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Research Article

Chemical and Biological Properties of *Elettaria cardamomum* Maton var. *minuscula* Essential Oil and Its Effect as Preservative to Shelf-Life Storage of Sous Vide Carrot Inoculated With *Pseudomonas aeruginosa*

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Microbial spoilage and contamination pose significant challenges to the shelf life and safety of minimally processed foods such as sous vide vegetables. Essential oils have emerged as promising natural preservatives due to their antimicrobial properties. In this study, *Elettaria cardamomum* Maton var. *minuscula* essential oil (ECMEO) was chemically characterized and its antimicrobial efficacy evaluated against various microorganisms through both vitro and in situ experiments. The antibiofilm activity of ECMEO, specifically against biofilm-forming *Pseudomonas aeruginosa*, was assessed using crystal violet staining and MALDI-TOF MS profiling. Additionally, insecticidal activity against *Megabruchidius dorsalis* was tested, and synergistic effects with mild thermal treatment on microbial growth were investigated. GC–MS analysis identified α-terpinyl acetate (40.9%) and 1,8-cineole (36.2%) as major components of ECMEO. The essential oil exhibited strong antimicrobial effects against several bacteria and yeasts, with minimum inhibitory concentrations between 0.33 and 0.56 mg/mL. ECMEO effectively inhibited microbial growth in vapor-phase assays and significantly reduced bacterial counts in vacuum-sealed sous vide carrots, including suppression of *P. aeruginosa* biofilms. Combined application with mild heat showed synergistic inhibition of bacterial growth. Furthermore, ECMEO demonstrated insecticidal activity against *M. dorsalis*. These findings suggest ECMEO

is a promising natural preservative for enhancing microbial safety and extending the shelf life of sous vide vegetables, with potential applications in food preservation and crop protection.

Keywords: antimicrobial activity in vitro; carrot model; in situ; *Pseudomonas aeruginosa*

1. Introduction

Bioactive compounds from medicinal and aromatic plants play a crucial role in combating antibiotic-resistant bacterial pathogens that cause serious infections. Due to these properties, such plants are commonly used in Mediterranean culinary traditions [1, 2]. The widespread and often excessive use of antibiotics in medical treatments has accelerated the emergence of multidrug-resistant bacteria, posing significant challenges in hospitals and communities alike. Consequently, medicinal and aromatic plants with antibacterial properties have been extensively studied as potential alternatives [3].

Cardamom (*Elettaria cardamomum* Maton), often called the "queen of spices," is a highly valued seasoning worldwide (Ashutosh [4]). Belonging to the Zingiberaceae family, its dried fruits are prized for culinary and medicinal uses [5]. Native to the tropical forests of southern India and Sri Lanka, commercial cultivation is now primarily centered in southern India [6]. This perennial aromatic plant is mainly harvested for its seeds, used whole or ground in spice blends. Cardamom flavoring is common in curry powders, sweets, baked goods, meats, teas, coffees, and confectionery products [7]. The essential oil of *E. cardamomum* is valued for its distinctive fragrance and is widely used in food and cosmetic industries as a flavoring and scent enhancer [4]. Beyond culinary uses, it exhibits medicinal properties including antispasmodic, antidiarrheal, cytotoxic, antioxidative, and antibacterial activities, such as relaxing intestinal smooth muscles [8–10]. Its essential oil inhibits growth of several Gram-negative bacteria, notably *Pseudomonas aeruginosa* and *Escherichia coli* [11]. *P. aeruginosa* poses serious risks to immunocompromised patients and is resistant to many antibiotics, while *E. coli* is a common gut resident often associated with gastrointestinal diseases [12–15]. Contamination of foods by *P. aeruginosa* has been linked to digestive tract infection outbreaks [16]. Additionally, cardamom essential oil shows insecticidal potential against species such as *Hyalomma anatolicum* ticks, *Tribolium castaneum*, and *Sitophilus oryzae* [17–19].

Among microorganisms of concern, *P. aeruginosa* is particularly significant [20]. Rinaldi et al. [21] demonstrated that sous vide carrots stored at 4°C for up to 10 days maintained microbial counts below 1 log CFU/g, indicating effective microbial control by thermal treatment. Similarly, [22]) reported that sous vide-cooked carrots stored for 30 days under refrigeration showed total aerobic plate counts under 1 log CFU/g. The growth behavior of *P. aeruginosa* has been widely studied, focusing on temperature effects and predictive modeling of its proliferation [23–25].

Consumers increasingly prefer minimally processed fruits and vegetables for convenience and natural freshness [26]. To extend shelf life and reduce bacterial contamination, post-harvest treatments such as washing and removal of damaged areas are applied [27]. Sanitizing agents in wash water can reduce bacterial loads, but their efficacy depends on microbial species, adhesion properties, and produce characteristics [28]. Plant diseases are also managed using natural biological chemicals and organic substances [29]. Studies by Stan et al. [30], Zhou et al. [31], Mandal and Domb [32], and Ben Miri [33] show that these biological compounds have antioxidant and antimicrobial effects that disrupt bacterial membranes and biosynthesis. Additionally, essential oils from certain spices contribute to reducing microbial activity [34].

In industrial and clinical settings, maintaining sterility is challenging due to microbial biofilms, which exhibit significantly higher resistance to antibiotics and disinfectants compared with planktonic bacteria [35, 36]. This resistance arises from several factors, including the protective exopolysaccharide matrix, the presence of dormant cells tolerant to antibiotics [37], and the expression of resistance genes specific to biofilm growth [38–41]. These complexities underscore the urgent need for innovative strategies to effectively disrupt and eradicate bacterial biofilms.

This study addresses the critical need to enhance microbial safety and extend the shelf life of minimally processed foods, specifically sous vide carrots. The primary objective was to chemically characterize the *Elettaria cardamomum* var. *minuscula* essential oil (ECMEO) and to comprehensively evaluate its antimicrobial activity against a spectrum of microorganisms through both in vitro and in situ assays. The antibiofilm effects of ECMEO, specifically against biofilm-forming *P. aeruginosa*, were assessed using crystal violet staining and MALDI-TOF MS profiling. Additionally, the insecticidal potential of ECMEO was assessed to explore its broader applications in food preservation and crop protection. The synergistic effects of ECMEO combined with mild thermal treatment on bacterial growth dynamics were also investigated. The findings support the potential use of ECMEO as a natural preservative capable of enhancing microbial safety and prolonging shelf life via both direct contact and vapor-phase antimicrobial mechanisms in sous vide products.

2. Material and Method

2.1. ECMEO. For this study, essential oil from ECMEO was obtained from Hanus Ltd. (Nitra, Slovakia). The essential oil was distilled from freshly harvested seeds cultivated in Guatemala, a region known for its favorable agroecological conditions. The ECMEO was delivered by the supplier in sealed amber glass vials under ambient conditions. Upon receipt, the samples were immediately stored at 4°C in a dark, temperature-controlled environment to prevent any potential degradation prior to analysis. No special transport conditions were required by the supplier due to the chemical stability of the oil.

2.2. Chemical Composition of the ECMEO. The chemical composition of ECMEO was analyzed using an Agilent 6890N gas chromatograph coupled with a 5975B quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, United States). Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; [5%-phenyl]-methylpolysiloxane stationary phase; Agilent Technologies, United States). A 10% (v/v) solution of ECMEO in hexane (HPLC grade, Sigma-Aldrich, St. Louis, MO, United States) was prepared by diluting the essential oil with hexane immediately prior to analysis. A 1-μL aliquot of this solution was injected in split mode with a split ratio of 40.8:1, and injector temperature set at 290°C. Helium 5.0 was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature programmed at 50°C with an increase of 3°C/min to 75°C (isothermal for 4 min), 5°C/min from 75°C to 120°C (isothermal for 2 min), and then 5°C/min to 290°C (resulting total run time, 57.33 min). The ion source and quadrupole temperatures were maintained at 230°C and 150°C, respectively. Mass spectra were acquired in electron ionization (EI) mode at 70 eV over the scan range 35–550 amu, with a solvent delay of 3.2 min. To obtain the retention times and mass spectra of individual *n*-alkanes (C_7–C_{35} mixture, Supelco, Bellefonte, PA, United States), the solvent delay time was set at 2.30 min, with the fact that the acquisition continued until the GC oven maintained a final temperature of 290°C for 8.00 min (resulting total run time, 65.33 min). The volatile constituents in the ECMEO were identified by comparing the recorded mass spectra with those in the Wiley7 and NIST libraries and by correlating the collected MS data and experimentally calculated Kovats retention indices with literature data. Compounds with concentrations higher than 0.1% were semi-quantified using the MSD ChemStation integrator (Agilent Technologies, Santa Clara, CA, United States) using their respective GC peak regions.

2.3. Bacterial and Yeast Species Tested for the Antimicrobial Assays. The antimicrobial activity of ECMEO was evaluated against a diverse panel of bacterial and yeast strains. Gram-positive bacteria included *Bacillus cereus* CCM (Czech Collection of Microorganism) 2010, *Listeria innocua* CCM 4030, *Staphylococcus epidermidis* CCM 4418, and *Streptococcus constellatus* CCM 4043. The yeast panel comprised *Candida tropicalis* CCM 8223, *Candida albicans* CCM 8186, *Candida glabrata* CCM 8270, *Candida krusei* CCM 8271, and *Candida parapsilosis* CCM 8260. Gram-negative bacteria included *Enterobacter aerogenes* CCM 2531, *K. pneumoniae* CCM 2318, *P. aeruginosa* CCM 1959, and *Shigella sonnei* CCM 1373. All microbial strains were sourced from the CCM, Brno, Czech Republic.

Additionally, *P. aeruginosa* strains were isolated from dairy processing environments to evaluate the antimicrobial and antibiofilm efficacy of ECMEO against environmental isolates with high biofilm-forming potential. Isolation was performed as follows: Samples were collected aseptically from surfaces and equipment in dairy facilities, transported to the laboratory under refrigerated conditions (4°C), and processed within 24 h. Samples were inoculated onto *Pseudomonas* selective agar (PA, Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and incubated at 30°C for 24–48 h. Presumptive *P. aeruginosa* colonies were initially identified based on colony morphology, pigmentation, and biochemical tests (oxidase positive, growth at 42°C), and their identity was subsequently confirmed by MALDI-TOF mass spectrometry (Bruker Daltonics, Bremen, Germany). Confirmed isolates were stored at −80°C in 20% glycerol until use. Cultivation of reference bacterial strains was performed in Mueller–Hinton broth (MHB) (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom), and yeasts were grown in Sabouraud dextrose broth (SDB) (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). Cultures were incubated at 37°C for bacteria and 25°C for yeasts, each for 24 h. Prior to antimicrobial testing, bacterial suspensions were adjusted to 0.5 McFarland standard (~1.5×10^8 CFU/mL) using a densitometer to ensure consistent inoculum density [42].

2.4. The Disc Diffusion Technique for Antimicrobial Activity. The antimicrobial activity of ECMEO was assessed by disc diffusion adapted from Vukovic et al. [10]. Mueller–Hinton agar (MHA) (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) was used for bacteria and Sabouraud dextrose agar (SDA) (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) for yeasts. Sterile 6-mm paper discs (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) were loaded with 10 μL of ECMEO and placed onto freshly inoculated agar plates. Plates were incubated for 24 h at 37°C (bacteria) or 25°C (yeasts). After incubation, inhibition zone diameters were measured with a digital caliper and reported as mean ± SD; no categorical strength scale was applied. Solvent-only discs served as negative controls; comparator agents included cefoxitin (Gram-negative), gentamicin (Gram-positive), and fluconazole (yeasts) (all Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) [10, 43].

2.5. The Minimum Inhibitory Concentration Assays. Minimum inhibitory concentrations of ECMEO were determined by broth microdilution in sterile 96-well microtiter plates (Corning Inc., Corning, NY, United States). Two-fold serial dilutions of ECMEO were prepared in MHB for bacterial strains and in SDB for yeasts (10 to 0.00488 mg/mL). Each well received 150 μL of a standardized microbial suspension (1×10^6 CFU/mL). Negative controls (broth + ECMEO without inoculum) and positive controls (broth + inoculum without ECMEO) were included on each plate. Plates were incubated at 37°C for 24 h (bacteria) or 25°C for 48 h (yeasts). Microbial growth was measured spectrophotometrically at 570 nm using a GloMax Multi Detection System (Promega Corporation, Madison, WI, United States). MIC endpoints (MIC_{50}/MIC_{90}) were defined as the lowest ECMEO concentrations yielding 50% and 90% growth inhibition relative to the growth control, respectively. All assays were performed in triplicate [42, 43].

2.6. Vapor Phase Antimicrobial Testing of Fruit and Vegetables. The vapor-phase antimicrobial activity of ECMEO was evaluated on fresh fruit and vegetable substrates including mango (*Mangifera indica*), peach (*Prunus persica*),

carrot (*Daucus carota*), and zucchini (*Cucurbita pepo*) [44, 45]. Fresh substrates were sourced from a local supermarket, Billa (Nitra, Slovakia). Upon receipt, samples were transported to the laboratory at 4°C and processed within 4 h. Fruits and vegetables were aseptically washed with sterile distilled water, cut into uniform 0.5 cm pieces, and air-dried under sterile conditions to remove surface moisture and contaminants. Prepared substrate pieces (approximately 1 g per sample) were placed individually into sterile 60 mm Petri dishes. Each dish was inoculated with 100 μL of standardized microbial suspension (approximately 1×10^6 CFU/mL) of the test strains as described in Section 2.3. ECMEO was diluted in ethyl acetate (HPLC grade, Sigma-Aldrich, St. Louis, MO, United States) at four concentrations: 500, 250, 125, and 62.5 μg/L. Filter paper discs (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) soaked with 20 μL of each ECMEO solution were placed inside the sealed Petri dishes, ensuring no direct contact with the substrates, to expose samples to the vapor phase. Ethyl acetate alone served as a negative control. Petri dishes were sealed with parafilm to create a closed environment and incubated at 37°C for 7 days. After incubation, microbial growth on substrates was quantified by image analysis using the ImageJ software (National Institutes of Health, Bethesda, MD, United States) to measure colony density. Standard microbiological plating and colony counting methods were used to validate the microbial load on samples. The antimicrobial efficacy of ECMEO vapor was assessed by comparing microbial growth on treated substrates versus controls, highlighting the potential application of ECMEO in food preservation [44, 45].

2.7. Antibiofilm Assay

2.7.1. Crystal Violet Analysis. The minimum biofilm inhibitory concentration (MBIC) of ECMEO against *P. aeruginosa* was determined using a crystal violet staining assay in sterile 96-well microtiter plates (Corning Inc., Corning, NY, United States). Overnight bacterial cultures grown in MHB were adjusted to approximately 1×10^6 CFU/mL and exposed to serial two-fold dilutions of ECMEO ranging from 100 to 0.049 mg/mL. Plates were incubated at 37°C for 24 h to allow biofilm formation in the presence of ECMEO. After incubation, planktonic cells were removed by gently washing wells three times with sterile phosphate-buffered saline (PBS). Biofilms were stained with 0.1% (*w*/*v*) crystal violet solution for 15 min at room temperature. Excess stain was removed by washing wells with distilled water, and plates were air-dried. The stained biofilm was solubilized with 95% ethanol, and absorbance was measured at 570 nm using a microplate reader (model and manufacturer, e.g., Glomax Multi Detection System, Promega Corporation, Madison, WI, United States). MBIC, $MBIC_{50}$, and $MBIC_{90}$ values were defined as the lowest ECMEO concentrations that inhibited 100%, 50%, and 90% of biofilm formation, respectively, compared with the untreated control wells. All assays were performed in triplicate [46].

2.7.2. MALDI-TOF MS Biotyper: An Important Tool for Biofilm Identification. Biofilm-associated protein degradation and changes were analyzed using a MALDI-TOF MicroFlex mass spectrometer (Bruker Daltonics, Bremen, Germany). Sterilized stainless steel coupons (approximately 10×10 mm) and standard glass microscope slides ($75 \times 25 \times 1$ mm) were used as substrates. The slides were placed vertically inside sterile 50 mL polypropylene tubes, fitting within the tube dimensions without bending or deformation. Stainless steel coupons were placed flat at the bottom of the tubes. Each tube contained 20 mL of MHB. Tubes were inoculated with 100 μL of *P. aeruginosa* suspension adjusted to approximately 1×10^6 CFU/mL. Experimental tubes were treated with ECMEO at a final concentration of 0.1% (*v*/*v*), prepared by dissolving ECMEO in ethyl acetate. Control tubes remained untreated. Tubes were incubated at 37°C under orbital shaking at $170 \times$ g for 3 days to allow biofilm formation on the coupon surfaces. After incubation, biofilms were gently removed from the stainless steel and glass surfaces by swabbing with sterile cotton swabs, and biofilm samples were incubated further at 37°C for 7, 9, 12, and 14 days. Planktonic cells from untreated controls were collected and analyzed alongside biofilm samples for comparative purposes. Protein spectra were acquired in linear positive ion mode using standard MALDI-TOF MicroFlex MS settings (mass range: [specify range], laser intensity, number of shots). Nineteen representative spectra per sample were selected based on signal quality and reproducibility. Euclidean distances between spectra were calculated using appropriate software, and a dendrogram was generated from the resulting distance matrix to assess differences in biofilm-associated protein degradation patterns and structural changes in the biofilm matrix [44, 45].

2.8. Combined Effects of ECMEO, Temperature, and Exposure Time on the Growth Dynamics of P. aeruginosa in a Bioreactor System. An experiment was carried out to investigate how heat and ECMEO influence the growth of *P. aeruginosa*, using a personal bioreactor system (RTS-1, Biosan, Riga, Latvia). The growth rate (GR) was monitored by measuring optical density (OD) at a wavelength of 850 nm. Initially, the bacteria were cultivated on MHA plates at 37°C for 24 h. After this, a single colony was selected and transferred into 80 mL of MHB, ensuring proper mixing. The broth was then divided into two 25 mL portions, placed in separate tubes with perforated caps, and incubated in bioreactors at 37°C. When the OD_{850} reached approximately 1, signifying the onset of exponential growth, 100 μL of ECMEO was added to one of the tubes, while the other tube served as a control and received no treatment. The bioreactors were gradually heated to 50°C, maintaining a rotation speed of 2000 rpm (one rotation per second). OD measurements were recorded at 5-min intervals for 20 min. The procedure was repeated at higher temperatures of 50°C, 55°C, 60°C, and 65°C to evaluate the combined influence of ECMEO and elevated temperatures on bacterial growth.

2.9. Sous Vide Experiment With Carrots. For this study, 2.5 kg of carrots (*Daucus carota*) were obtained from a certified supplier in the Slovak Republic. Once cooled, the carrots were transferred to the microbiology lab, where they were cut into 5 g segments using a sterilized knife and cutting

board. On Days 1 and 7, 483 5-g samples were allocated to the treatment and control groups. Each sample was treated with a 10-mg/mL solution of ECMEO mixed with rapeseed oil, then vacuum-sealed individually using a Concept vacuum packer (Concept, Choceň, Czech Republic). Both vacuum-sealed and non-sealed parts were included as control samples. To simulate the presence of *P. aeruginosa* without compromising the carrots, 100 μL of *P. aeruginosa* and 10 mg/mL ECMEO were applied to the samples. The samples were gently mixed for about 1 min before being sealed [44, 45]. Samples were then subjected to sous vide cooking at temperatures of 50°C, 55°C, 60°C, and 65°C for 5, 10, 15, and 20 min to evaluate the combined effect of ECMEO and heat treatment on microbial growth. After cooking, 100 μL of each sample was inoculated onto plate count agar (PCA), violet red bile lactose agar (VRBL), and *Pseudomonas* agar plates. PCA plates were incubated at 30°C, while VRBL and *Pseudomonas* agar plates were incubated at 37°C for 24 h. Colonies were further processed and identified by MALDI-TOF MS Biotyper (Figure 1). Overview of the sous vide experimental procedure and microbiological analysis of carrot samples are as follows:

A. Sample grouping
   - C — Control samples without vacuum packaging
   - CV — Control samples with vacuum packaging
   - EO — Samples treated with 10 mg/mL ECMEO and vacuum-packed
   - PS — Samples inoculated with *P. aeruginosa* and vacuum-packed
   - PS + EO — Samples inoculated with *P. aeruginosa* and treated with ECMEO, then vacuum-packed

B. Packaging process: individual vacuum sealing of each 5 g carrot sample using Concept vacuum packer (Concept, Choceň, Czech Republic).

C. Temperature and time treatments: sous vide cooking at 50°C, 55°C, 60°C, and 65°C for 5, 10, 15, and 20 min to evaluate combined effects on microbial growth.

D. Microbial inoculation and plating on selective media: PCA, VRBL, and *Pseudomonas* agar.

E. Incubation conditions: PCA plates incubated at 30°C; VRBL and *Pseudomonas* agar plates incubated at 37°C for 24 h.

F. Identification of bacterial colonies using MALDI-TOF MS Biotyper.

2.10. Insecticidal Activity. The insect *Megabruchidius dorsalis* Fahreus, 1839, was utilized as the test organism to determine the insecticidal effectiveness of ECMEO. *M. dorsalis* is considered dangerous because it is a notorious pest of stored legumes, causing significant damage to crops by feeding on seeds and reducing their market value, which can lead to economic losses in agriculture. To carry out the experiment, groups of 50 *M. dorsalis* beetles were placed in Petri dishes, which were pre-lined with sterile filter paper. The ECMEO was prepared by diluting it with 0.1% polysorbate, resulting in varying concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.12%. A 100-μL aliquot of each ECMEO solution was applied to sterile filter paper discs and placed inside sealed Petri dishes. The dishes were enclosed with parafilm and maintained at room temperature for 24 h. The control groups received 100 μL of 0.1% sodium polysorbate solution. After exposure, the insects were assessed for mortality, and the numbers of live and dead individuals were recorded. The experiment was conducted in triplicate using independently prepared samples [42].

2.11. Statistical Analysis. All data are presented as mean ± standard deviation (SD) from triplicate experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD post hoc test, with significance set at $p \leq 0.05$ (CoStat v6.451; CoHort Software, Pacific Grove, CA, United States). Graphical outputs were generated using JMP Pro 17.0 (SAS Institute, Cary, NC, United States).

3. Results and Discussion

3.1. Chemical Composition of ECMEO. GC–MS analysis was performed to identify the volatile compounds present in ECMEO and to assess its chemical profile. The findings, detailed in Table 1, show the distribution of the identified components across various chemical classes, with their corresponding percentages. A total of 24 components were detected, comprising 99.9% of the essential oil. The oil is predominantly made up of monoterpenes (98.3%), with the highest concentrations found in the monoterpene ester α-terpinyl acetate and the monoterpene ether 1,8-cineole, representing 40.9% and 36.2% of the total content, respectively. Other monoterpene hydrocarbons, such as α-pinene, sabinene, and limonene, were present at lower levels (2.1%, 4.7%, and 3.4%, respectively). Additionally, three sesquiterpene compounds were detected, making up 1.6% of the total oil.

Previously, Vukovic et al. [10] also reported high quantities of oxygenated monoterpenes α-terpinyl acetate (37.3%) and 1,8-cineole (28.6%) in cardamom essential oil. Similarly, Savan and Küçükbay [7], during analysis of ECMEO distilled from dried fruits, among 67 detected components (96.9% of the oil), observed α-terpinyl acetate (40.7%), 1,8-cineole (25.6%), and linalool (6.4%) as the major constituents. Also, Menon et al. [47] found 1,8-cineole (28.4%) and α-terpinyl acetate (21.3%) as the most abundant compounds, while [11]) reported the prevalence of α-terpinyl acetate (44.3%), 1,8-cineole (10.7%), α-terpineol (9.8%), and linalool (8.6%). To investigate variations in both yield and chemical composition, Gopalakrishnan and Narayanan [48] employed supercritical carbon dioxide extraction under different conditions, such as pressure, temperature, and moisture levels. They observed that the extraction conditions had an impact on the concentration of primary constituents like 1,8-cineole and α-terpinyl acetate. In a similar vein,

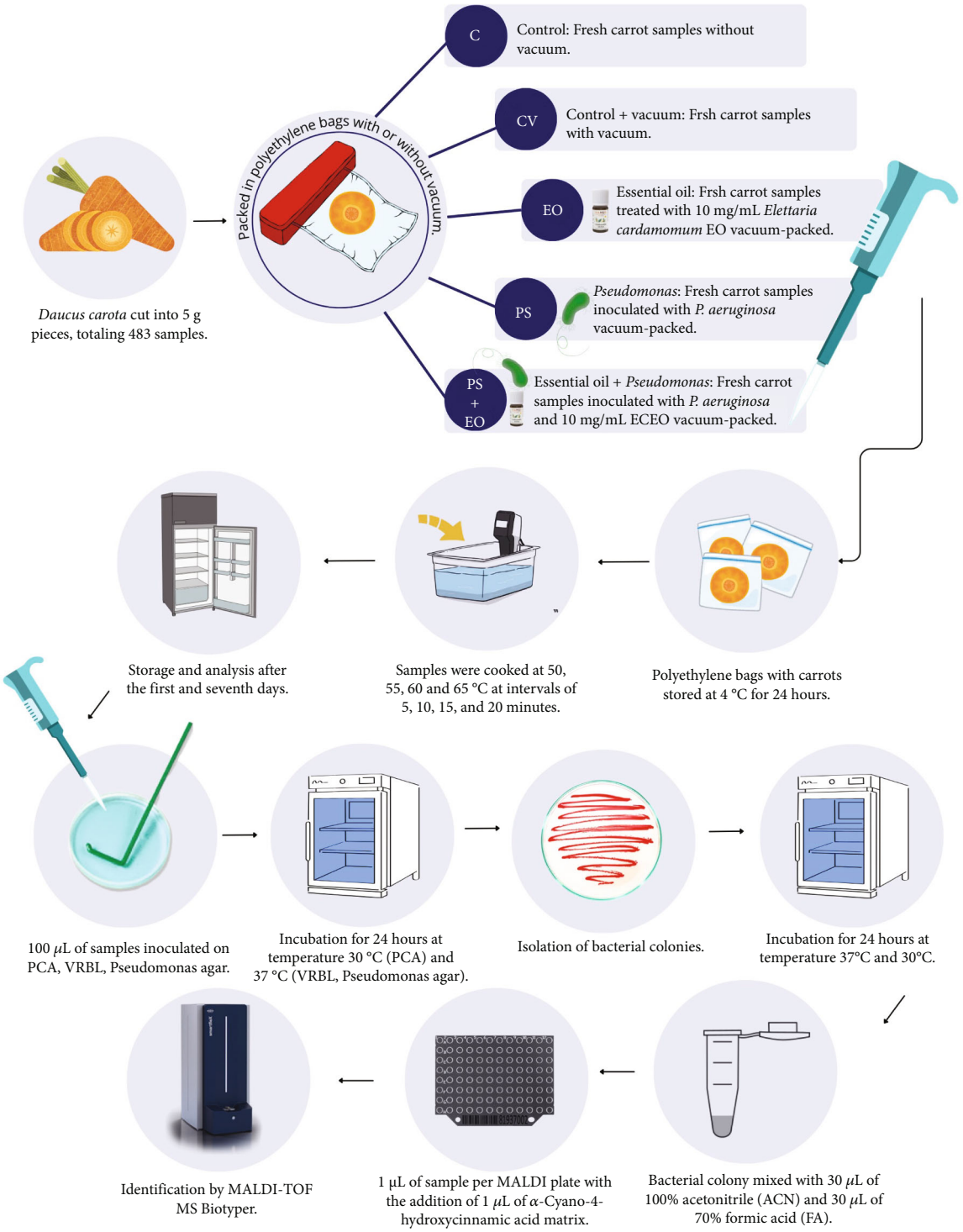


Figure 1: The steps involved sous vide samples preparation of carrot and microbiological analysis with identification of microbiota.

Sultana et al. [49] pointed out that different environmental factors influenced the oil's chemical composition. In their analysis, the oil was primarily composed of 97.6% monoterpenes, with 1,8-cineole (89.6%), cis-ocimene (3.7%), and α-terpinene (2.2%) being the key compounds. Notably, 1,8-cineole was found in all samples except one exposed to sunlight, where its concentration varied between 29.4% in the heated oil and 89.4% in the untreated oil. When exposed to heat (110°C), new compounds like linalool acetate, nerol, citronellol, limonene-1,2-epoxide, thujyl alcohol, and trans-pinocarveol were generated. Kumara et al. [50] reported a high concentration of monoterpene ether 1,8-cineole in Malabar cardamom essential oil sample, while Mysore variety contains more monoterpene ester α-terpinyl acetate. Contrary to abovementioned data, Husain et al. [51] reported 1,8-cineole (74%) as the dominant component in

TABLE 1: Chemical composition and classes of identified compounds in *E. cardamomum* Maton var. *minuscula* essential oil.

No.	Compound[a]	%[b]	KI[c] (Lit.)	KI[c] (Calc.)
1	α-Thujene	0.3	930	927
2	α-Pinene	2.1	939	935
3	Sabinene	4.7	975	973
4	β-Pinene	0.5	979	977
5	β-Myrcene	1.6	990	988
6	α-Terpinene	tr	1017	1018
7	*o*-Cymene	0.5	1026	1027
8	Limonene	3.4	1029	1033
9	1,8-Cineole	36.2	1031	1038
10	γ-Terpinene	0.5	1059	1064
11	*p*-Mentha-3,8-diene	0.3	1072	1075
12	α-Terpinolene	0.2	1088	1087
13	Linalool	0.9	1096	1099
14	4-Terpineol	1.3	1177	1182
15	α-Terpineol	1.7	1188	1195
16	Octanol acetate	tr	1213	1210
17	Linalool formate	tr	1216	1215
18	Car-3-en-2-one	2.2	1248	1248
19	δ-Terpinyl acetate	tr	1317	1313
20	α-Terpinyl acetate	40.9	1349	1353
21	Geranyl acetate	1.0	1381	1379
22	β-Selinene	0.7	1490	1488
23	α-Selinene	0.2	1498	1495
24	Germacrene B	0.7	1561	1561
Total			99.9	
Class of compounds		*Number of compounds*		*%*
Nonterpenic compounds		1		tr
Esters		1		tr
Monoterpenes		20		98.3
Monoterpene hydrocarbons		11		14.1
Monoterpene alcohols		3		3.9
Monoterpene ketones		1		2.2
Monoterpene ethers		1		36.2
Monoterpene esters		4		41.9
Sesquiterpenes		3		1.6
Sesquiterpene hydrocarbons		3		1.6

[a]Identified compounds.
[b]Percentage composition.
[c]Literature and calculated values of retention indices on HP-5MS column.
[d]tr-percentage amounts less than 0.1%.

E. cardamomum fruits essential oil, which is in agreement with Abdullah et al. [52] who found that composition and ratio of 1,8-cineole and α-terpinyl acetate vary depending on the plant's geographical origin and stage of growth.

3.2. Antimicrobial Properties

3.2.1. Disc Diffusion Methods Assay for Antimicrobial Activity. The antimicrobial potential of ECMEO was assessed using the disc diffusion method against five yeast strains, four Gram-positive (G^+), and four Gram-negative (G^-) bacterial strains, including biofilm-forming *P. aeruginosa*. Among G^+ bacteria, *L. innocua* and *S. constellatus* exhibited the highest susceptibility, each with inhibition zones of 17.33 mm. In the G^- group, *E. aerogenes* showed the most pronounced response (12.33 mm). Notably, ECMEO also inhibited *C. glabrata* and *C. krusei*, with zones of 8.33 mm, and suppressed *P. aeruginosa* biofilm-forming strains (12.33 mm). These findings indicate that ECMEO possesses broad-spectrum antimicrobial and antibiofilm activity. However, conventional antibiotics exhibited greater overall efficacy. Importantly, *L. innocua*, *P. aeruginosa*, and *C. albicans* demonstrated notable resistance to cefoxitin, gentamicin, and fluconazole, respectively (Table 2).

Numerous studies have confirmed the broad-spectrum antimicrobial properties of cardamom essential oil (ECMEO). Snoussi et al. [9] reported strong inhibitory effects of ECMEO against various foodborne, cariogenic, and clinically significant microorganisms, including 16 yeast species and 49 bacterial strains (16 Gram-positive and 33 Gram-negative), based on inhibition zone diameters, MIC, and MBC/MFC values. Vukovic et al. [10] found ECMEO particularly effective against *Pseudomonas fluorescens* and *C. glabrata*, showing inhibition zones of 13.33 and 11.33 mm, respectively. Similarly, Gradinaru et al. [53] demonstrated that green cardamom oil inhibited *Staphylococcus aureus* ATCC 25923 at 6.25 mg/mL, though 1,8-cineole alone exhibited greater potency against MRSA isolates (MIC 1.25 mg/mL vs. Six.25 mg/mL for the oil). MIC values for green cardamom oil ranged from 0.023 to 0.046 mg/mL, with highest inhibition zones observed against Gram-positive bacteria (21.77 mm), cariogenic bacteria (19.51 mm), and fungi (39.5 mm) [9]. Additional evidence from Teneva et al. [54] reported inhibition zones of 8-10 mm and MICs ranging from 60 to > 600 ppm against *Salmonella* spp., *S. aureus* ATCC 6538P, *Proteus vulgaris*, and *E. coli* ATCC 25922. Mutlu-Ingok and Karbancioglu-Guler [55] further confirmed the efficacy of green cardamom oil against *Campylobacter jejuni* and *C. coli*.

The antimicrobial efficacy observed in our study, particularly against *P. aeruginosa*, aligns with the established mechanisms of action of ECMEO's main constituents, α-terpinyl acetate and 1,8-cineole. These compounds integrate into bacterial lipid bilayers, increasing membrane permeability and disrupting membrane potential, leading to leakage of intracellular components and subsequent cell death, which correlates with the low MIC values recorded in our assays [56].

Moreover, 1,8-cineole is known to inhibit bacterial efflux pumps, preventing the expulsion of toxic substances and enhancing bacterial susceptibility to ECMEO [57]. The synergistic action of α-terpinyl acetate and 1,8-cineole likely destabilizes bacterial membranes and impairs vital cellular functions, explaining the potent antimicrobial activity observed [58].

These synergistic or additive effects suggest a complex mode of action that may vary among bacterial strains. To elucidate these interactions and their impact on bacterial

TABLE 2: Antimicrobial activity of ECMEO assessed by disc diffusion method.

Microorganism	Inhibition zone ECMEO (mm)	Inhibition zone ATB (mm)
Gram-positive bacteria		
Bacillus cereus CCM 2010	15.67 ± 0.58^{a}	30.67 ± 0.57^{ab}
Listeria innocua CCM 4030	17.33 ± 0.58^{a}	31.33 ± 0.58^{a}
Staphylococcus epidermidis CCM 4418	16.33 ± 0.58^{a}	30.33 ± 0.59^{ab}
Streptococcus constellatus CCM 4043	17.33 ± 0.57^{a}	30.67 ± 0.58^{ab}
Gram-negative bacteria		
Enterobacter aerogenes CCM 2531	12.33 ± 0.58^{b}	30.33 ± 0.57^{ab}
Klebsiella pneumoniae CCM 2318	11.67 ± 0.59^{bc}	30.67 ± 0.58^{ab}
Pseudomonas aeruginosa CCM 1959	10.33 ± 0.56^{c}	31.33 ± 0.57^{a}
Shigella sonnei CCM 1373	12.67 ± 0.58^{b}	30.67 ± 0.58^{ab}
Yeast		
Candida albicans CCM 8136	7.67 ± 0.58^{d}	30.33 ± 0.60^{ab}
Candida glabrata CCM 8270	8.33 ± 0.57^{d}	29.67 ± 0.58^{ab}
Candida krusei CCM 8271	8.33 ± 0.59^{d}	27.67 ± 0.59^{c}
Candida parapsilosis CCM 8260	7.33 ± 0.58^{d}	29.33 ± 0.58^{bc}
Candida tropicalis CCM 8264	8.33 ± 0.59^{d}	29.33 ± 0.59^{bc}
Biofilm forming bacteria (BFB)		
Pseudomonas aeruginosa	12.33 ± 0.58^{b}	30.67 ± 0.58^{ab}

Note: Values represent mean ± standard deviation (SD) from three independent replicates. Different superscript letters within a column indicate statistically significant differences ($p \leq 0.05$).
Abbreviation: ATB, antibiotics (fluconazole for yeasts; cefoxitin for Gram-negative, gentamicin for Gram-positive bacteria).

physiology, further studies using checkerboard assays, time-kill kinetics, and transcriptomic analyses under ECMEO exposure are warranted [59].

Overall, our MIC and inhibition zone results for *P. aeruginosa* are consistent with previously published data [9, 55], reinforcing ECMEO's potential as a natural antimicrobial agent. While standard antibiotics demonstrated higher efficacy in our tests, ECMEO presents promise as a complementary or preventive antimicrobial, particularly relevant for food preservation and plant protection applications.

Based on our results, it can be concluded that ECMEO essential oil exhibits broad-spectrum antimicrobial activity against yeast, Gram-positive, and Gram-negative bacteria, including biofilm-forming *P. aeruginosa*. The observed inhibitory effects, particularly on *Listeria innocua* and *S. constellatus*, correspond with the known mechanisms of α-terpinyl acetate and 1,8-cineole, which disrupt bacterial membranes and interfere with bacterial defense systems. Despite conventional antibiotics showing greater efficacy, ECMEO holds significant promise as a natural antimicrobial agent for food preservation and plant protection applications.

3.2.2. Minimal Inhibition Concentration. ECMEO exhibited strong antimicrobial activity, showing the highest efficacy against Gram-positive bacteria, particularly *S. constellatus* and *Staphylococcus epidermidis*. It also demonstrated considerable activity against Gram-negative bacteria, with slightly higher MIC values for species such as *E. aerogenes* and *K. pneumoniae*. Among yeast species, it was most effective against *C. tropicalis*. The essential oil was also active against biofilm-forming *P. aeruginosa*, although its effectiveness was slightly lower compared with Gram-positive bacteria. These findings suggest that ECMEO is most potent against Gram-positive bacteria and biofilm-forming bacteria, while showing moderate activity against Gram-negative bacteria and yeasts. Detailed results from the minimum inhibitory concentration assay are presented in Table 3.

According to the classification by Aligiannis et al. [60], essential oils with MIC values between 0.05 and 0.5 mg/mL are considered strongly active, those between 0.6 and 1.5 mg/mL moderately active, and values above 1.5 mg/mL indicate weak activity. Based on this, ECMEO exhibited strong antimicrobial activity, with MIC_{50} values ranging from 0.332 to 0.558 mg/mL and MIC_{90} values between 0.360 and 0.580 mg/mL. These results align with findings from [10], who reported strong activity of cardamom essential oil against biofilm-forming *Pseudomonas fluorescens*. The antimicrobial action of essential oils is generally attributed to their phenolic compounds disrupting microbial cell membranes [61]. In ECMEO, these phenolics likely contribute significantly to the observed activity, while the presence of less active major components may explain the reduced efficacy against certain fungi or bacteria, as observed by Singh et al. [11]. Additionally, synergistic effects among the oil's components may enhance its antifungal properties [62].

Based on our MIC results, ECMEO demonstrates strong antimicrobial potential, particularly against Gram-positive and biofilm-forming bacteria, supporting its prospective application as a natural preservative in food systems and as an alternative to synthetic antimicrobials.

3.2.3. Antimicrobial Analysis in Vapor Phase. A study was performed to assess the antimicrobial properties of *E. cardamomum* essential oil in the vapor phase using fruit and vegetable models. The same microbial strains tested in the in vitro analysis were also used in this experiment. The outcomes for the fruit model are detailed in Table 4 and Figure 2a,b, while the results for the vegetable model are provided in Table 5 and Figure 3a,b.

Consistent with the in vitro results, ECMEO exhibited the greatest vapor-phase antimicrobial efficacy against Gram-positive (G^+) bacteria, followed by Gram-negative (G^-) strains and yeasts in the fruit-based model. At a

TABLE 3: Minimal inhibition concentration (MIC_{50} and MIC_{90}) in milligrams per milliliter of ECMEO.

Microorganism	MIC_{50} (mg/mL)	MIC_{90} (mg/mL)
Gram-positive bacteria		
Bacillus cereus CCM 2010	0.343 ± 0.015^{c}	0.364 ± 0.012^{c}
Listeria innocua CCM 4030	0.360 ± 0.013^{c}	0.373 ± 0.016^{c}
Staphylococcus epidermidis CCM 4418	0.340 ± 0.016^{c}	0.365 ± 0.017^{c}
Streptococcus constellatus CCM 4043	0.332 ± 0.011^{c}	0.360 ± 0.014^{c}
Gram-negative bacteria		
Enterobacter aerogenes CCM 2531	0.435 ± 0.016^{b}	0.458 ± 0.015^{b}
Klebsiella pneumoniae CCM 2318	0.442 ± 0.013^{b}	0.462 ± 0.010^{b}
Pseudomonas aeruginosa CCM 1959	0.443 ± 0.012^{b}	0.460 ± 0.010^{b}
Shigella sonnei CCM 1373	0.450 ± 0.025^{b}	0.472 ± 0.021^{b}
Yeast		
Candida albicans CCM 8136	0.558 ± 0.021^{a}	0.580 ± 0.020^{a}
Candida glabrata CCM 8270	0.540 ± 0.005^{a}	0.566 ± 0.009^{a}
Candida krusei CCM 8271	0.548 ± 0.017^{a}	0.569 ± 0.014^{a}
Candida parapsilosis CCM 8260	0.550 ± 0.022^{a}	0.573 ± 0.025^{a}
Candida tropicalis CCM 8264	0.524 ± 0.010^{a}	0.542 ± 0.009^{a}
Biofilm forming bacteria (BFB)		
Pseudomonas aeruginosa	0.354 ± 0.023^{c}	0.374 ± 0.024^{c}

Note: Data are the mean (± SD) of three samples. Different letters in each column refer to significant differences ($p \leq 0.05$).

concentration of 500 μg/L, ECMEO achieved its strongest inhibitory effects, reducing *Bacillus subtilis* growth on mango by 95.92%. Similar reductions were observed for *Listeria innocua* (95.65%) and *Staphylococcus epidermidis* (95.54%). Among G^- strains, *P. aeruginosa* was the most sensitive, with growth inhibition reaching 96.00% at 500 μg/L. Importantly, this inhibitory effect persisted at lower concentrations (250, 125, and 62.5 μg/L), demonstrating potent activity even at submaximal doses. In peach samples, ECMEO maintained strong antimicrobial activity against G^+ bacteria, particularly *L. innocua* (95.67% inhibition). Other G^+ strains showed similarly high inhibition rates at 500 μg/L. Among G^- bacteria, *E. aerogenes* and *S. sonnei* exhibited the highest sensitivity, with growth reductions of 86.67% and 86.59%, respectively. Across both fruit models, the highest ECMEO concentration consistently produced the most pronounced inhibitory effect on *P. aeruginosa*.

The antimicrobial effects of ECMEO in the vapor phase on vegetables were evaluated using different models. Among these, the carrot model exhibited the strongest antimicrobial activity, with significant inhibition of Gram-positive bacteria: *Bacillus cereus* (95.60%), *Listeria innocua* (95.48%), *S. constellatus* (93.49%), and *Staphylococcus epidermidis* (93.43%) at the highest concentration of 500 μg/L. The carrot model also showed the highest antibacterial effect against biofilm-forming *P. aeruginosa* (97.33% inhibition). Similarly, the zucchini model demonstrated potent antibacterial activity at 500 μg/L, with 97.33% inhibition of biofilm-forming *P. aeruginosa*, followed by 94.52% inhibition of *B. cereus* and 94.15% inhibition of *S. constellatus*. Significant inhibitory effects were also observed against other Gram-positive and Gram-negative bacteria. Overall, as presented in Figures 2a,b and 3a,b, the percentage of microbial inhibition increased with rising ECMEO concentrations across all food models.

Essential oils have been extensively studied for controlling postharvest diseases in fresh produce, especially through vapor-phase applications. For example, thyme essential oil vapor effectively controlled *Monilinia fructicola* and *Botrytis cinerea* in sweet cherries [63, 64]. Similar antimicrobial effects have been demonstrated against anthracnose in mangoes [65] and avocados [66]. Furthermore, Liu et al. [67] showed that fumigation with thyme oil inhibited both mycelial growth and conidial germination of *M. fructicola* in plums and apricots.

Additionally, vapors of eucalyptus (*Eucalyptus globulus*) and cinnamon *Cinnamomum zeylanicum*) oils have exhibited antibacterial properties, reducing spoilage in tomatoes and strawberries [68]. However, Cindi et al. [69] reported that cinnamon oil vapor was less effective than thyme oil in controlling *Monilinia laxa* in peaches.

These findings align with our observations on the vapor-phase antimicrobial activity of *E. cardamomum* essential oil, supporting its potential application for postharvest protection in fruits and vegetables.

The demonstrated antimicrobial activity of ECMEO in the vapor phase particularly within in situ fruit models highlights its potential as a non-contact preservation method. This approach is especially valuable for minimally processed and vacuum-packed foods, where direct application of antimicrobials can negatively impact texture, flavor, or other sensory attributes. The ability of ECMEO vapors to significantly reduce microbial loads without direct surface contact suggests effective integration into active packaging systems, such as essential oil-impregnated films or sachets, to maintain microbial safety during cold storage and distribution [70].

The vapor-phase antimicrobial activity observed under sous vide conditions is primarily due to the volatility and physicochemical characteristics of ECMEO's main components, α-terpinyl acetate and 1,8-cineole [71]. These volatile monoterpenes evaporate within vacuum-sealed packaging, migrate through the headspace, and partition onto food surfaces or interact with packaging materials, enabling microbial inhibition without direct contact. Their moderate vapor pressure and lipophilicity facilitate diffusion and solubility in microbial membranes, thereby enhancing antimicrobial efficacy [72].

Furthermore, interactions between essential oil volatiles and polymer packaging films can influence the concentration and availability of active compounds at the food interface, modulating the overall preservative effect [73]. This integration of ECMEO vapor-phase antimicrobial activity with packaging technology offers a promising strategy to extend shelf life and improve the safety of minimally processed foods.

Based on our results, it can be concluded that ECMEO in vapor phase exhibits strong antibacterial activity even at lower concentrations, indicating its potential for use in active packaging or sous vide packaging, where direct

TABLE 4: In situ evaluation of the antimicrobial efficacy of ECMEO vapor phase in a fruit-based model system.

Food model	Microorganisms	Concentration of ECMEO in μg/L			
		62.5	125	250	500
Mango					
Gram-positive bacteria	*Bacillus cereus*	56.47 ± 1.55^{b}	65.37 ± 0.70^{b}	78.06 ± 1.29^{b}	95.92 ± 2.18^{a}
	Listeria innocua	54.92 ± 2.55^{b}	65.25 ± 2.29^{b}	76.69 ± 2.89^{b}	95.65 ± 1.08^{a}
	Staphylococcus epidermidis	55.16 ± 1.41^{b}	65.00 ± 3.13^{b}	74.92 ± 1.52^{b}	95.54 ± 1.10^{a}
	Streptococcus constellatus	55.00 ± 3.27^{b}	65.66 ± 1.91^{b}	75.76 ± 2.81^{b}	94.19 ± 1.28^{a}
Gram-negative bacteria	*Enterobacter aerogenes*	45.74 ± 2.10^{c}	64.29 ± 2.28^{b}	75.15 ± 2.94^{b}	85.34 ± 2.27^{b}
	Klebsiella pneumoniae	45.00 ± 2.49^{c}	66.33 ± 2.34^{b}	74.81 ± 4.05^{b}	84.49 ± 2.90^{b}
	Pseudomonas aeruginosa	43.70 ± 2.06^{c}	64.50 ± 0.99^{b}	73.04 ± 1.62^{b}	86.92 ± 2.12^{b}
	Shigella sonnei	45.71 ± 2.17^{c}	66.14 ± 1.66^{b}	75.21 ± 3.24^{b}	85.16 ± 2.12^{b}
Yeast	*Candida albicans*	-6.70 ± 2.80^{d}	24.23 ± 2.27^{c}	34.77 ± 2.70^{c}	44.51 ± 1.95^{c}
	Candida glabrata	-6.30 ± 2.62^{d}	24.36 ± 2.55^{c}	35.06 ± 3.22^{c}	46.16 ± 2.13^{c}
	Candida krusei	-6.26 ± 1.99^{d}	24.34 ± 2.56^{c}	33.97 ± 2.42^{c}	44.92 ± 3.33^{c}
	Candida parapsilosis	-7.89 ± 1.39^{d}	25.77 ± 1.84^{c}	33.26 ± 1.62^{c}	42.37 ± 1.12^{c}
	Candida tropicalis	-6.26 ± 1.57^{d}	24.30 ± 2.62^{c}	34.22 ± 2.97^{c}	44.89 ± 3.41^{c}
BFB	*Pseudomonas aeruginosa*	67.00 ± 3.41^{a}	76.62 ± 2.32^{a}	87.27 ± 2.53^{a}	96.00 ± 1.64^{a}
Peach					
Gram-positive bacteria	*Bacillus cereus*	44.71 ± 1.00^{b}	64.63 ± 1.17^{b}	75.89 ± 4.19^{b}	94.93 ± 1.70^{a}
	Listeria innocua	46.44 ± 2.51^{b}	64.12 ± 1.35^{b}	77.34 ± 1.53^{b}	95.67 ± 1.92^{a}
	Staphylococcus epidermidis	44.48 ± 1.13^{b}	65.85 ± 2.14^{b}	76.70 ± 2.32^{b}	94.60 ± 3.28^{a}
	Streptococcus constellatus	44.74 ± 1.01^{b}	64.41 ± 1.22^{b}	74.96 ± 1.74^{b}	94.15 ± 3.22^{ab}
Gram-negative bacteria	*Enterobacter aerogenes*	37.12 ± 2.28^{c}	65.78 ± 1.92^{b}	74.99 ± 3.48^{b}	86.67 ± 2.75^{bc}
	Klebsiella pneumoniae	34.00 ± 2.16^{c}	65.21 ± 2.38^{b}	74.95 ± 3.74^{b}	84.96 ± 1.65^{c}
	Pseudomonas aeruginosa	33.60 ± 2.00^{c}	65.36 ± 2.64^{b}	75.89 ± 2.30^{b}	83.35 ± 2.06^{c}
	Shigella sonnei	34.31 ± 2.80^{c}	65.58 ± 2.95^{b}	73.12 ± 0.58^{b}	86.59 ± 3.27^{c}
Yeast	*Candida albicans*	-6.77 ± 2.21^{d}	12.82 ± 0.64^{c}	23.97 ± 2.63^{c}	45.77 ± 2.05^{d}
	Candida glabrata	-8.03 ± 0.81^{d}	14.37 ± 2.18^{c}	26.04 ± 1.28^{c}	46.11 ± 2.68^{d}
	Candida krusei	-8.48 ± 1.57^{d}	16.02 ± 2.29^{c}	23.96 ± 0.53^{c}	46.11 ± 2.72^{d}
	Candida parapsilosis	-8.06 ± 2.03^{d}	13.70 ± 1.11^{c}	24.37 ± 3.18^{c}	46.48 ± 2.67^{d}
	Candida tropicalis	-8.06 ± 2.29^{d}	12.74 ± 2.66^{c}	24.29 ± 1.60^{c}	44.34 ± 1.53^{d}
BFB	*Pseudomonas aeruginosa*	65.19 ± 1.53^{a}	75.15 ± 3.25^{a}	86.29 ± 3.20^{a}	97.37 ± 2.63^{a}

Note: Values represent mean ± standard deviation (SD) from three independent replicates. Different superscript letters within each column (for each fruit model: mango and peach) indicate statistically significant differences ($p \leq 0.05$).

contact with food is undesirable. The mechanism of action relies on the volatility of its main components (α-terpinyl acetate, 1,8-cineole), which disperse within a vacuum-sealed environment, penetrate food surfaces and microbial membranes, effectively disrupting microbial life processes without direct contact. This study presents a novel perspective on the synergistic effect of ECMEO vapor combined with thermal processing (sous vide), enabling efficient control of microbial contamination with minimal impact on the sensory properties of food. The results also support the potential implementation of ECMEO in innovative preservation systems that extend shelf life and enhance the safety of minimally processed products.

3.3. Antibiofilm Activity of ECMEO Against Pseudomonas Aeruginosa. The antibiofilm potential of ECMEO was assessed using crystal violet staining and MALDI-TOF MS Biotyper analysis. As shown in Table 3, the MBICs ($MBIC_{50}$ and $MBIC_{90}$) against *P. aeruginosa* were 0.354 ± 0.023 mg/mL and 0.374 ± 0.024 mg/mL, respectively. To investigate molecular changes induced by ECMEO within biofilms, protein spectra were acquired using MALDI-TOF MS Biotyper. Comparative spectral profiles were generated from untreated planktonic cultures and non-exposed biofilm controls under identical conditions, allowing differentiation of chemical responses triggered by the essential oil.

The results revealed distinct alterations in mass spectral profiles of biofilms, reflected by variations in spectral peaks. Notably, the ECMEO-treated group showed more pronounced peaks on Days 7 and 12 compared with planktonic cells. Early time points (Days 3 and 5) are presented in Figure 4a,b, and the mid-phase Day 9 is shown in Figure 4d. Significant changes on glass and plastic surfaces were also observed on Days 9, 12, and 14 (Figures 4c, 4e,

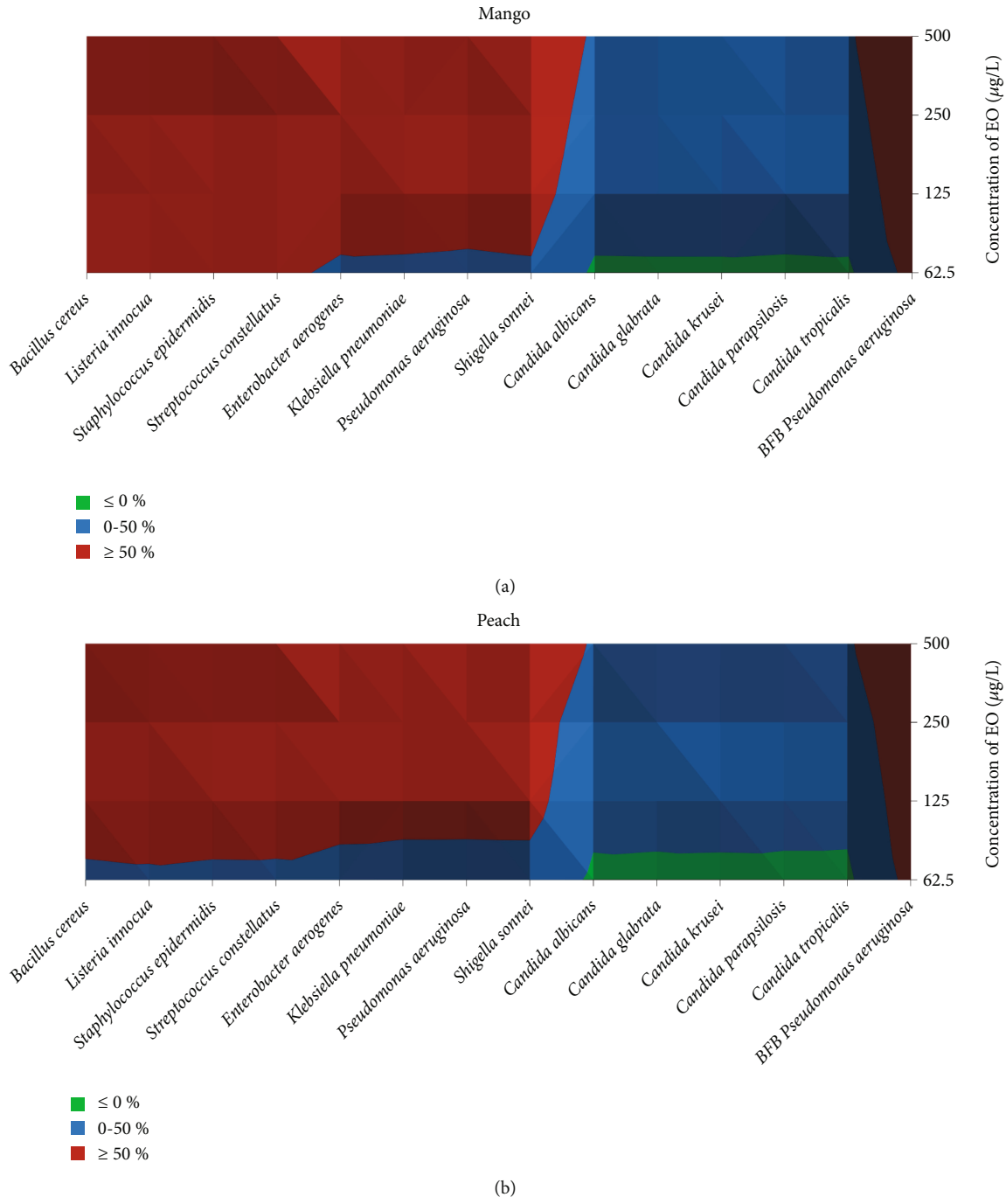


Figure 2: Isometric visualization of the data presented in Table 4 showing in situ vapor-phase antimicrobial activity of ECMEO in fruit models: (a) mango and (b) peach. Color coding indicates the level of microbial growth inhibition: green (≤ 0%, promicrobial effect), blue (0%–50%, moderate inhibition), and red (≥ 50%, strong inhibition).

and 4f). These findings suggest that ECMEO disrupts biofilm stability, particularly on glass and plastic, which may contribute to biofilm suppression.

Cardamom essential oil has demonstrated notable antibacterial and antibiofilm activity, particularly against Gram-negative pathogens. Abdullah et al. [52] reported that the oil inhibited the growth of *P. aeruginosa* and *E. coli* at a concentration of 1%, with measurable antibiofilm effects observed even at lower concentrations (0.015%–0.125 % *v*/*v*), especially in suppressing *E. coli* biofilm development. The oil was also effective against *Salmonella* Typhimurium, underscoring its potential as a natural antimicrobial agent. Previous studies have highlighted essential oils' ability to disrupt biofilm formation or exert direct antibacterial effects [74–77]. In a comprehensive screening, 61 commercial essential oils were tested for their effects on biofilm production in clinical *P. aeruginosa* strains, demonstrating variable but promising antibiofilm properties. Specific constituents

TABLE 5: In situ evaluation of the antimicrobial efficacy of ECMEO vapor phase in a vegetable-based model system.

Food model	Microorganisms	Concentration of ECMEO in μg/L			
		62.5	125	250	500
Carrot					
Gram-positive bacteria	*Bacillus cereus*	34.37 ± 1.47^{c}	56.30 ± 0.73^{b}	75.15 ± 1.57^{b}	95.60 ± 2.81^{a}
	Listeria innocua	35.10 ± 0.61^{c}	55.77 ± 1.01^{b}	73.12 ± 2.98^{bc}	95.48 ± 2.68^{a}
	Staphylococcus epidermidis	34.03 ± 3.34^{cd}	54.92 ± 2.70^{b}	75.22 ± 1.59^{b}	93.43 ± 2.17^{ab}
	Streptococcus constellatus	33.80 ± 0.98^{cd}	53.33 ± 0.94^{b}	76.29 ± 3.69^{b}	93.49 ± 2.36^{ab}
Gram-negative bacteria	*Enterobacter aerogenes*	27.03 ± 2.12^{de}	44.59 ± 2.07^{c}	65.69 ± 2.86^{c}	84.11 ± 4.80^{c}
	Klebsiella pneumoniae	26.39 ± 4.18^{e}	43.92 ± 2.78^{c}	66.13 ± 3.19^{c}	85.94 ± 2.78^{bc}
	Pseudomonas aeruginosa	23.40 ± 3.89^{e}	43.61 ± 0.38^{c}	65.22 ± 3.07^{c}	83.86 ± 0.61^{c}
	Shigella sonnei	25.50 ± 2.51^{e}	42.63 ± 0.70^{c}	64.89 ± 3.29^{c}	84.51 ± 3.17^{c}
Yeast	*Candida albicans*	55.44 ± 1.68^{b}	45.78 ± 2.06^{c}	28.92 ± 5.51^{d}	-7.32 ± 2.22^{d}
	Candida glabrata	55.60 ± 1.22^{b}	45.29 ± 2.26^{c}	23.07 ± 1.86^{d}	-8.63 ± 1.61^{d}
	Candida krusei	55.44 ± 1.60^{b}	44.74 ± 1.01^{c}	23.97 ± 2.44^{d}	-4.96 ± 2.33^{d}
	Candida parapsilosis	55.67 ± 1.92^{b}	44.57 ± 1.21^{c}	23.30 ± 1.20^{d}	-8.84 ± 0.99^{d}
	Candida tropicalis	55.34 ± 1.71^{b}	45.04 ± 1.28^{c}	25.03 ± 1.60^{d}	-6.70 ± 1.78^{d}
BFB	*Pseudomonas aeruginosa*	66.33 ± 3.19^{a}	75.88 ± 2.81^{a}	86.78 ± 1.92^{a}	97.33 ± 2.40^{a}
Zucchini					
Gram-positive bacteria	*Bacillus cereus*	35.00 ± 2.74^{c}	53.85 ± 1.23^{b}	74.26 ± 0.51^{b}	94.52 ± 1.07^{ab}
	Listeria innocua	33.67 ± 1.74^{c}	54.96 ± 2.65^{b}	73.15 ± 0.61^{b}	93.56 ± 2.06^{ab}
	Staphylococcus epidermidis	33.78 ± 0.73^{c}	52.41 ± 1.12^{b}	77.03 ± 3.13^{b}	93.82 ± 1.48^{ab}
	Streptococcus constellatus	35.03 ± 3.29^{c}	54.41 ± 1.48^{b}	73.37 ± 2.71^{b}	94.15 ± 2.67^{ab}
Gram-negative bacteria	*Enterobacter aerogenes*	24.54 ± 4.07^{d}	43.48 ± 1.61^{c}	64.16 ± 0.78^{c}	84.93 ± 1.28^{c}
	Klebsiella pneumoniae	23.11 ± 1.25^{d}	45.47 ± 1.51^{c}	63.19 ± 1.59^{c}	85.86 ± 3.22^{c}
	Pseudomonas aeruginosa	24.03 ± 3.35^{d}	46.07 ± 1.64^{c}	66.07 ± 2.78^{c}	88.95 ± 1.13^{bc}
	Shigella sonnei	25.37 ± 3.29^{d}	44.54 ± 1.07^{c}	65.66 ± 1.91^{c}	84.26 ± 3.07^{c}
Yeast	*Candida albicans*	55.41 ± 2.21^{b}	44.66 ± 1.06^{c}	22.56 ± 0.95^{d}	-7.08 ± 1.58^{d}
	Candida glabrata	53.63 ± 0.96^{b}	44.56 ± 2.94^{c}	24.03 ± 1.01^{d}	-7.07 ± 1.57^{d}
	Candida krusei	56.33 ± 1.81^{b}	44.56 ± 1.02^{c}	25.70 ± 1.86^{d}	-6.92 ± 2.09^{d}
	Candida parapsilosis	56.28 ± 1.86^{b}	44.86 ± 1.41^{c}	23.34 ± 1.53^{d}	-7.40 ± 1.55^{d}
	Candida tropicalis	55.00 ± 2.35^{b}	45.07 ± 1.21^{c}	27.12 ± 2.10^{d}	-6.78 ± 1.11^{d}
BFB	*Pseudomonas aeruginosa*	67.32 ± 1.71^{a}	77.03 ± 2.61^{a}	86.99 ± 2.18^{a}	97.34 ± 2.41^{a}

Note: Values represent mean ± standard deviation (SD) from three independent replicates. Different superscript letters within each column (for each vegetable model: carrot and zucchini) indicate statistically significant differences ($p \leq 0.05$).

such as eucalyptol have been reported to inhibit biofilms in *Staphylococcus aureus* and *P. aeruginosa* [78], while linalool modulates biofilm development in both species as well [79].

A dendrogram constructed using MSP distances visually represented the similarity of biofilm structures. In the later stages of biofilm formation (Days 7, 12, and 14), biofilms treated with ECMEO (PAECP and PAECEO) exhibited shorter MSP distances, clearly distinguishing them from both control and planktonic biofilms, as shown in the dendrogram (Figure 5). The substantial differences in MSP distances indicate that the protein profiles of control biofilms were dissimilar to those of the ECMEO-treated biofilms. Mass spectra analysis revealed that early-stage biofilms (planktonic cells on Days 3, 5, and 9) had smaller MSP distances, more similar to control groups. However, as the experiment progressed, MSP distances in the treated groups increased, peaking on Days 3, 5, and 12. These findings suggest that ECMEO treatment significantly affects *P. aeruginosa* biofilm homeostasis.

These alterations in biofilm structure and protein profiles may be attributed to ECMEO's interference with quorum sensing systems such as las, rhl, and pqs, which are critical regulators of biofilm formation and maturation in *P. aeruginosa*. By disrupting these signaling pathways, ECMEO likely inhibits the synthesis of exopolysaccharides and other matrix components essential for biofilm stability [80, 81]. Monoterpenes like α-terpinyl acetate and 1,8-cineole, major constituents of ECMEO have been shown to modulate these systems, leading to biofilm matrix destabilization and increased bacterial susceptibility. Such interference results in the compromised biofilm architecture observed in our MALDI-TOF MS analyses.

The study demonstrated that ECMEO significantly inhibits the biofilm formation of *P. aeruginosa*. MALDI-

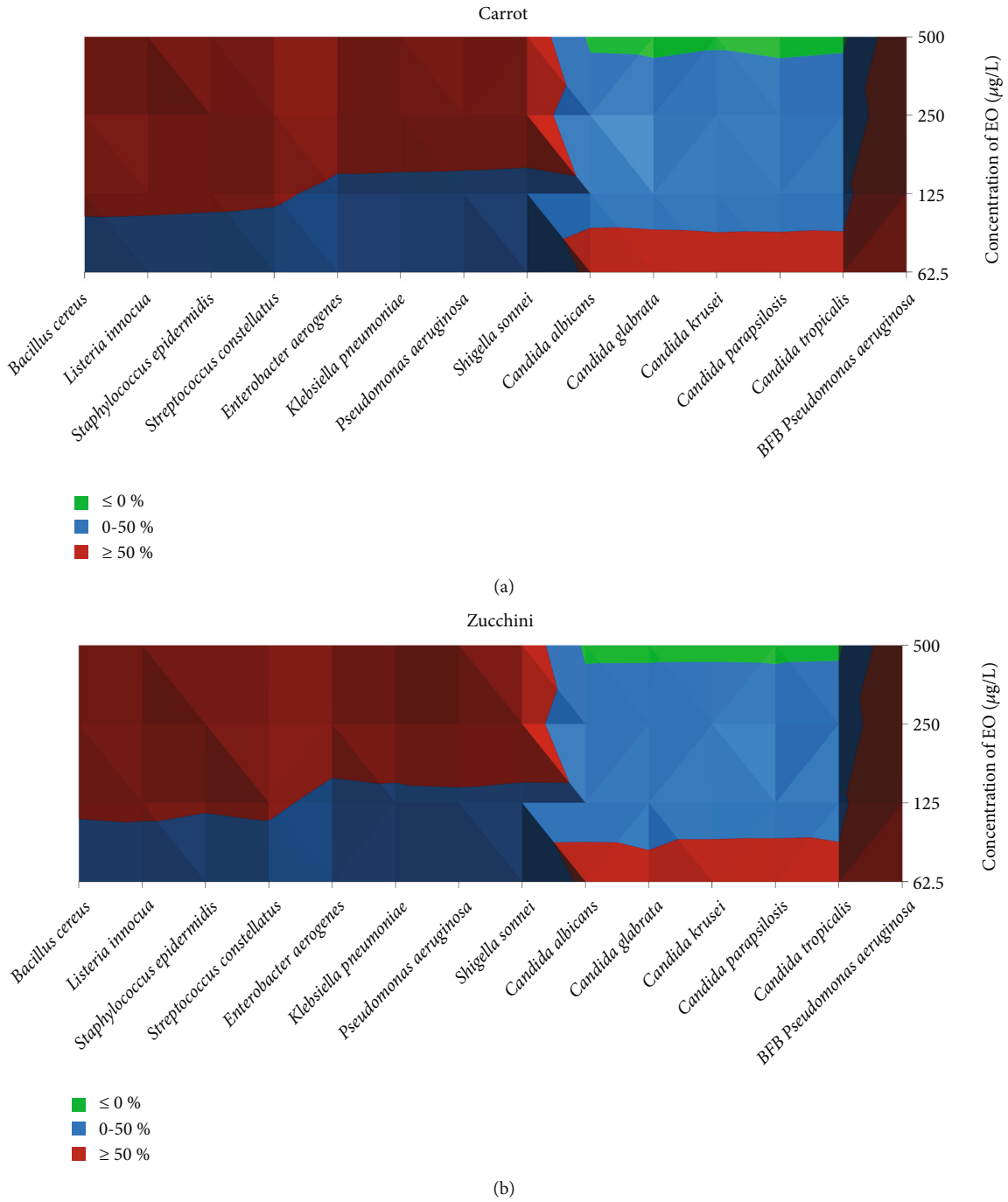
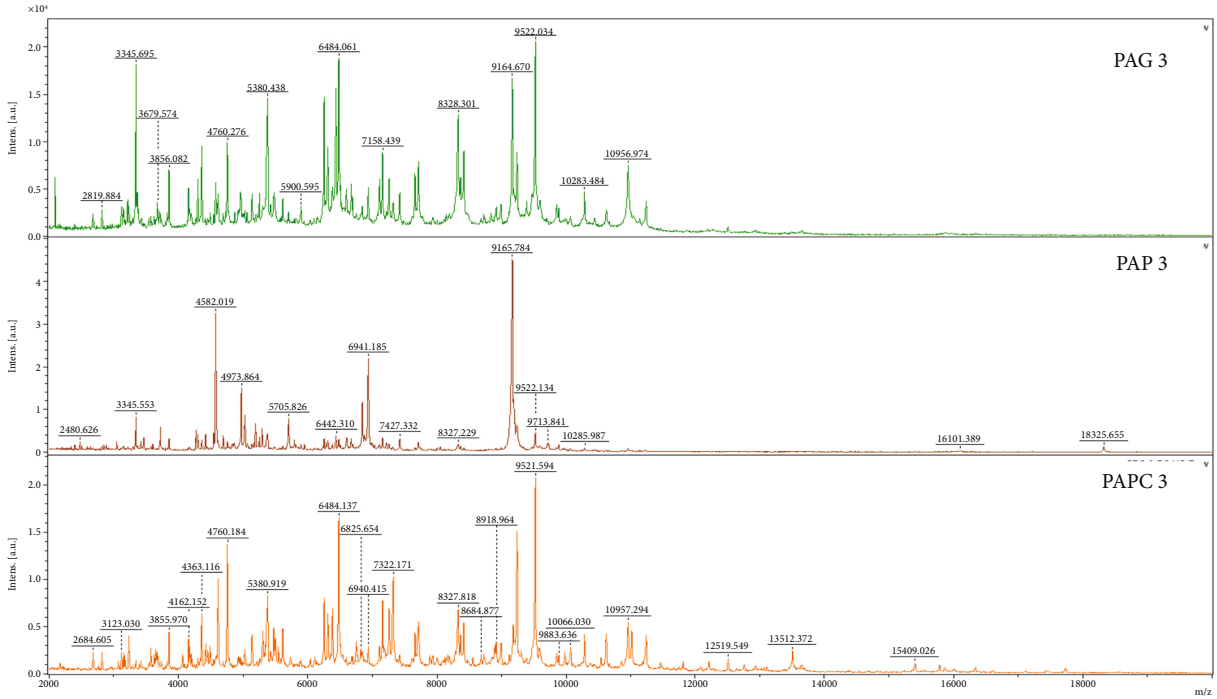


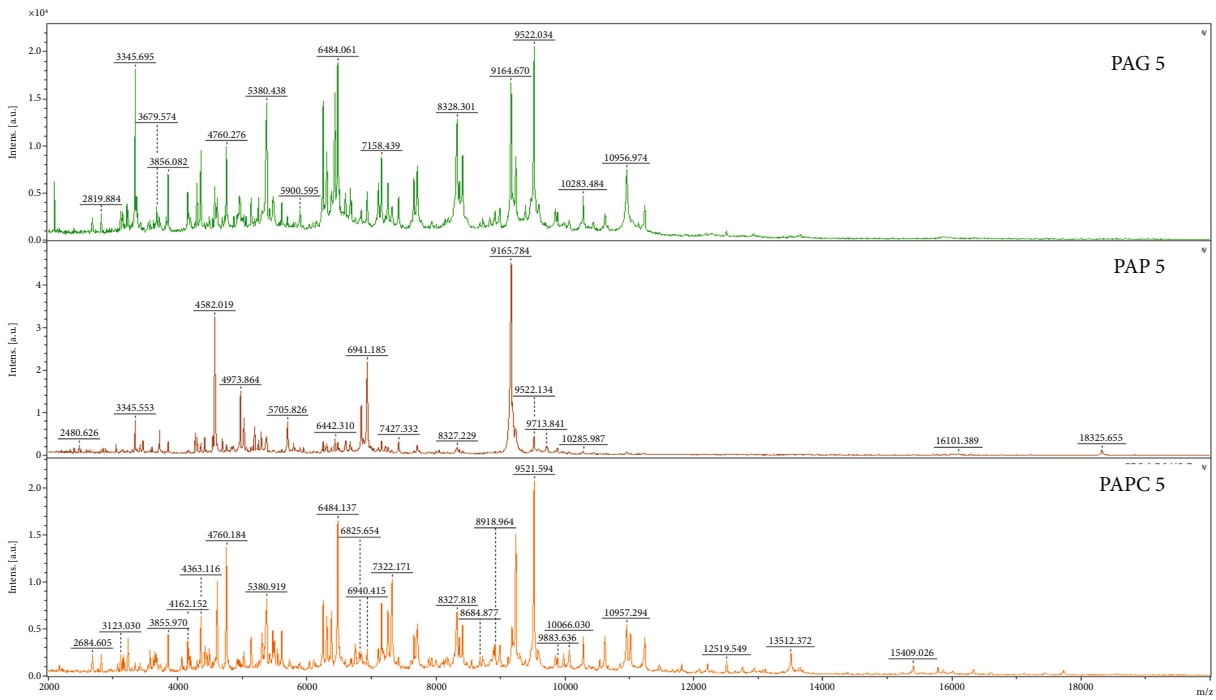
Figure 3: Isometric visualization of the data presented in Table 4 showing in situ vapor-phase antimicrobial activity of ECMEO in vegetable models: (a) carrot and (b) zucchini. Color coding indicates the level of microbial growth inhibition: green (≤ 0%, promicrobial effect), blue (0%–50%, moderate inhibition), and red (≥ 50%, strong inhibition).

TOF MS analysis revealed pronounced changes in the spectral profiles of biofilms treated with ECMEO, especially on glass and plastic substrates during long-term observation. MSP-based dendrograms showed marked differences between control biofilms and those treated with ECMEO, with the effect intensifying over time, indicating a prolonged impact of the oil on the biofilm. This work provides a novel insight into the antibiofilm activity of ECMEO. The results highlight the practical potential of ECMEO as a natural agent for controlling pathogen biofilms in food industry and medicine.

3.4. Kinetic Growth of Pseudomonas Aeruginosa. The influence of temperature and ECMEO on *P. aeruginosa* growth dynamics was evaluated using a personal bioreactor system (RTS-1, Biosan, Riga, Latvia), by monitoring changes in OD at 850 nm (OD_{850}) and calculating GRs. Essential oil was introduced at 6.62 h, when both groups had an OD of

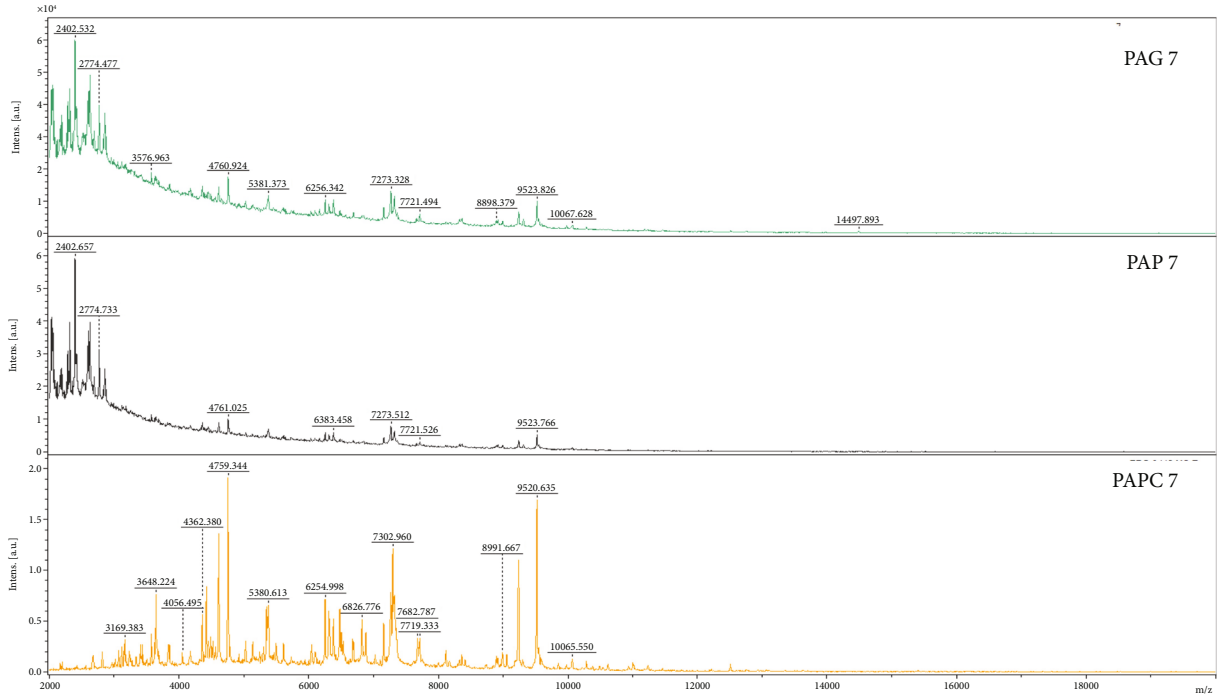


(a)

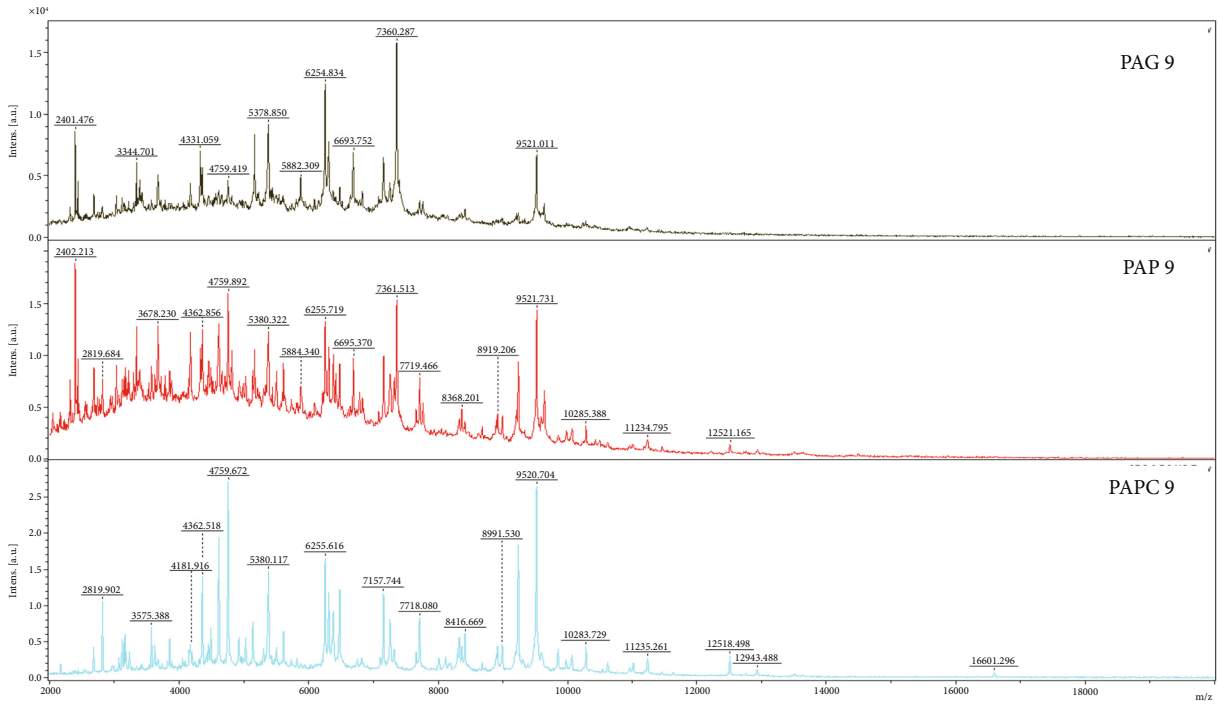


(b)

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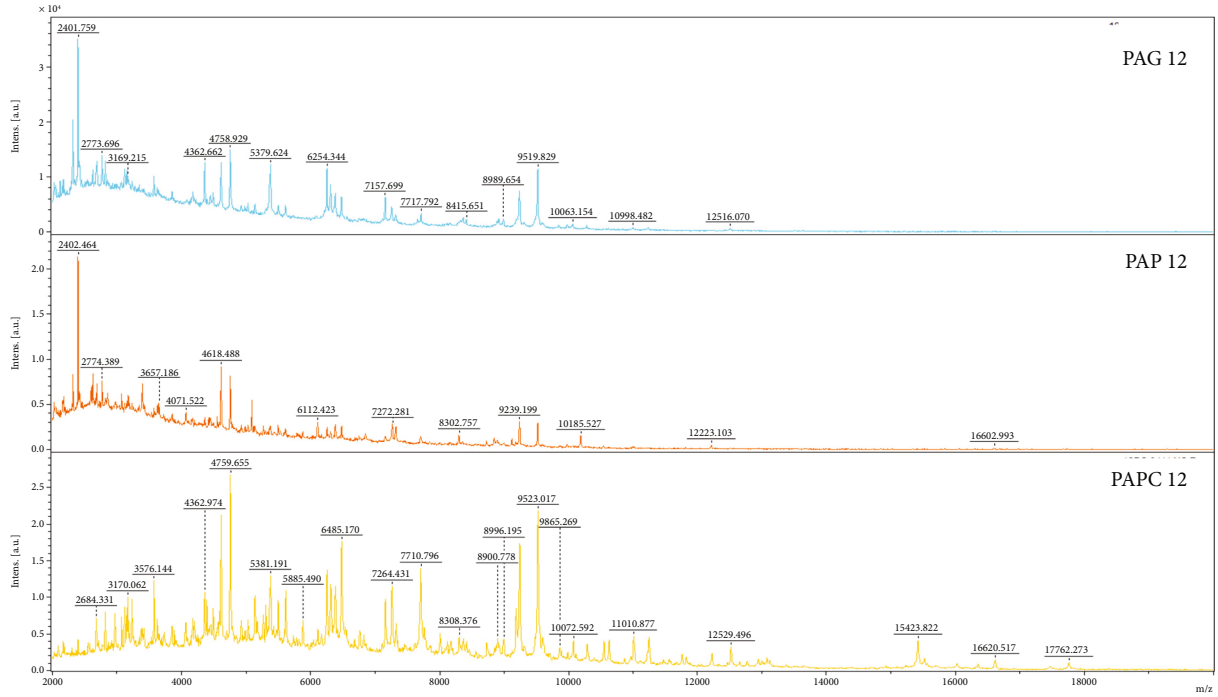


(c)

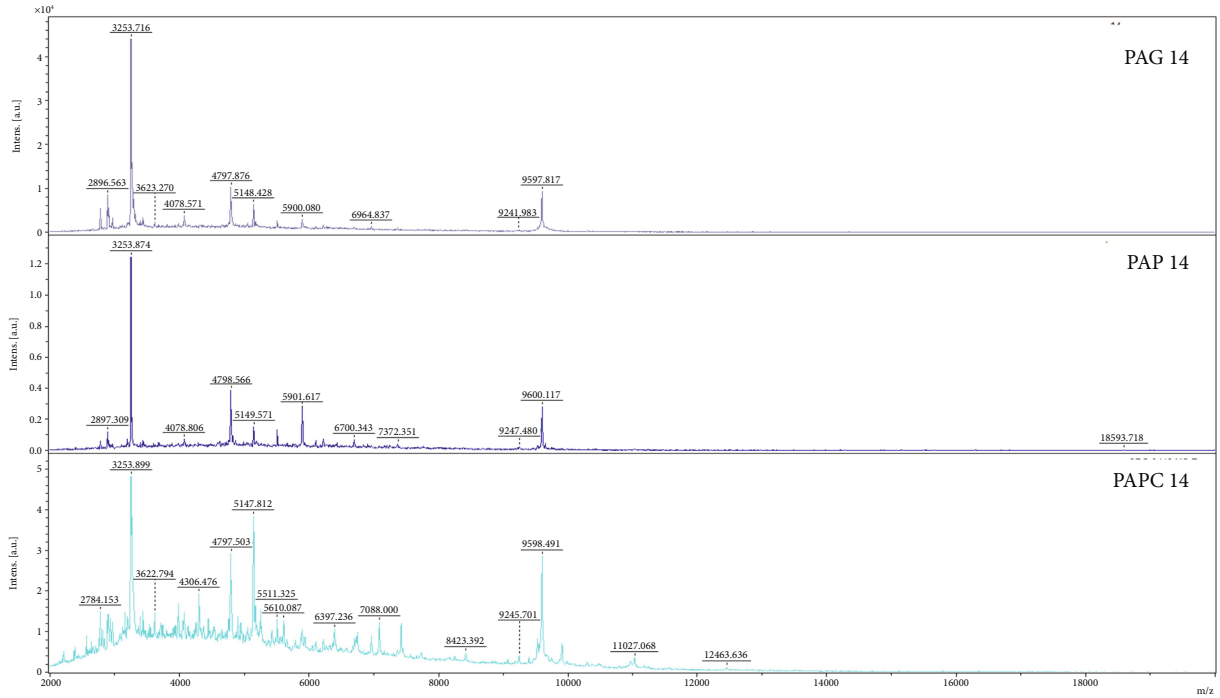


(d)

Figure 4: Continued.



(e)



(f)

Figure 4: Representative MALDI-TOF mass spectra of *Pseudomonas aeruginosa* collected at different incubation time points: (a) Day 3, (b) Day 5, (c) Day 7, (d) Day 9, (e) Day 12, and (f) Day 14. Abbreviations: PA, *P. aeruginosa*; G, glass surface; P, plastic surface; PC, planktonic cells.

about 1 (Figure 6a,b). At 37°C, both the EO-treated and control groups followed similar growth patterns, with the treated group reaching an OD of 1.15 and the control group 1.04. After the EO addition, the treated group exhibited a significant increase in OD (1.52) and GR (2.17), while the control group grew more slowly, reaching an OD of 0.92 and a negative growth rate of −0.58. When the temperature was increased to 50°C, the growth of the EO-treated group declined further, with an OD of 0.77 and GR of −2.17, whereas the control group maintained a higher OD (1.03)

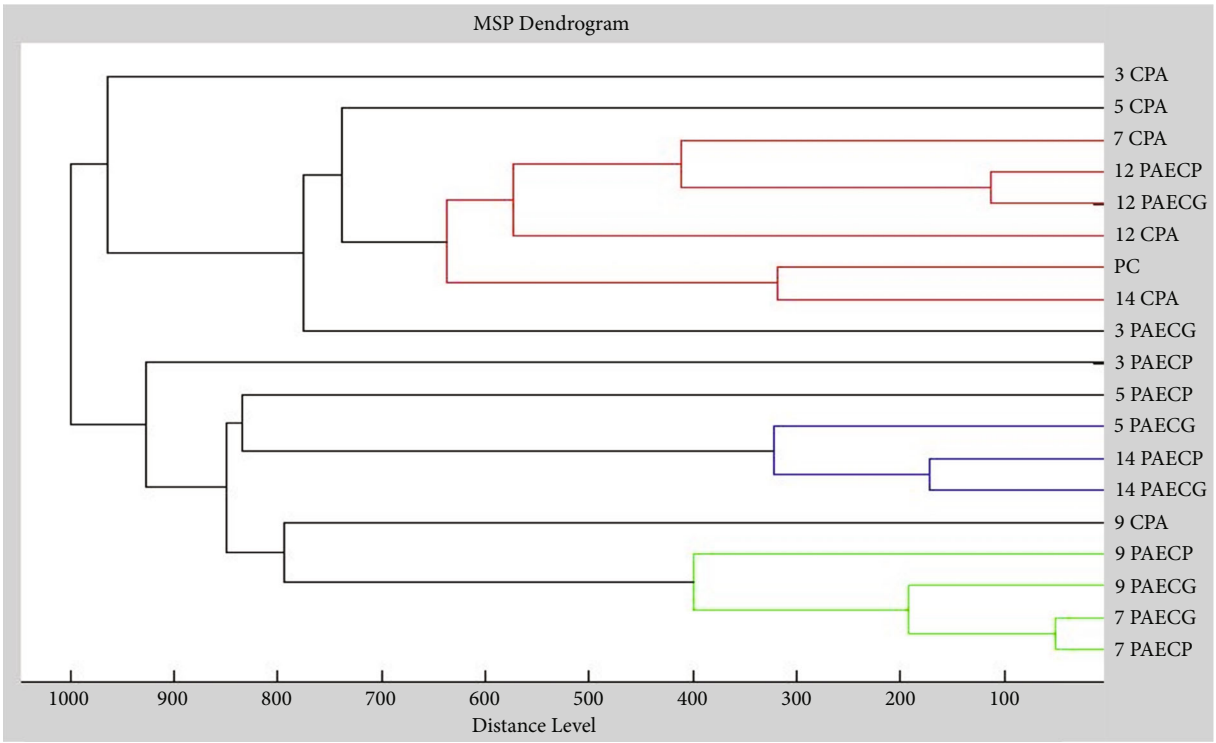


Figure 5: Dendrogram of *P. aeruginosa* based on MALDI-TOF MS peak profiles (MSPs) of planktonic cells and biofilms under control and ECMEO-treated conditions. Abbreviations: PA, *P. aeruginosa*; EC ,- *Elettaria cardamomum* essential oil; G, glass surface; P, plastic surface; PC, planktonic cells.

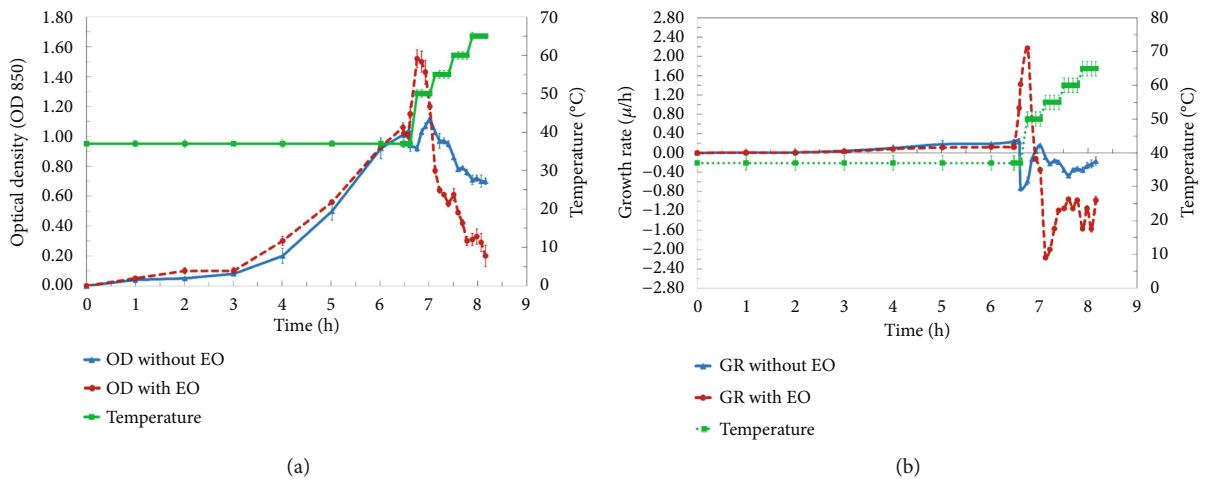


Figure 6: Comparison of *Pseudomonas aeruginosa* growth over time at different temperatures in ECMEO-treated (with EO) and untreated samples (without EO): (a) optical density at 850 nm and (b) growth rate (μ/h).

and a slower decline in GR (−0.09). At 60°C and 65°C, bacterial growth was suppressed in both groups, but the EO-treated group showed more pronounced inhibition, with OD dropping to 0.2 and GR falling to −1.57 at 65°C, compared with the control group, which had an OD of 0.7 and GR of −0.17. These findings suggest that the synergistic effect of elevated temperature and cardamom essential oil markedly suppresses the growth of *P. aeruginosa*. The findings suggest that EO possesses strong antimicrobial properties, especially when used in conjunction with heat treatment, showing potential for controlling bacterial growth under thermal stress.

P. aeruginosa is a Gram-negative bacterium primarily affecting immunocompromised patients, causing bloodstream, respiratory, and urinary tract infections. Due to its high antibiotic resistance, it is classified as a critical pathogen by the WHO [82]. Biofilm formation further enhances its resistance, creating a need for alternative decontamination

methods. While sterilization is effective at extreme temperatures (> 150°C), lower temperatures (50°C–65°C) are more relevant in clinical and food industry settings. Controlled heat exposure has been shown to reduce biofilms; however, data on the effects of moderate temperatures remain limited. Our study demonstrated that the combination of ECMEO and elevated temperature significantly inhibits the growth of *P. aeruginosa*. Kinetic analysis using OD (OD_{850}) revealed clear deviations from standard growth patterns, including delayed lag phase and reduced maximum growth rate, particularly at temperatures above 55°C with ECMEO treatment. These results suggest that ECMEO exerts bacteriostatic effects by impairing membrane integrity and disrupting quorum sensing pathways critical for adaptation under stress. The combined mild heat stress and essential oil components create a hostile microenvironment inhibiting proliferation and survival of this biofilm-forming pathogen [83, 84]. The observed synergistic effect between ECMEO and mild heat treatment likely involves multiple cellular targets. Mild heat can increase membrane fluidity, thereby enhancing permeability and making bacterial membranes more susceptible to disruption by ECMEO components such as α-terpinyl acetate and 1,8-cineole. Additionally, heat stress may cause partial protein denaturation and impair bacterial repair mechanisms, further sensitizing cells to the antimicrobial action of ECMEO. These combined effects create a hostile microenvironment that inhibits bacterial growth and biofilm formation more effectively than either treatment alone [85, 86]. Thermal conditions in sous vide processing affect the volatility and stability of ECMEO constituents, particularly α-terpinyl acetate and 1,8-cineole. Moderate heat can increase volatilization rates, enhancing the concentration of active compounds in the packaging headspace and thus their antimicrobial efficacy. However, excessive heat may lead to degradation or loss of key components, potentially diminishing activity. Kinetic and thermodynamic studies suggest that these effects follow Arrhenius-type behavior, necessitating careful balance during sous vide treatments to maximize antimicrobial potential while preserving EO integrity [85, 87].

The substantial resistance of *P. aeruginosa* biofilms to antibiotics underscores the need for alternative approaches. According to Araby and El-Tablawy [88], 92% of *P. aeruginosa* isolates were resistant to azithromycin, cefepime, and tobramycin [58]. Azithromycin, a macrolide antibiotic, is often ineffective due to intrinsic resistance. Cefepime, a fourth-generation cephalosporin, and tobramycin, an aminoglycoside, are standard treatments, particularly in cystic fibrosis patients. Some isolates showed complete resistance to all tested antibiotics, highlighting treatment challenges [89].

Rosemary essential oil has demonstrated strong antibacterial effects, with inhibition zones between 13 and 30 mm, including isolates showing > 20 mm susceptibility [88]. These findings support our observations that essential oils, particularly combined with mild heat, effectively inhibit *P. aeruginosa* growth. Similarly, ECMEO exhibited pronounced antimicrobial activity with a reported MIC of 0.5 mg/mL, accelerating bacterial killing relative to untreated controls. This evidence highlights cardamom essential oil's promising potential in heat-assisted preservation strategies [83].

Although ECMEO exhibited strong antimicrobial activity against *P. aeruginosa*, the potential for selection of resistant subpopulations or induction of adaptive stress responses cannot be excluded. Bacteria exposed to essential oils may activate diverse defense mechanisms, including efflux pumps, biofilm remodeling, and stress response regulons, which can contribute to tolerance or resistance development. To better understand these adaptive processes, genomic and proteomic analyses of ECMEO-treated *P. aeruginosa* are recommended, as they can reveal changes in gene and protein expression related to membrane integrity, efflux systems, and oxidative stress pathways [90–92]. Future studies should focus on these molecular adaptations to assess the long-term efficacy and safety of ECMEO as an antimicrobial agent. Based on our results, we can conclude that ECMEO significantly inhibits the growth of *P. aeruginosa*, especially when combined with mild temperature elevation. At 37°C, bacterial growth was only slightly affected by the essential oil; however, at temperatures of 50°C and above, a pronounced suppression of growth was observed in the ECMEO-treated group compared with the control. The strongest inhibitory effect was recorded at 65°C, where the lowest OD values and negative growth rates were measured. These findings suggest a synergistic effect between heat and ECMEO, enhancing antimicrobial efficacy against *P. aeruginosa*. This effect supports the potential use of ECMEO as an adjunct preservative in thermally processed foods, such as sous vide technology, which is further investigated in this study.

3.5. Carrot Microbiology Assay. Across the various sampling intervals, microbial counts in samples stored at 0°C remained relatively constant, with no statistically significant differences observed. This suggests that the bacteriostatic potential of the essential oil may have been masked by the low-temperature storage conditions, limiting its observable antimicrobial effect. However, this hypothesis cannot be fully excluded, as lower temperatures might increase bacterial resistance [93]. During storage, mesophilic bacterial populations remained steady, with initial counts ranging from 2×10^6 to 5×10^6 CFU/g, and a slight increase to 1.5×10^6 CFU/g after 14 days. Lactic acid bacteria counts were also stable, ranging from 1×10^5 to 2.4×10^5 CFU/g [94]. Yeast populations (2.1×10^6 to 2.2×10^6 CFU/g) and Enterobacteriaceae (ranging from 1.9×10^5 to 9.7×10^5 CFU/g) showed similar trends. Psychrotrophic bacteria increased from 2×10^4 to 8×10^5 CFU/g over 14 days. No significant differences in microbial growth were observed between control samples and those treated with lemon, rosemary, or melissa essential oils under these conditions [94]. Variability in antibacterial activity among essential oils may be due to differences in their phenolic compound composition [93]. Essential oils are believed to inhibit microorganisms via multiple mechanisms, including disruption of cell membranes leading to depletion of cellular energy, and inhibition of enzymatic processes [94].

Microbiological evaluation of raw carrots inoculated with *P. aeruginosa* revealed initial total viable count (TVC) levels of 1.96 log CFU/g and an absence of coliforms on Day 0. Over a 7-day period, TVC values in untreated and vacuum-packed samples ranged from 1.32 to 2.25 log CFU/g. In contrast, samples treated with ECMEO alone exhibited consistently lower microbial loads (1.21–1.62 log CFU/g), while the ECMEO + *P. aeruginosa* group maintained even lower TVC levels (1.05–1.93 log CFU/g). These results underscore the antimicrobial potential of ECMEO, particularly when applied in combination with vacuum packaging. Notably, in the presence of a known biofilm-forming pathogen (*P. aeruginosa*), ECMEO treatment resulted in a measurable reduction of microbial counts. This suggests that ECMEO not only acts as an immediate antimicrobial agent but also confers protective effects throughout storage. The observed reduction supports its integration into minimally processed food systems as a natural strategy to mitigate spoilage and pathogen growth (Figure 7).

On Day 1, coliform bacteria (CB) were undetectable in both the vacuum-packed control and ECMEO-treated samples (Figure 8), suggesting that initial processing and packaging alone provided adequate microbial suppression. However, by Day 7, slight CB growth was observed in the control group, with counts ranging from 1.03 to 1.18 log CFU/g depending on the thermal treatment conditions. In samples inoculated with *P. aeruginosa*, CB counts were significantly higher, ranging from 1.44 to 2.00 log CFU/g on Day 1 and increasing further to 1.55–2.16 log CFU/g by Day 7. These findings indicate that *P. aeruginosa* not only persists but may facilitate coliform proliferation under favorable conditions. Importantly, the combination of ECMEO and *P. aeruginosa* inoculation resulted in substantially lower CB counts throughout the storage period. On Day 1, values ranged from 1.31 to 1.78 log CFU/g and further declined to 1.13–1.36 log CFU/g on Day 7. This highlights the effectiveness of ECMEO in mitigating the microbial risk associated with coliforms, even under bacterial challenge and mild thermal conditions. Taken together, these results support the hypothesis that ECMEO acts synergistically with thermal treatment to suppress microbial growth and enhance the microbial safety of vacuum-packed carrots. The pronounced reduction in CB emphasizes its potential role in reducing spoilage and improving the hygienic quality of minimally processed vegetables during cold storage.

In the sous vide carrot model, the presence of *P. aeruginosa* was confirmed only in the inoculated groups, while non-inoculated controls remained free of contamination throughout storage (Figure 9). On Day 1, bacterial counts in the inoculated group without ECMEO ranged from 1.36 to 2.14 log CFU/g, decreasing slightly by Day 7 (1.06–1.49 log CFU/g), likely due to refrigeration. In contrast, the group treated with both ECMEO and *P. aeruginosa* exhibited significantly lower counts at both time points (1.08–1.29 log CFU/g on Day 1), with the greatest reductions observed after longer exposures (15–20 min) at 50°C–55°C. These results indicate a synergistic antimicrobial effect of ECMEO and mild thermal treatment. The oil also demonstrated inhibitory effects when applied alone. Overall, ECMEO enhanced microbiological safety by limiting *P. aeruginosa* growth, a pathogen known for its resistance and biofilm formation, confirming its potential as a natural preservative for sous vide vegetables.

Based on the results from microbiological tests on raw carrot slices, we can state that during storage at 4°C, there were no significant changes in microbial counts, likely due to growth suppression by the low temperature, which may have masked the antibacterial effect of ECMEO. Initial values of TVCs, lactic acid bacteria, yeasts, and Enterobacteriaceae remained stable throughout the monitored days. The application of ECMEO together with vacuum packaging resulted in a significant reduction of microbial contamination, particularly in samples inoculated with *P. aeruginosa*, confirming the antibacterial potential of ECMEO. The results also indicated a synergistic effect between ECMEO and mild heat treatment (50°C–65°C), which significantly limited the growth of *P. aeruginosa* in the sous vide model. Thus, the oil can be stated as suitable natural preservative for minimally processed vegetable products.

3.6. Identification of Microbiota Isolated From Fresh and Sous Vide Treated Carrot. The microbial profiling of sous vide-treated carrots, assessed via MALDI-TOF mass spectrometry, revealed a broad taxonomic diversity among the isolates. In total, 127 bacterial isolates were successfully identified with score values ≥ 2.0, indicating high-confidence species-level identification. These isolates were distributed across 16 species, representing eight genera and nine families. The most frequently encountered taxa belonged to the Enterobacteriaceae and Bacillaceae families, which are commonly associated with plant surfaces and soil environments. On Day 1 of the experiment, *Bacillus licheniformis* and *Enterococcus faecalis* were the most prevalent species, each accounting for 15% of the isolates across experimental groups. Other notable species included *Bacillus cereus*, *Bacillus safensis*, *Bacillus simplex*, *Pseudomonas putida*, and *Ralstonia pickettii*, each constituting approximately 8% of the total microbial community. These results are summarized in Figure 10. The detection of these diverse bacterial communities highlights the complexity of the carrot microbiome and underscores the need for effective antimicrobial strategies such as the use of ECMEO to mitigate the presence of both spoilage organisms and potential opportunistic pathogens in minimally processed vegetables.

The taxonomic composition of microbial communities identified from sous vide carrot samples on Day 1 is illustrated in Figure 11. A total of 269 isolates were characterized using MALDI-TOF MS, with identification scores up to 2. These isolates were classified into 23 species, 14 genera, and 16 families. The dominant microbial families included Bacillaceae and Pseudomonadaceae. Among the species, *P. aeruginosa* was most prevalent (18%), followed by *Enterococcus faecalis* (9%) and *Pantoea agglomerans* (6%). These findings highlight the early microbial colonization patterns of minimally processed carrots and emphasize the importance of early intervention strategies to control spoilage and pathogenic organisms in ready-to-eat vegetables.

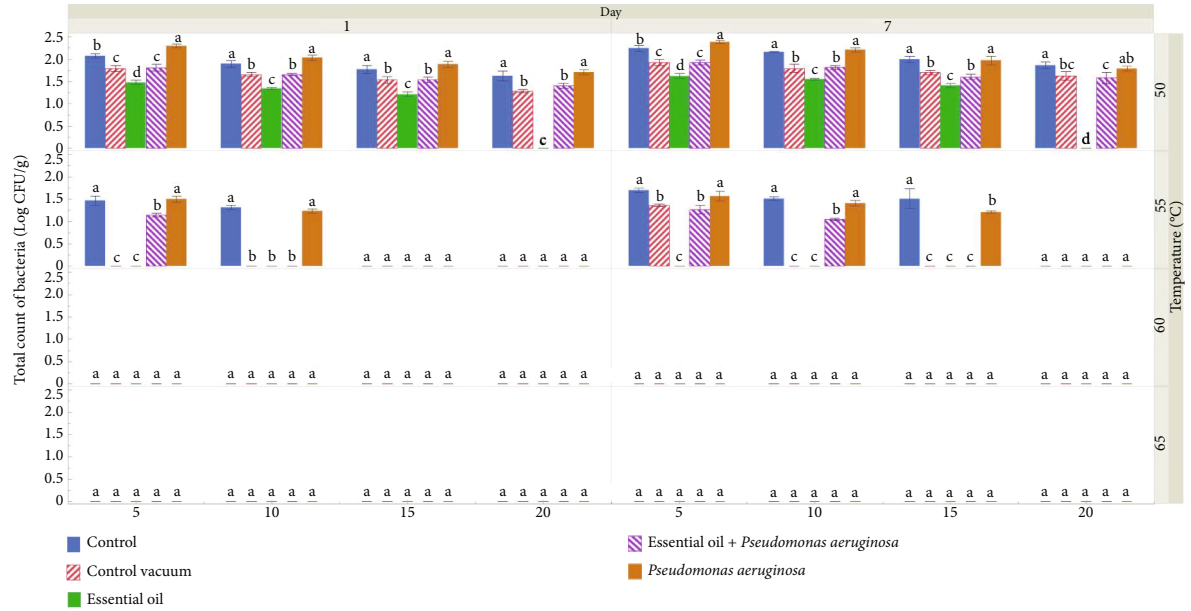


FIGURE 7: Total bacterial counts (log CFU/g) in samples treated at temperatures ranging from 50°C to 65°C for 5 to 20 min, assessed on Day 1 and Day 7 of storage. Data represent the mean values ± standard deviation (SD) from three replicates. Different letters within each treatment group indicate statistically significant differences ($p \leq 0.05$). Treatment groups: control: fresh sample packed in polyethylene bags and stored at 4°C; control vacuum: fresh sample vacuum-packed in polyethylene bags and stored at 4°C; essential oil: fresh sample treated with ECMEO (10 mg/mL), vacuum-packed, and stored at 4°C; *Pseudomonas aeruginosa*: fresh sample inoculated with *P. aeruginosa*, vacuum-packed, and stored at 4°C; essential oil + *P. aeruginosa*: fresh sample treated with ECMEO (10 mg/mL) and inoculated with *P. aeruginosa*, vacuum-packed, and stored at 4°C.

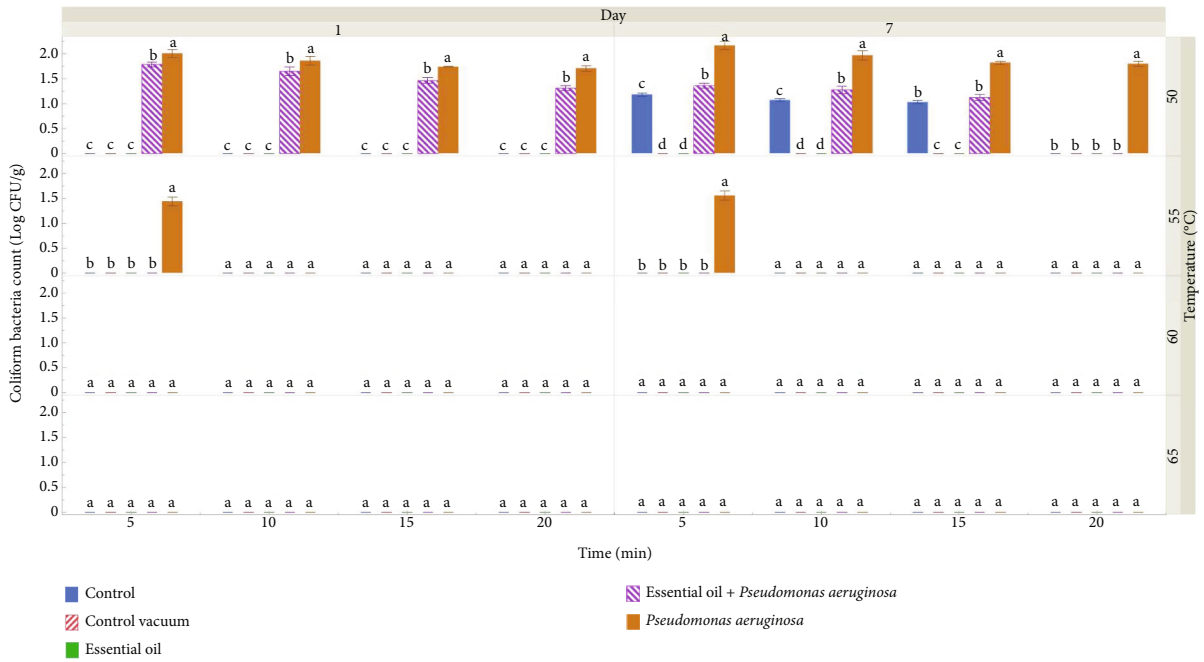


FIGURE 8: The coliform bacteria count (log CFU/g) in the carrot samples subjected to various treatments (temperatures between 50°C and 65°C for 5 to 20 min) were evaluated on Day 1 and Day 7 of storage. The data represent the mean values ± standard deviation (SD) of three replicates. Significant differences ($p \leq 0.05$) within each treatment group are denoted by different letters. The following treatments were included: control: fresh carrot samples packed in polyethylene bags and stored at 4°C; control vacuum: fresh carrot samples vacuum-packed in polyethylene bags and stored at 4°C; essential oil: samples treated with 10 mg/mL ECMEO, vacuum-packed, and stored at 4°C; *Pseudomonas aeruginosa*: samples inoculated with *P. aeruginosa*, vacuum-packed, and stored at 4°C; essential oil + *Pseudomonas aeruginosa*: samples treated with 10 mg/mL ECMEO and inoculated with *P. aeruginosa*, vacuum-packed, and stored at 4°C.

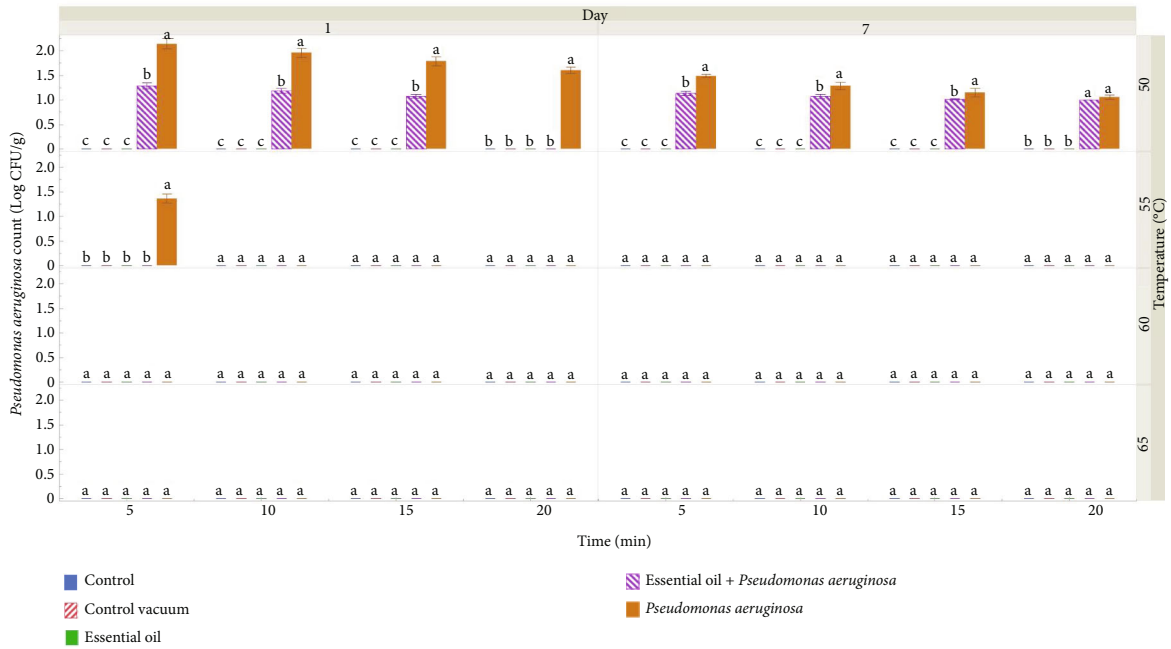


FIGURE 9: The *P. aeruginosa* count (log CFU/g) in the carrot samples subjected to various treatments (temperatures between 50°C and 65°C for 5 to 20 min) were evaluated on Day 1 and Day 7 of storage. The data represent the mean values ± standard deviation (SD) of three replicates. Significant differences ($p \leq 0.05$) within each treatment group are denoted by different letters. The following treatments were included: control: fresh carrot samples packed in polyethylene bags and stored at 4°C; control vacuum: fresh carrot samples vacuum-packed in polyethylene bags and stored at 4°C; essential oil: samples treated with 10 mg/mL ECMEO, vacuum-packed, and stored at 4°C; *Pseudomonas aeruginosa*: samples inoculated with *P. aeruginosa*, vacuum-packed, and stored at 4°C; essential oil + *Pseudomonas aeruginosa*: samples treated with 10 mg/mL ECMEO and inoculated with *P. aeruginosa*, vacuum-packed, and stored at 4°C.

Microbial species, genera, and families were identified from the seventh day of carrot sous vide preservation, as illustrated in Figure 12. A total of 270 isolates were detected by mass spectrometry, with each category reaching a maximum numerical value of 2. These isolates were categorized into 13 taxa, 13 families, and 24 species. The Pseudomonadaceae and Enterococcaceae families were the most prevalent. The most commonly isolated species from the sous vide carrot model during the 7-day study were *P. aeruginosa* (25%), *Enterococcus faecalis* (8%), *Bacillus idriensis*, *Pantoea agglomerans*, *Pseudomonas brassicacearum*, *Pseudomonas kilonensis*, and *Pseudomonas putida* (all 5%).

The microbial analysis of fresh and sous vide-treated carrots revealed notable shifts in microbial populations throughout the study. Fresh produce, especially vegetables, can harbor bacterial pathogens like *Listeria monocytogenes*, *Salmonella*, and *E. coli*, which may contaminate crops during cultivation, harvesting, or distribution [95]. Foodborne illnesses linked to contaminated fruits and vegetables, with *Salmonella* and *E. coli* O157:H7 being common culprits, have increased in recent years [96]. Bıyıklı et al. [97] noted the presence of thermophilic and mesophilic bacteria in sous vide vegetables, which was confirmed in our study, with species like *P. aeruginosa* and *E. faecalis*, known to thrive in moderate temperatures [98]. Our findings showed that, at Day 0, the microbial community in fresh carrots was primarily composed of bacteria from the *Enterobacteriaceae* and *Bacillaceae* families, with *B. licheniformis* and *E. faecalis* being the most prevalent. These results align with Valero et al. [99], who also observed high levels of *Bacillus* species in raw carrots. On Day 1 of sous vide treatment, *P. aeruginosa* became more prominent, which was similarly observed by Zavadlav et al. [100] in heat-treated vegetables. *P. aeruginosa* is known for its strong survival mechanisms, making it a common inhabitant of preserved foods and a potential contributor to spoilage under certain conditions. By Day 7, there was a noticeable shift, with *P. aeruginosa* continuing to dominate, followed by other *Pseudomonas* species. This supports the findings of Balali et al. [101], who reported the persistence of *Pseudomonas* species in stored foods and their role in organic matter degradation. The detection of *Bacillus* species, such as *Bacillus idriensis*, indicates their ability to withstand sous vide processing temperatures and survive in a dormant state until conditions for growth are favorable [102]. While the sous vide method effectively reduces pathogenic bacteria (with *E. coli* present in 7.1% of whole vegetables and 11.4% of freshly cut samples), it is crucial for food safety to start with high-quality, minimally contaminated produce and incorporate essential oils for enhanced antimicrobial protection [103].

Overall, this study underscores the dynamic shifts in microbial populations that occur during the sous vide process. While initial microbial counts are dominated by mesophilic bacteria such as *Bacillus* and *Enterococcus*, the treatment allows for the survival of thermophilic species like *P. aeruginosa*. The persistence of these species through the seven-day storage period indicates their resilience to thermal processing and highlights the importance of controlling



Figure 10: A Krona chart was used to visualize the taxonomic distribution of microbial isolates recovered from sous vide carrot samples on Day 0, revealing the diversity of species, genera, and families present at the initial stage of the experiment.

microbial populations in sous vide-treated foods for food safety and quality.

Based on the results, it can be stated that MALDI-TOF MS analysis identified a wide taxonomic diversity of microorganisms on both fresh and sous vide processed carrot slices. At the beginning of the experiment, *Bacillus licheniformis* and *Enterococcus faecalis* dominated, but during storage and thermal treatment, the presence of *P. aeruginosa* became increasingly pronounced, being most abundant on Day 7 of the observation. The presence of *P. aeruginosa* and other species indicates their ability to survive under sous vide conditions, confirming their resistance to thermal processing and capability to persist in a dormant state. The findings confirm dynamic changes in the microbial community during storage, highlighting the need for effective antimicrobial strategies, such as the use of plant essential oils (e.g., ECMEO), to ensure the quality and safety of minimally processed vegetable products.

3.7. Insecticidal Activity of ECMEO. Table 6 summarizes the insecticidal activity of ECMEO against *M. dorsalis*. The strongest effects were recorded at ECMEO concentrations of 12.5%, 25%, 50%, and 100%, with significant mortality observed. In contrast, the lower concentrations of 6.25% and 3.125% did not produce notable repellent effects. Specifically, a 12.5% concentration led to a measurable reduction in *M. dorsalis* presence, while 25% caused mortality in 60% to 80% of the insect population.

The results from the insecticidal bioassay of ECMEO against *M. dorsalis* reveal significant insecticidal effects at higher concentrations, with the highest efficacy observed at 12.5%, 25%, 50%, and 100%. These findings align with



Figure 11: A Krona chart was used to visualize the taxonomic distribution of microbial isolates recovered from sous vide carrot samples on Day 1, revealing the diversity of species, genera, and families present at the initial stage of the experiment.

previous research demonstrating the insecticidal potential of essential oils from various plant species. For instance, Wanna et al. [19] reported notable insect mortality following exposure to plant-based essential oils, emphasizing their potential role as natural pest control agents. The strong insecticidal performance of ECMEO is likely attributed to its high content of bioactive monoterpenes, primarily α-terpinyl acetate and 1,8-cineole. These compounds have been shown to interfere with the nervous system of insects, leading to neurotoxic and fumigant effects that impair physiological processes essential for survival [104, 105]. Wang et al. [105] further highlight the importance of both dosage and exposure duration in determining the fumigant efficacy of essential oils. In our study, ECMEO concentrations of 25% and 50% achieved up to 80% mortality in *M. dorsalis*, which is comparable to the 96% mortality reported by Wanna et al. [19] at a concentration of 300 μL/L after 168 h. Conversely, lower concentrations of ECMEO (3.125% and 6.25%) did not exhibit significant repellent or insecticidal activity. These results suggest that while ECMEO possesses potent insecticidal properties, its effectiveness is concentration-dependent and potentially species-specific. Given its efficacy and natural origin, ECMEO represents a promising alternative to synthetic insecticides for integrated pest management, particularly in postharvest protection strategies. The dual efficacy of ECMEO, evidenced by its significant antimicrobial and insecticidal effects, highlights its versatility as a natural bioactive agent. Its effectiveness against *P. aeruginosa*, a resilient spoilage and pathogenic microorganism, and *M. dorsalis*, a pest of stored legumes, supports its integration into holistic food preservation strategies. This dual-functionality not only reduces reliance on synthetic chemicals but also aligns with current trends in sustainable and clean-label food technologies. Future applications may include its use in grain storage, active packaging, and integrated pest management systems, where both microbial and insect threats must be addressed concurrently [83, 104].

Based on the results, it can be concluded that ECMEO exhibits significant insecticidal activity against *M. dorsalis*,



Figure 12: A Krona chart was used to visualize the taxonomic distribution of microbial isolates recovered from sous vide carrot samples on Day 7, revealing the diversity of species, genera, and families present at the initial stage of the experiment.

TABLE 6: Insecticidal potential of ECMEO against *M. dorsalis*.

Concentration (%)	Number of living individuals	Number of dead individuals	Insecticidal activity (%)
100	0	100	100.00 ± 0.00
50	10	90	90.00 ± 0.00
25	20	80	80.00 ± 0.00
12.5	40	60	60.00 ± 0.00
6.25	70	30	30.00 ± 0.00
3.125	90	10	10.00 ± 0.00
Control group	100	0	0.00 ± 0.00

with efficacy notably increasing at concentrations of 12.5% and above. Lower concentrations showed no significant effect. This insecticidal activity is mainly attributed to the content of α-terpinyl acetate and 1,8-cineole, which interfere with the insect's nervous system. Therefore, ECMEO represents a promising natural alternative for integrated pest management, and its combination with antibacterial properties supports its application in sustainable food technologies.

4. Conclusion

This study demonstrated that ECMEO exhibits potent antimicrobial and antibiofilm activity, especially against *P. aeruginosa*, a pathogen known for its resistance and biofilm formation. GC–MS analysis identified α-terpinyl acetate and 1,8-cineole as dominant bioactive compounds responsible for this effect. ECMEO significantly reduced microbial loads in sous vide-treated carrots, achieving up to 97.33% inhibition of biofilm-forming *P. aeruginosa* at 500 μg/L in vapor phase. The synergy between ECMEO and mild heat (50°C–65°C) further enhanced antimicrobial efficacy, delaying bacterial growth and reducing OD and growth rates by

up to 60% compared with controls. Additionally, ECMEO exhibited strong insecticidal activity against *M. dorsalis*, causing up to 80% mortality at 25% concentration. These findings highlight ECMEO's potential as a natural preservative and bioactive agent for minimally processed foods, offering a dual function in microbial and pest control. The demonstrated synergy with thermal processing in sous vide applications represents a novel approach for enhancing food safety and shelf life. Future research should focus on molecular mechanisms underlying ECMEO's antimicrobial action, resistance development in target pathogens, and optimization of delivery systems such as EO-impregnated packaging films to maximize efficacy and stability.

Data Availability Statement

All data supporting the findings of this study are contained within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Miroslava Kačániová: conceptualization, data curation, writing – original draft, methodology, investigation, formal analysis, supervision, project administration, funding acquisition. **Zhaojun Ban**: data curation, writing – original draft, formal analysis. **Li Li**: data curation, writing – original draft, formal analysis. **Alessandro Bianchi**: visualization, software, data curation, writing – original draft. **Margarita Terentjeva**: review and editing, data curation, formal analysis. **Anis Ben Hsouna**: data curation, writing – original draft, formal analysis. **Rania Ben Saad**: data curation, writing – original draft, formal analysis. **Joel Horacio Elizondo-Luevano**: review and editing, data curation, formal analysis. **Stefania Garzoli**: review and editing, supervision, methodology.

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