.85
			Total	98.25
Rosemary	1	2.93	α-pinene	0.04
	2	3.32	Camphene	tr
	3	4.13	ß-pinene	<0.1
	4	6.44	ß-Myrcene	<0.1
	5	8.43	α-Phellandrene	0.13
	6	8.66	α-Terpinene	0.16
	7	8.80	1,8-Cineole	0.24
	8	17.98	ɣ-Terpinene	0.30
	9	18.60	α-Terpinolene	<0.1
	10	19.23	Linalool	70.82
	11	19.53	Camphor	0.40
	12	21.73	trans-Pinocamphone	0.10
	13	22.42	Borneol	<0.1
	14	22.64	Terpinen-4-ol	0.20
	15	26.41	α-Terpineol	0.23
	16	26.41	Myrtenol	<0.1
	17	28.14	Verbenone	0.17
	18	37.18	Tricyclo[4.2.1.1(2,5)]decane	8.03
	19	37.18	trans-Bornyl acetate	12.98
	20	38.66	trans-Caryophyllene	0.36
	21	39.74	(+)-Aromadendrene	0.18
	22	40.42	(-)-Caryophyllene	0.16
	23	42.61	Caryophylla-3,8(13)-dien-5ß-ol	<0.1
			Total	94.75
Green tea	1	22.19	2-Ethyl-1-hexanol	0.26
	2	23.66	Linalool	27.30
	3	28.31	Linalool oxide IV	1.10
	4	28.77	Linalool oxide III	4.26
	5	30.53	Geraniol	5.46
	6	32.02	Phenethyl alcohol	7.86
	7	31.23	Benzyl alcohol	10.11
	8	14.65	Dodecane	1.23
	9	16.26	Styrene	0.42
	10	17.4	Tridecane	13.42
	11	20.11	Tetradecane	3.35
	12	29.77	Octadecane	1.40
	13	33.03	Jasmone	6.79
	14	36.55	Perhydrofarnesyl acetone	7.60
	15	23.43	Benzaldehyde	0.71
	16	33.23	2-Acetyl-1H-pyrrole	5.52
			Total	96.81
Basil	1	4.09	α-Pinene	0.68
	2	4.46	Camphene	0.86
	3	5.18	ß-Pinene	1.09
	4	5.55	ß-Myrcene	0.80
	5	6.76	1,8-Cineol	13.36
	6	7.16	trans-β Ocimene	1.58
	7	7.46	c-Terpin	1.77
	8	10.25	Linalool	43.23
	9	11.42	Camphor	0.66
	10	11.58	Myrtenol	1.30
	11	14.95	α-Cubebene	4.86
	12	18.10	Eugenol	8.41

Table 3. *Cont.*

EO	Peak Number	Retention Time (min)	Compounds	Area (%)
Basil	13	18.44	Methyl cinnamate	4.17
	14	18.86	iso-Caryophyllene	3.03
	15	19.77	α-Caryophyllene	1.71
	16	20.67	Azulene	1.28
	17	21.77	α-Farnesene	1.66
	18	22.37	Germacrene B	1.59
	19	22.77	Germacrene D	0.80
	20	23.47	Naphthalene	1.97
			Total	94.80
Mustard	1	4.36	2,4 Dimethy-l-1heptene	0.92
	2	7.76	Stearic acid, 3 (octadecyloxy) propyl ester	0.30
	3	8.77	Decane	0.63
	4	8.98	Nonane, 2,6-dimethyl	0.44
	5	9.10	Heptane, 5-ethyl 2-methyl	0.56
	6	11.06	2-Undecanethiol, 2methyl	0.57
	7	11.19	Cyclooctane, 1,4-dimethyl,trans	0.79
	8	14.77	Dodecane	1.26
	9	17.20	Anethole	0.38
	10	17.61	2-Isopropyl-5-methyl-1-heptanol	1.22
	11	17.84	Dichloroacetic acid, tridecyl ester	1.07
	12	18.07	1 Docosene	0.84
	13	20.31	Tetradecane	0.95
	14	22.02	Hexadecane, 2,6,11,15 tetra methyl	0.31
	15	22.31	1 Iodo 2 methyl undecane	0.39
	16	23.16	Pentadecane	0.78
	17	23.26	Phenol, 2,6-bis (1,1-dimethylethyl)	1.82
	18	23.78	Benzoic acid, 4-ethoxy, ethyl ester	19.51
	19	23.89	1 Hexadecanol,3,7,11,15 tetramethyl	0.32
	20	24.12	1Hexadecanol, 2 methyl	0.43
	21	24.39	Acetic acid, 3,7,11,15tetramethylhexadecyl ester	0.45
	22	24.65	1Dodecanol, 2hexyl	0.36
	23	25.82	Nonadecane	1.15
	24	27.89	Heptadecane	1.31
	25	28.20	Benzene, 1,1′(2butene1,4diyl) bis	0.33
	26	28.90	Trichloroacetic acid, hexadecyl ester	0.57
	27	29.03	Tert-Hexadecanethiol	0.40
	28	29.66	Octadecane	0.75
	29	31.43	Z,Z,Z 4,6,9-Nonadecatriene	0.56
	30	31.60	Tetradecane, 2,6,10-trimethyl	0.91
	31	32.60	Phthalic acid, isobutyl octadecyl ester	0.49
	32	33.46	Octacosane	0.37
	33	35.53	cis Vaccenic acid	0.60
	34	37.93	Glycerol 1 palmitate	3.68
	35	38.13	Eicosanoic acid	0.36
	36	39.86	Propyl octadeca-9,12-dienoate	0.41
	37	40.15	Alpha-phenyl-alpha-tropylacetaldehyde tosylhydrazone	6.91
	38	40.51	Butyl 9,12-octadecadienoate	14.29
	39	40.58	9,12-Octadecadienoic acid (Z,Z), 2 hydroxy 1 (hydroxymethyl) ethyl ester	10.38
	40	40.66	6,9,12,15Docosatetraenoicacid, methyl ester	2.70
	41	40.74	1Heptatriacotanol	1.92
	42	40.90	Hexadecanoic 1 (hydroxymethyl)1,2ethanediyl ester	3.09
	43	41.26	Phthalic acid, di(2propylpentyl) ester	1.14
	44	41.95	Stigmasterol	2.07
	45	44.01	çSitosterol	5.28
	46	44.60	Squalene	0.65
	47	45.65	dMannitol, 1decylsulfonyl	0.42
	48	46.54	Linoleic acid ethyl ester	0.96
	49	48.54	çTocopherol	1.25
			Total	97.27
Marjoram	1	4.33	Camphene	0.68
	2	5.91	p-Cymene	3.34
	3	7.25	c-Terpinene	3.69
	4	8.30	trans-Sabinene hydrate	37.95

Table 3. *Cont.*

EO	Peak Number	Retention Time (min)	Compounds	Area (%)
Marjoram	5	9.50	Dehydro Sabinene ketone	1.18
	6	9.50	3-Isothujenol	0.86
	7	10.71	Terpinen-4-ol	18.89
	8	11.14	α-Terpineol	6.48
	9	12.96	Linalyl acetate	1.30
	10	13.40	Bornyl acetate	0.53
	11	13.90	3-Thujanyl acetate	2.35
	12	21.76	Spathulenol	8.67
	13	21.90	Caryophyllene oxide	8.67
			Total	94.60

* tr indicates less than 0.01.

From the GC-MS analysis of the tested EOs, the monoterpene alcohols were identified as the major constituents in most of the tested EOs. This suggests that monoterpene alcohols may contribute significantly to their antibacterial activity, as has been observed with similar EOs in previous studies [63]. Bassolé et al. investigated the antibacterial effects of the primary monoterpene alcohols found in EOs. Their findings revealed that the major components exhibited notable inhibitory effects on test bacteria, including *L. monocytogenes*, *E. aerogenes*, *E. coli*, and *P. aeruginosa*. Among the five key compounds analyzed, thymol and carvacrol demonstrated the strongest antibacterial activities, followed by eugenol. In contrast, linalool showed moderate activity, while menthol exhibited relatively limited antibacterial effects. Additionally, paired combinations of *Lippia multiflora*, *Mentha x piperita*, and *Ocimum basilicum* EOs displayed synergistic effects in inhibiting *E. faecalis* [64]. Similarly, a study by Gallucci et al. reported that carvacrol and thymol exhibited potent bactericidal activity against all the tested strains, including *E. coli*, *S. aureus*, and *B. cereus* [65]. In another study evaluating the antimicrobial activity of basil oil, components such as linalool (64.35%) were found to inhibit all tested strains of *S.* Enteritidis [66].

4. Conclusions

This study demonstrated that EOs are effective natural sanitizers for reducing bacterial contamination in eggshells and improving their shelf life. Nine out of the ten EOs tested exhibited significant antibacterial activity against a range of foodborne pathogens, with rosemary, cinnamon, clove, and peppermint showing the highest levels of efficacy in both liquid and vapor phases. Rosemary EO was particularly potent, with strong inhibition against *S. aureus*, *L. monocytogenes*, and *E. faecalis*, while cinnamon EO showed the highest activity against *S.* Enteritidis and *E. coli*. In vapor phase testing, rosemary, thyme, clove, and cinnamon EOs achieved inhibition zones ranging from 21 to 33 mm, and peppermint EO effectively inhibited *P. aeruginosa*. The use of EOs significantly reduced the bacterial load on eggs during the storage period, with clove EO demonstrating the most consistent antimicrobial effect. Although some decline in egg quality was observed over time, EOs helped preserve better external and internal egg quality compared to the dry control. GC-MS analysis identified monoterpene alcohols as the dominant compounds in most EOs, which may contribute to their antimicrobial properties. Overall, these findings support the use of EOs as effective natural agents for enhancing food safety and quality preservation during storage.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/pr13010224/s1, Figure S1: GC-MS chromatograms of the essential oils: (A) clove, (B) peppermint, (C) cinnamon, (D) thyme, (E) rosemary, (F) basil, (G) mustard, (H) marjoram, and (I) green tea.

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