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Sous Vide Vacuum-Packed *Ipomoea batatas*: Exploring the Antimicrobial and Antibiofilm Effects of *Elettaria cardamomum* Essential Oil Against *Salmonella enterica*

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ABSTRACT

Cardamom, a highly valued spice, is predominantly cultivated in Guatemala and India. The plant is notable for its potent essential oil and unique, pleasant aroma. One of the many potential uses of cardamom essential oil lies within the culinary industry. This study aimed to assess the antimicrobial properties of the essential oil of cardamom (ECEO) in relation to its chemical profile. To achieve this, its antimicrobial activity was evaluated in vitro using the disk diffusion method and determination of minimum inhibitory concentration (MIC). The most abundant components were α-terpinyl acetate and 1,8-cineole. The findings revealed that gram-positive bacteria were the most sensitive to ECEO, as demonstrated by both methodologies. In situ testing showed that ECEO exhibited strong antimicrobial effects against *Bacillus subtilis* and *Pseudomonas aeruginosa*, which were identified in fruit and vegetable models. Additionally, ECEO was shown to inhibit biofilm formation, as observed in *Salmonella enterica*. Microbiological assessments further highlighted the exceptional efficacy of ECEO against *S. enterica*. These findings, in conjunction with prior research, indicate that ECEO possesses highly potent antimicrobial properties, making it a promising candidate for use as a preservative in sweet potatoes.

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1 | Introduction

Aromatic and medicinal plants contain a diverse array of bioactive compounds that are utilised to combat the proliferation of drug-resistant pathogenic bacteria and fungi responsible for severe and potentially fatal diseases [1, 2]. Due to their antibacterial properties, spices and aromatic plants play a significant role in the preparation of Mediterranean cuisine. They demonstrate efficacy against a wide range of harmful gram-positive and gram-negative bacteria, including genera such as *Listeria*, *Vibrio*, *Staphylococcus*, *Pseudomonas*, *Salmonella*, *Bacillus* and *Micrococcus*, many of which exhibit multidrug resistance [3–5]. The growing prevalence of multidrug resistance in both community- and hospital-acquired infections is largely attributed to the excessive use of antibiotics by consumers for treating infections.

Aromatic and medicinal plants, known for their antibacterial properties, have long been a subject of scientific interest [1]. The emergence of multidrug-resistant bacteria has spurred efforts to discover new antibiotics [6]. Essential oils (EOs), extracted from aromatic plants and utilised for their therapeutic benefits, have been considered as viable alternatives to traditional antibiotics [7]. Several studies have revealed that various *Salmonella* species can form biofilms through different chemical signalling mechanisms [8, 9]. These biofilms, formed by *Salmonella*, are highly resistant to environmental stressors such as low water activity or disinfectants, providing the microorganism with a better chance of surviving outside the host and enabling it to infect new hosts [10]. Consequently, biofilm formation enhances the microorganism's resistance to bactericidal treatments [11, 12]. Research has also shown that *Salmonella* can form biofilms on non-living surfaces like plastic, rubber, cement, glass and stainless steel [13, 14]. The large surface area of fruits and vegetables makes them especially susceptible to colonisation by *Salmonella* [15, 16]. These food products are produced in large volumes both indoors and outdoors and are distributed in raw or minimally processed forms. Numerous studies indicate that *Salmonella* contamination can occur at multiple stages throughout the production chain, including cutting, washing, packaging and transportation, as well as during cultivation and harvesting in the field [17].

The CEEO is abundant in monoterpenoid compounds, such as α-terpinyl acetate, 1,8-cineole (28.94%–34.91%), α-terpineol (12.47%–14.89%), sabinene (11.17%–13.50%), nerol (3.69%–6.10%), β-linalool (1.43%–2.97%) and α-pinene (1.15%–2.42%). These key components have been shown to possess various pharmacological and therapeutic properties, including antiviral, antibacterial, antidiabetic, anti-inflammatory, antioxidant and gastroprotective effects [18, 19].

Elettaria cardamomum, a plant from the ginger family (Zingiberaceae), is primarily valued for its seeds. This perennial shrub can grow up to eight feet in height and possesses thick, fleshy lateral roots [20]. Cardamom is commonly used as a spice in cooking, with the darker seeds being extracted from the seed pods and ground into a powder. The main cultivation regions for cardamom include Southern India, Tanzania, Guatemala and Sri Lanka, with Morocco also producing cardamom for culinary purposes. Known as the "Queen of Spices," cardamom has been used in India for centuries [21]. It is a popular ingredient in a variety of foods such as coffee, cakes, bread and curries, and is also used to flavour sweet dishes and beverages [21]. In addition to its culinary applications, both the seeds and EO are used to flavour various foods, including meats, frozen desserts, sweets, baked goods, puddings, condiments, relishes and sauces. Cardamom is also an essential spice in Moroccan tagine, commonly used in meat dishes. In traditional Chinese and Indian medicine, cardamom is utilised to alleviate intestinal gas and support digestion [21]. Thanks to its calming properties, it is also found in massage oils, lotions, soaps, detergents and perfumes [21]. Cardamom EO, along with other natural antimicrobial agents and antibacterial inorganic compounds, is a potent tool against harmful microorganisms and is used in food packaging and edible coatings [22, 23].

This study was motivated by the beneficial biological properties of the EO of *E. cardamomum* (ECEO) and its metabolites. It offers a detailed analysis of the oil's chemical composition and biological activity. The research highlights the advantages of *E. cardamomum* and explores the bioactive effects of ECEO, particularly its antimicrobial (both in vitro and in situ) and antibiofilm properties. Additionally, the study aims to provide valuable insights into the potential use of ECEO as a natural preservative, focusing on its application in vacuum-packed preserved foods, such as sous vide sweet potatoes (*Ipomoea batatas*) contaminated with *Salmonella enterica* subsp. *enterica* strain.

2 | Material and Methods

2.1 | Essential Oil

The essential oil (ECEO) of *E. cardamomum* used in this study was sourced from Hanus s.r.o. (Nitra, Slovakia). The fresh seeds of *E. cardamomum* were steam-distilled to obtain the EO, with the plants being grown in India. For analysis, the ECEO was stored in the dark at 4°C.

2.2 | Chemical Composition of the ECEO

GC/MS analysis of the essential oil was performed using a 6890N GC system coupled with a 5975B quadrupole mass detector (Agilent Technologies, Santa Clara, CA, USA) and equipped with an HP5-MS capillary fused silica column (30 m length, 250 μm inner diameter and 0.25 μm film thickness of (5%-phen yl)-methylpolysiloxane). One microlitre of 10% hexane solution of the ECEO sample was injected in split mode (split ratio 40.8:1; split/splitless injector temperature 290°C) and eluted with He 5.0 carrier gas (flow rate of 1 mL/min) under the following temperature programme: 50°C–75°C (increasing rate 3°C/min), held 4 min at 75°C, 75°C–120°C (increasing rate 5°C/min), held 2 min at 120°C and 120°C–290°C (increasing rate 5°C/min). The temperatures of MS ion source and MS quadrupole were set at 230°C and 150°C, respectively. For a mass scan range from 35 to 550 amu and a solvent delay time of 3.20 min, mass spectra were acquired at 70 eV for a total run time of 57.33 min. For the purpose of recording the retention times of the n-alkane mixture (C_7–C_{35}) and further experimental calculations of Kovats retention indices of volatiles, slight changes were made to the chromatographic conditions. Recording of mass spectra started

2.30 min after injection and continued until the GC oven maintained a final temperature of 290°C for 8.00 min (total run time 65.33 min).

The identification of volatile compounds in the essential oil sample was conducted by comparing the recorded mass spectra with those in the Wiley7 and NIST library and by matching the obtained MS data and experimentally determined retention indices with literature data [24]. Compounds with concentrations > 0.1% were semiquantified based on their corresponding GC peak areas using the MSD ChemStation integrator (Agilent Technologies, Santa Clara, CA, USA).

2.3 | Antimicrobial Assays

2.3.1 | Bacteria and Yeasts Tested

The study evaluated the antibacterial properties of the EO using a range of bacterial strains, including gram-negative (G^-) organisms: *S. enterica* subsp. *enterica* CCM 3807, *Pseudomonas aeruginosa* CCM 1959, *Yersinia enterocolitica* CCM 7204T and *Escherichia coli* CCM 3953. Additionally, yeast strains such as *Candida albicans* CCM 8186, *Candida glabrata* CCM 8270, *Candida krusei* CCM 8271, *Candida parapsilosis* CCM 8260 and *Candida tropicalis* CCM 8223 were included in the evaluation. For gram-positive (G^+) bacteria, the strains tested were *Staphylococcus aureus* susp. *aureus* CCM 4423, *Listeria monocytogenes* CCM 4699, *Bacillus subtilis* subsp. *Spizizenii* CCM 1999 and *Streptococcus constellatus* CCM 4043. All bacterial and yeast samples were sourced from the Czech Collection of Microorganisms (CCM) in Brno, Czech Republic. The biofilm-forming *S. enterica* was isolated from milk production environments for the study of its antibacterial and antibiofilm activities. Before testing, the bacterial and yeast cultures were incubated for 24 h at 37°C or 25°C, respectively, using Mueller Hinton Broth (MHB, Oxoid, Basingstoke, UK) and Sabouraud Dextrose Broth (SDB, Oxoid, Basingstoke, UK). On the day of testing, the inoculum's optical density (OD) was adjusted to match the 0.5 McFarland standard [25].

2.3.2 | Disc Diffusion Method

To evaluate microbial sensitivity to the EO, the disc diffusion method was used, applying the same microbial strains as in the earlier experiment. The bacterial inocula were cultivated on Mueller Hinton Agar (MHA, Oxoid, Basingstoke, UK), while the yeast inocula were grown on Sabouraud Dextrose Agar (SDA, Oxoid, Basingstoke, UK). Small discs (6 mm in diameter) were soaked with a solution of ECEO. The bacterial and yeast cultures were incubated for 24 h at 37°C and 25°C, respectively. The antibacterial effectiveness of the EO was assessed by measuring the zone of inhibition, categorising mild inhibition as 0–5 mm, moderate inhibition as 5–10 mm and strong inhibition as more than 10 mm. Controls were set up with clear blank discs and standard antibiotics or antifungal agents (gentamicin for G^+ bacteria, cefoxitin for G^- bacteria and fluconazole for yeasts; Oxoid, Basingstoke, UK). The disc diffusion assays were performed in triplicate with three independent biological replicates to ensure reproducibility and statistical reliability of the results.

2.3.3 | Minimal Inhibition Concentration

As outlined earlier, the minimal inhibitory concentration (MIC) values (MIC_{50} and MIC_{90}) were determined using standard procedures. First, 50 μL of the microbial inoculum was added to each well of a 96-well microtiter plate. Various concentrations of ECEO, ranging from 10 to 0.00488 mg/mL in Mueller Hinton Broth (MHB), were then introduced. Negative and positive controls were prepared by adding MHB and Sabouraud Dextrose Broth (SDB) with EO at the correct concentrations, as well as MHB and SDB with the inoculum. The bacterial growth was then measured using a spectrophotometer (Glomax, Promega Inc., Madison, WI, USA), which determined the OD at 570 nm. The MIC_{50} and MIC_{90} values were defined as the lowest ECEO concentrations that inhibited 50% and 90% of microbial growth, respectively. This experiment was conducted three times independently.

2.3.4 | Antimicrobial Assay of Fruit and Vegetables in Vapour Phase

The antibacterial effects of ECEO in the vapour phase were evaluated using a selection of gram-positive and gram-negative bacterial and yeast strains on apple, pear, sweet potato and carrot substrates [26]. These substrates were cut into 0.5 mm pieces, thoroughly washed, dried and then placed in 60 mm Petri dishes, where they were inoculated with the microbial strains. ECEO was dissolved in ethyl acetate at varying concentrations (500, 250, 125 and 62.5 μg/mL), with filter papers soaked in ethyl acetate as controls. The Petri dishes were then sealed and incubated at 37°C for a period of 7 days. The microbial colony density was measured using the ImageJ programme [27], and growth in the vapour phase was assessed following standard microbiological techniques for bacteria and yeasts. The vapour-phase antimicrobial assay was performed in triplicate with three independent biological replicates to ensure reproducibility and statistical reliability of the results.

2.4 | Antibiofilm Assay

2.4.1 | Crystal Violet Study

In a study conducted by Kačániová et al. [28], the minimal biofilm inhibitory concentration (MBIC) was assessed by incubating bacterial suspensions in Mueller Hinton Broth at 37°C. The ECEO was applied to the bacterial inoculum at two different dilutions, ranging from 100 to 0.049 mg/mL, in microtiter plates. After 24 h, the wells were cleaned and stained, and the extent of colour development was measured at 570 nm. The MBIC, $MBIC_{50}$ and $MBIC_{90}$ were determined based on the concentrations that inhibited 100%, 50% and 90% of biofilm formation, respectively.

2.4.2 | MALDI-TOF MS Biotyper: A Valuable Instrument in Biofilms Detection

The degradation of proteins during biofilm formation was analysed using the Bruker Daltonics MALDI-TOF MicroFlex device.

For this, glass and stainless-steel slides were placed into 50-mL polypropylene tubes containing 100 μL of a biofilm-forming *S. enterica* inoculum and 20 mL of MHB. Ethyl acetate was added to the experimental tubes to achieve a 0.1% concentration, while the control tubes were left untreated. After 3 days of shaking at 170 *g*, biofilms were removed from the surfaces of the glass and stainless-steel slides. The samples were then incubated at 37°C for 7, 9, 12 and 14 days. In addition, planktonic cells from control samples that did not contain ECEO were also analysed. MALDI-TOF mass spectrometry in linear positive mode was used to acquire protein spectra, and 19 typical global spectra were selected for calculating Euclidean distances. This distance matrix was then used to generate dendrograms [26].

2.5 | Investigation of the Impact of ECEO, Temperature and Duration on the Growth Behaviour of *S. enterica* in a Regulated Bioreactor Environment

To investigate the impact of heat and ECEO on the growth dynamics of *S. enterica*, a controlled experiment was conducted using a personal bioreactor (RTS-1, Biosan, Riga, Latvia). Bacterial growth was monitored by measuring OD at 850 nm. The strain was initially cultured on MHA at 37°C for 24 h. A single colony from this culture was transferred into 60 mL of MHB and thoroughly mixed to ensure uniform distribution. The broth was then divided into two 22 mL samples, which were placed into perforated-cap tubes and incubated in separate bioreactors at 37°C. Upon reaching an OD_{850} of around 1, indicative of the exponential growth phase, 1% ECEO was added to one tube, while the other remained untreated as a control. The bioreactors were then gradually heated to 50°C, with continuous rotation at 2000 rpm (one rotation per second). OD measurements were recorded every 5 min for a total of 20 min. This procedure was repeated at 55°C, 60°C and 65°C to assess the combined effects of EO and elevated temperatures on bacterial growth inhibition. The temperature range of 50°C–65°C was selected to reflect common sous vide cooking conditions, balancing food safety and food quality preservation. The 1% (w/v) concentration of ECEO was chosen based on preliminary antimicrobial efficacy tests and practical relevance. The "exponential phase" was defined at OD850 = 1.0 based on the growth curve analysis conducted under the experimental conditions.

2.6 | Sous Vide Sweet Potato Experiment

A total of 2.5 kg of sweet potatoes (*Ipomoea batatas*) was sourced from an approved vendor in the Slovak Republic for this study. After being cooled, the sweet potatoes were transported to the microbiological lab, where they were sliced into five-gram portions using a sterile knife. From three whole sweet potatoes, a total of 480 five-gram samples were prepared, with 240 assigned to the treated and control groups on day 1 and another 240 for day 7. Each sample was individually vacuum-sealed using a Concept vacuum packer after being treated with a 1% *v/w* ECEO solution mixed with rapeseed oil. The control samples included both vacuum-sealed and unpackaged portions. To replicate the presence of *S. enterica* without compromising the sweet potatoes, samples were inoculated with 100 μL of *S. enterica* and 1% *v/w* ECEO. Prior to sealing, the samples were gently mixed for approximately 1 min [27].

The following information was available for consideration during the evaluation process:

i. Control: Fresh sweet potato samples were stored at 4°C in plastic bags and subsequently subjected to a temperature range of 50°C–65°C for a duration of 5–25 min.
ii. Control + vacuum: Fresh sweet potato samples were vacuum-packed in polyethylene bags and maintained at 4°C. They were then incubated at 50°C–65°C for 5–25 min.
iii. EO: After being vacuum-packed, fresh sweet potatoes were treated with 1% ECEO and kept at 4°C. After that, they were cooked for 5–25 min at 50°C–65°C.
iv. *S. enterica*: Fresh sweet potatoes that had been vacuum-packed and treated with *S. enterica* were stored at 4°C until they were exposed to the bacteria for 5–25 min at 50°C–65°C.
v. *S. enterica* + EO: Vacuum-packed fresh potato treated with *S. enterica* and containing 1% ECEO was stored at 4°C before being treated for 5–25 min at 50°C–65°C.

On the first day of the experiment, raw, uncooked sweet potatoes were used to create control samples, which were then macerated for 24 h following the experimental procedure. During this period, the EO from the first set of samples and *S. enterica* from the second set were introduced, mixed gently and blended. The samples were prepared using a CASO SV1000 sous vide apparatus from a company based in Arnsberg, Germany. Before cooking sous vide, the samples were grouped and carefully cooked to a set temperature and duration. Once cooked, the samples were packaged in high-barrier polyethylene vacuum-sealed bags, designed to protect them from moisture and temperature variations, ranging from −30°C to +100°C. These bags, made from impermeable material with a thickness between 40 and 200 μm, ensure a long shelf life while preserving the product's quality and organoleptic properties. They are free of plasticisers such as bisphenol A and contain no microplastics. Additionally, when stored in refrigerators or freezers, the bags maintain their integrity for multiple years.

2.7 | Microbiological Analyses of Samples and Identification of Microorganisms

Microbiological analysis was conducted on sweet potato samples from day 1 to day 7 of the study. A subset of samples was examined again on days 1 and 7. Five grams of sweet potato were weighed, placed into a sterile stomacher bag and diluted to a 10^{-1} concentration with 45 mL of peptone water. The mixture was then homogenised for 20 min using a stomacher. A 0.1 mL aliquot from the dilution was placed onto Plate Count Agar (PCA), and the samples were incubated in a GFL 3031 shaking incubator (GLS, Burgwedel, Germany) for 30 min. To assess coliform bacteria, Violet Red Bile Lactose Agar (VRBL, Oxoid, Basingstoke, UK) was used, with incubation at 37°C for 24–48 h. Total viable counts (TVCs) were determined using PCA,

incubated at 30°C for 48–72 h. *S. enterica* was enumerated by inoculating 0.1 mL of the sample onto Xylose-Lysine Deoxycholate (XLD, Oxoid, Basingstoke, UK) agar and incubating at 37°C for 24 h. Afterward, the media were processed to quantify the total *Salmonella*, coliforms, and live bacteria.

For the identification of microorganisms isolated from the sweet potato samples, the MALDI-TOF MS Biotyper (Bruker Daltonics, Bremen, Germany) and reference libraries were used. A stock solution was prepared by combining 50% acetonitrile, 47.5% water and 2.5% trifluoroacetic acid. To prepare 1 mL of the stock solution, 500 µL of acetonitrile, 475 µL of filtered water and 25 µL of 10% trifluoroacetic acid were mixed. The "HCCA matrix portioned" solution was created by combining the stock solution with the organic solvent in a 250 µL Eppendorf flask. Matrix materials were supplied by Aloqence Science (Vrable, Slovakia), and sample preparation followed the protocols described in previous studies [27].

2.8 | Statistical Analysis

The data are presented as mean values ± standard deviation (SD), and each evaluation was carried out in triplicate. A one-way ANOVA was conducted, and then Tukey's HSD test was run at $p \leq 0.05$ of significance (CoStat version 6.451, CoHort Software, Pacific Grove, CA, USA). Finally, the JMP Pro 17.0 software programme (SAS Institute, Cary, NC, USA) was used for the graphic elaborations.

3 | Results

3.1 | Chemical Composition of ECEO

The chemical composition and the percentage of each class of identified compounds in the analysed *E. cardamomum* essential oil sample are presented in Table 1. Table 1 shows that 42 volatile compounds were identified, representing 99.8% of the oil.

The most abundant compounds are from the class of oxygenated monoterpenes (76.2%). Among them, monoterpene ether 1,8-cineole (36.9%) and monoterpene ester α-terpinyl acetate (37.5%) were observed in the highest concentrations. The monoterpene hydrocarbon class, comprising 17 identified compounds, accounts for 13.8% of the oil. It should also be emphasised that sesquiterpene hydrocarbons, as well as non-terpene compounds, were found in low concentrations (2.6% and 0.2%, respectively).

3.2 | Disc Diffusion Method

The disc diffusion method was utilised to assess the antibacterial properties of ECEO, employing a total of five yeast strains, encompassing four gram-positive (G^+) and four gram-negative (G^-) bacteria, along with *S. enterica* biofilm-forming bacteria (BFB). The findings demonstrated that ECEO exhibited the highest antibacterial activity against gram-negative *S. enterica* (14.33 mm), yeasts *C. tropicalis* (11.67 mm) and gram-positive

TABLE 1 | Chemical composition and classes of identified compounds in *Elettaria cardamomum* essential oil.

No	Compound[a]	%[b]	KI[c] (lit.)	KI[c] (calc.)
1	α-Thujene	0.4	930	927
2	α-Pinene	2.4	939	935
3	α-Fenchene	tr[d]	952	951
4	Sabinene	4.3	975	973
5	β-Pinene	0.5	979	978
6	β-Myrcene	2.7	990	989
7	1-Octanal	0.1	998	1004
8	α-Phellandrene	tr	1002	1007
9	δ-3-Carene	tr	1011	1009
10	α-Terpinene	0.6	1017	1018
11	*o*-Cymene	0.3	1026	1029
12	Limonene	0.4	1029	1032
13	1,8-Cineole	36.9	1031	1038
14	(*E*)-β-Ocimene	tr	1050	1052
15	γ-Terpinene	1.1	1059	1064
16	*p*-Mentha-3,8-diene	0.5	1072	1075
17	α-Terpinolene	0.4	1088	1087
18	Linalool	0.9	1096	1099
19	4,8-Dimethyl-1,3,7-nonatriene	0.2	1110	1115
20	β-Phellandrene	0.1	1029	1127
21	*cis*-β-Terpineol	tr	1144	1146
22	*trans*-Linalool oxide	tr	1174	1174
23	4-Terpineol	1.7	1177	1183
24	α-Terpineol	1.6	1188	1195
25	Octanol acetate	0.1	1213	1210
26	Linalool formate	0.3	1216	1215
27	Car-3-en-2-one	2.5	1248	1249
28	Geranial	0.3	1267	1266
29	δ-Terpinyl acetate	0.2	1317	1313
30	Methyl geranate	0.2	1324	1323
31	α-Terpinyl acetate	37.5	1349	1348
32	Geranyl acetate	1.2	1381	1380
33	(*E*)-Methyl cinnamate	tr	1378	1385
34	β-Elemene	0.3	1390	1390
35	γ-Elemene	0.1	1436	1434
36	*trans*-Muurola-3,5-diene	tr	1453	1450

(Continues)

TABLE 1 | (Continued)

No	Compound[a]	% [b]	KI[c] (lit.)	KI[c] (calc.)
37	Germacrene D	tr	1481	1481
38	β-Selinene	0.9	1490	1489
39	α-Selinene	0.3	1498	1495
40	Epizonarene	tr	1501	1501
41	δ-Amorphene	0.1	1512	1512
42	Germacrene B	0.9	1561	1562
Total		99.8		

Class of compounds	Number of compounds	%
Nonterpenic compounds	3	0.2
Aldehydes	1	0.1
Esters	2	0.1
Monoterpenes	30	97.0
Monoterpene hydrocarbons	17	13.8
Monoterpene alcohols	5	4.2
Monoterpene ketones	1	2.5
Monoterpene aldehydes	1	0.3
Monoterpene ethers	1	36.9
Monoterpene esters	5	39.3
Sesquiterpenes	9	2.6
Sesquiterpene hydrocarbons	9	2.6

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

L. monocytogenes (17.33 mm), *S. aureus* and *S. constellatus* (16.67 mm). It has been demonstrated that ECEO exhibits 15.67 mm of antibacterial activity against the biofilm-forming gram-negative bacterium *S. enterica*. In addition, it was demonstrated that antibiotics were more effective against bacteria than ECEO. Nonetheless, the most potent antibiotic resistance against *Streptococcus pneumoniae, S. enterica, E. coli*, *B. subtilis*, *S. constellatus* and *C. krusei* was demonstrated by cefoxitin, gentamicin and fluconazole (Table 2).

3.3 | Minimal Inhibition Concentration

The broth microdilution method was selected for the determination of the lowest inhibitory concentrations, herein referred to as MIC_{50} and MIC_{90}. This was undertaken to enhance comprehension of ECEO's antibacterial activity. The study observed that ECEO exerted its most significant impact on gram-positive species inhibition. Specifically, the lowest MIC_{50} (0.249 and 0.256 mg/mL) and MIC_{90} (0.307 and 0.303 mg/mL) were observed in *S. constellatus* and *B. subtilis*, respectively. In contrast,

TABLE 2 | Disc diffusion method antimicrobial activity of ECEO in mm.

Microorganism	Inhibition zone	ATB
G-negative bacteria		
Salmonella enterica CCM 3807	14.33 ± 0.58^{bc}	30.67 ± 0.58^{abc}
Pseudomonas aeruginosa CCM 1959	12.67 ± 0.59^{cd}	30.33 ± 0.59^{bc}
Yersinia enterocolitica CCM 7204 T	12.33 ± 0.57^{de}	30.33 ± 0.56^{bc}
Escherichia coli CCM 3953	12.33 ± 0.58^{de}	30.67 ± 0.58^{abc}
G-positive bacteria		
Staphylococcus aureus CCM 4423	16.67 ± 0.58^{a}	30.33 ± 0.57^{bc}
Listeria monocytogenes CCM 4699	17.33 ± 0.59^{a}	30.67 ± 0.58^{abc}
Bacillus subtilis subsp. *Spizizenii* CCM 1999	16.33 ± 0.57^{a}	32.33 ± 0.57^{a}
Streptococcus constellatus CCM 4043	16.67 ± 0.58^{a}	31.67 ± 0.56^{ab}
Yeast		
Candida albicans CCM 8136	10.33 ± 0.58^{f}	24.67 ± 0.59^{f}
Candida tropicalis CCM 8264	11.67 ± 0.57^{def}	27.67 ± 0.58^{de}
Candida glabrata CCM 8270	10.67 ± 0.58^{ef}	27.33 ± 0.57^{e}
Candida krusei CCM 8271	11.33 ± 0.59^{def}	30.33 ± 0.59^{bc}
Candida parapsilosis CCM 8260	10.33 ± 0.59^{f}	28.33 ± 0.58^{de}
Biofilm-forming bacteria (BFB)		
Salmonella enterica	15.67 ± 0.56^{ab}	29.33 ± 0.58^{cd}

Note: Data are the mean (± SD) of 3 samples. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$).
Abbreviation: ATB, antibiotics.

Gram-negative *P. aeruginosa* and *E. coli* exhibited the lowest MIC_{90} (0.366 and 0.377 mg/mL) and MIC_{50} (0.345 and 0.356 μg/mL). The most favourable MIC results were obtained by the yeast strain *C. tropicalis*, which had MIC_{50} and MIC_{90} values of 0.463 and 0.442 mg/mL. The specific outcomes of the MIC test are displayed in Table 3.

TABLE 3 | Minimal inhibition concentration (MIC_{50} and MIC_{90}) of ECEO in mg/mL.

Microorganism	MIC_{50}	MIC_{90}
G-negative bacteria		
Salmonella enterica CCM 3807	0.367 ± 0.020^{b}	0.387 ± 0.009^{ab}
Pseudomonas aeruginosa CCM 1959	0.356 ± 0.012^{b}	0.377 ± 0.005^{ab}
Yersinia enterocolitica CCM 7204T	0.367 ± 0.011^{b}	0.376 ± 0.002^{ab}
Escherichia coli CCM 3953	0.345 ± 0.020^{b}	0.366 ± 0.018^{ab}
G-positive bacteria		
Staphylococcus aureus CCM 4423	0.271 ± 0.006^{c}	0.310 ± 0.056^{b}
Listeria monocytogenes CCM 4699	0.267 ± 0.011^{c}	0.307 ± 0.059^{b}
Bacillus subtilis subsp. *Spizizenii* CCM 1999	0.256 ± 0.011^{c}	0.307 ± 0.059^{b}
Streptococcus constellatus CCM 4043	0.249 ± 0.017^{c}	0.303 ± 0.063^{b}
Yeast		
Candida albicans CCM 8186	0.471 ± 0.013^{a}	0.443 ± 0.059^{a}
Candida tropicalis CCM 8264	0.463 ± 0.022^{a}	0.442 ± 0.064^{a}
Candida glabrata CCM 8270	0.467 ± 0.010^{a}	0.440 ± 0.057^{a}
Candida krusei CCM 8271	0.474 ± 0.006^{a}	0.452 ± 0.067^{a}
Candida parapsilosis CCM 8260	0.477 ± 0.008^{a}	0.450 ± 0.066^{a}
Biofilm-forming bacteria (BFB)		
Salmonella enterica	0.339 ± 0.010^{b}	0.354 ± 0.022^{b}

Note: Data are the mean (± SD) of 3 samples. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$).

3.4 | Antimicrobial Activity on Vapour Phase

In the subsequent investigation, fruit and vegetables were employed as food models for an in situ antimicrobial evaluation. Furthermore, a selection of microorganisms was utilised in vitro. The antibacterial activity in situ results from the fruit model are displayed in Table 4 and Figure 1a,b. A comparison of the in vitro assay with the ECEO vapour phase revealed that the former was generally less effective in suppressing gram-positive bacteria in the apple model than gram-negative bacteria. The findings indicated that the highest dose of ECEO (500 μg/L) was the most effective (95.45%) in suppressing *B. subtilis* progression in the apple model. The highest doses of ECEO that were tested (95.22% and 94.78%, respectively) were found to be effective in reducing the growth of *S. aureus* and *L. monocytogenes* in the apple model (Table 3). A notable degree of inhibition was observed at the highest dose (500 μg/L) when compared to the other gram-negative bacteria tested; nevertheless, ECEO was most effective at preventing biofilm-forming *Y. enterocolitica* (79.33%) at this concentration. In addition, ECEO exhibited limited antibacterial efficacy against strains of *Candida*. In the model utilising pears (ECEO), *S. aureus* exhibited a 77.95% reduction in activity at a concentration of 500 μg/L. In the in situ growth of the yeast model, ECEO exhibited a weak inhibitory effect. In the context of gram-positive strains, ECEO demonstrated optimal inhibitory efficacy against *S. enterica* (66.11%), attaining this effect at a dosage of 500 μg/L. The findings further demonstrated that when ECEO was administered at the highest concentration observed among the pear model yeasts, it was most effective in inhibiting the growth of *C. tropicalis* (44.91%). Furthermore, at the highest tested dose, ECEO significantly reduced *S. enterica* biofilm. It is notable to observe that yeast demonstrated pro-yeast growth at the highest doses. This finding suggests that the elevated concentrations of ECEO may have promoted the growth of both G^+ and G^- bacteria. Compared to the apple model, the efficacy of ECEO was significantly lower in the inhibition of single microorganisms.

Next, the effect of ECEO on the vegetable models was investigated (Table 5 and Figure 2a,b). Sweet potatoes and carrots were selected as model foods. Sweet potatoes were also investigated in another experiment in which the antimicrobial activity of ECEO was monitored in a sous vide sweet potato model with *S. enterica*. On the sweet potato model, ECEO efficacy was mainly observed for *S. aureus* at the highest concentration (97.41%). The same results were obtained for other G^+ bacteria. Slightly lower activity was recorded against G^- bacteria, with ECEO being the most effective against *P. aeruginosa* (87.29%). Similar results to those obtained with G^- bacteria were also obtained with the biofilm-forming *S. enterica*. Compared to the yeast bacteria in the vegetable model, they showed lower inhibition at the highest concentration. However, in comparison with the fruit model, pro-microbial growth was detected on vegetables for individual microorganisms only in carrots for yeasts.

3.5 | Antibiofilm Activity

The bacteria *S. enterica* possesses the capacity to survive and develop increased resistance to antibiotic medicines through the production of biofilms, either individually or collectively. The crystal violet assay was utilised to evaluate the antibiofilm activity of ECEO against *S. enterica*, yielding an $MBIC_{50}$ of 0.339 mg/mL and an $MBIC_{90}$ of 0.354 mg/mL (Table 3). The antibiofilm effects of ECEO on glass and stainless-steel surfaces against *S. enterica* were assessed using mass spectrometry and the MALDI-TOF MS Biotyper. The control samples, which comprised untreated biofilm and planktonic cells, exhibited a comparable trend in their spectra. Consequently, the representative spectrum of the control group was designated as the

TABLE 4 | In situ analysis of the antimicrobial activity (%) of the vapour phase of ECEO in fruit model.

Food model	Microorganisms	Concentration of ECEO in μg/L			
		62.5	125	250	500
Apple					
G⁻ negative bacteria	*Salmonella enterica*	46.00 ± 2.51^{c}	57.03 ± 2.38^{b}	66.95 ± 1.61^{b}	75.33 ± 1.06^{b}
	Pseudomonas aeruginosa	45.11 ± 3.34^{c}	52.32 ± 2.92^{b}	64.07 ± 0.71^{b}	74.30 ± 0.74^{b}
	Yersinia enterocolitica	45.95 ± 2.89^{c}	54.66 ± 2.93^{b}	65.74 ± 2.10^{b}	79.33 ± 6.03^{b}
	Escherichia coli	45.04 ± 1.18^{c}	56.47 ± 2.34^{b}	67.09 ± 2.35^{b}	75.56 ± 1.27^{b}
G⁺ positive bacteria	*Staphylococcus aureus*	55.33 ± 2.51^{b}	74.78 ± 3.61^{a}	85.24 ± 1.66^{a}	95.22 ± 0.78^{a}
	Listeria monocytogenes	54.10 ± 3.40^{b}	72.78 ± 3.20^{a}	85.56 ± 2.06^{a}	94.78 ± 1.82^{a}
	Bacillus subtilis subsp. *Spizizenii*	66.11 ± 2.18^{a}	76.69 ± 2.20^{a}	84.76 ± 1.81^{a}	95.45 ± 2.18^{a}
	Streptococcus constellatus	63.81 ± 0.76^{a}	74.62 ± 3.25^{a}	85.04 ± 1.32^{a}	94.26 ± 4.13^{a}
Yeast	*Candida albicans*	−16.38 ± 2.76^{d}	−6.33 ± 1.07^{c}	24.59 ± 3.94^{c}	34.96 ± 2.69^{c}
	Candida tropicalis	−14.52 ± 2.17^{d}	−7.51 ± 1.68^{c}	24.26 ± 3.07^{c}	33.93 ± 2.47^{c}
	Candida glabrata	−15.66 ± 1.91^{d}	−7.06 ± 1.77^{c}	24.70 ± 3.10^{c}	33.33 ± 1.08^{c}
	Candida krusei	−14.60 ± 1.89^{d}	−6.96 ± 1.61^{c}	24.04 ± 2.02^{c}	33.96 ± 3.42^{c}
	Candida parapsilosis	−15.82 ± 2.45^{d}	−6.20 ± 1.62^{c}	25.03 ± 2.64^{c}	32.83 ± 1.66^{c}
BFB	Biofilm-forming *S. enterica*	45.19 ± 2.29^{c}	56.55 ± 1.94^{b}	64.59 ± 3.26^{b}	75.25 ± 1.05^{b}
Pear					
G⁻ negative bacteria	*Salmonella enterica*	33.61 ± 2.92^{b}	44.66 ± 2.01^{b}	54.85 ± 2.62^{b}	66.11 ± 1.60^{b}
	Pseudomonas aeruginosa	33.75 ± 0.71^{b}	43.59 ± 2.03^{b}	54.41 ± 3.19^{b}	65.30 ± 2.16^{b}
	Yersinia enterocolitica	35.33 ± 2.73^{b}	42.59 ± 1.14^{b}	54.55 ± 2.04^{b}	65.71 ± 2.00^{b}
	Escherichia coli	36.11 ± 2.49^{b}	45.77 ± 2.72^{b}	55.13 ± 3.06^{b}	64.93 ± 2.68^{b}
G⁺ positive bacteria	*Staphylococcus aureus*	46.07 ± 2.73^{a}	55.37 ± 1.72^{a}	64.59 ± 3.22^{a}	77.95 ± 1.22^{a}
	Listeria monocytogenes	46.07 ± 0.62^{a}	56.69 ± 2.04^{a}	65.56 ± 0.17^{a}	75.33 ± 3.77^{a}
	Bacillus subtilis subsp. *Spizizenii*	44.56 ± 1.90^{a}	53.16 ± 0.34^{a}	66.76 ± 2.92^{a}	74.92 ± 3.56^{a}
	Streptococcus constellatus	45.97 ± 2.41^{a}	54.07 ± 1.12^{a}	67.43 ± 1.80^{a}	76.44 ± 2.26^{a}
Yeast	*Candida albicans*	−13.60 ± 2.00^{c}	−7.08 ± 1.47^{c}	25.66 ± 2.21^{c}	43.26 ± 2.30^{c}
	Candida tropicalis	−15.70 ± 2.88^{c}	−7.50 ± 1.68^{c}	24.63 ± 2.21^{c}	44.91 ± 1.76^{c}
	Candida glabrata	−16.78 ± 1.93^{c}	−8.09 ± 1.15^{c}	25.03 ± 3.51^{c}	42.89 ± 2.42^{c}
	Candida krusei	−15.56 ± 3.82^{c}	−7.63 ± 2.07^{c}	24.60 ± 2.17^{c}	43.39 ± 0.25^{c}
	Candida parapsilosis	−15.65 ± 3.31^{c}	−7.69 ± 1.89^{c}	25.70 ± 2.16^{c}	44.16 ± 1.31^{c}
BFB	Biofilm-forming *S. enterica*	35.33 ± 2.26^{b}	45.96 ± 2.43^{b}	54.67 ± 2.20^{b}	67.77 ± 0.84^{b}

Note: Data are the mean (± SD) of 3 samples. Different letters in each column (for each fruit model: apple and pear) refer to significant differences (Tukey, $p \leq 0.05$).

spectrum of the planktonic cells. Subsequently, comparative analyses were carried out on glass surfaces and on stainless-steel surfaces in the ECEO-supplemented groups. Specifically, the experimental groups and the planktonic control spectrum differed significantly at the commencement of the experiment (3 days). The form of the mass spectrum and the number of peaks also demonstrated these variations. As illustrated in Figure 3A, the spectrum derived from the stainless-steel surface exhibited a greater number of peaks in comparison to the trial spectrum derived from the glass surface. A comparison of the experiment with the control plankton spectrum, which exhibited similar peak shapes and numbers primarily on steel surfaces, revealed that these differences persisted until day 5 (Figure 3B). When the spectra displayed a considerable lack of uniformity on day 14 (Figure 3D–F), this pattern of differences persisted (Figure 3F).

The MSP separations were utilised to construct a dendrogram, which illustrated the similarity between the biofilm structures of the control and experimental groups (Figure 4). In general,

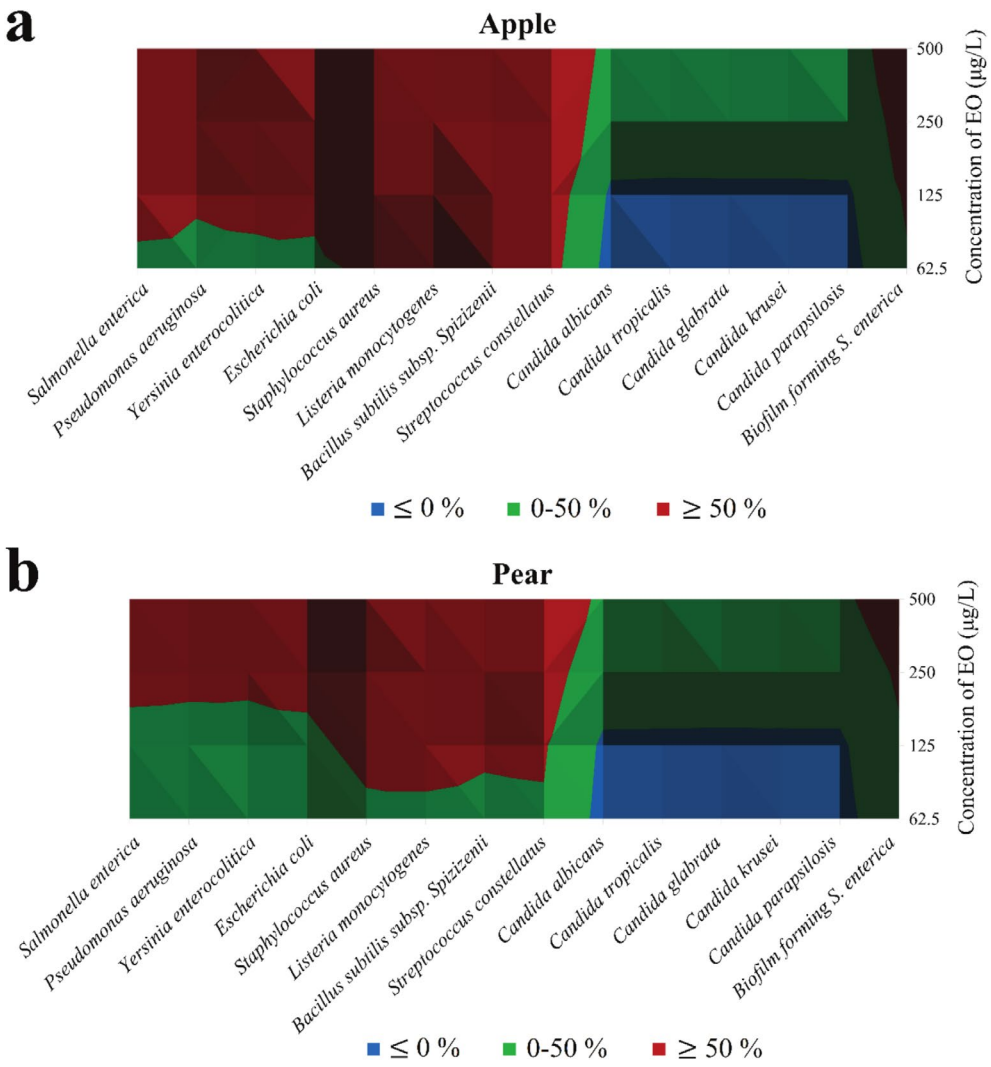


FIGURE 1 | Isometric elaboration of the data of Table 4 (In situ analysis of the antimicrobial activity of the vapour phase of ECEO in fruits model): (a) Apple; (b) Pear. Blue ≤0%; Green: 0%–50%; Red: ≥50%.

the early biofilm stages of the test groups on days 3 and 12 (SEECSS, SEECP) showed the shortest MSP distances compared to the control groups (3, 5, 7 CSE). The MSP distances did not increase as the exposure time of the experimental groups to ECEO increased. Throughout the experiment, the maximum MSP distance was observed daily in the experimental group. The most significant fluctuations were seen, particularly in the experimental group on glass and stainless-steel surfaces and the control planktonic cells (PC). ECEO was found to affect the homeostasis of the *S. enterica* biofilm and contributed to its suppression at the start and end of the experiment. These claims are consistent with the findings of the mass spectra analysis.

3.6 | Kinetics Growth of *S. enterica*

The combined effect of ECEO and temperature on the growth of *S. enterica* was evaluated by monitoring the OD (OD850) and growth rate (GR) (Figure 5). OD850 = 1.0 was considered the exponential growth phase, as described in the Methods section. EO was introduced after 4.34 h (1.15 OD—log phase), with subsequent observations highlighting its effect at increasing temperatures. At 37°C, both treated and untreated samples showed stable growth, with OD reaching 1.32 in the EO-treated group and 1.15 in the control group shortly after the addition of EO. The growth rate (GR) peaked at 0.59 in the treated group compared to 0.09 in the control group. However, with increasing temperature, bacterial growth decreased in both groups. At 50°C, OD decreased faster in the untreated samples, with a GR of −0.12 compared to −0.6 in the treated group. This trend continued at 55°C, where treated samples showed greater growth suppression (OD 0.72, GR −0.78) compared to the control (OD 0.89, GR −0.24). At 60°C and 65°C, both OD and GR decreased further, with the EO-treated group consistently showing stronger inhibition.

Overall, the combination of EO and elevated temperatures effectively suppressed the growth of *S. enterica*, with treated samples exhibiting greater heat sensitivity compared to controls. This highlights the potential of ECEO as an additional antimicrobial agent under heat stress (Figures 6 and 7).

TABLE 5 | In situ analysis of the antimicrobial activity (%) of the vapour phase of ECEO in vegetable models.

Food model	Microorganisms	Concentration of ECEO in μg/L			
		62.5	125	250	500
Sweet potato					
G-negative bacteria	*Salmonella enterica*	24.59 ± 3.73^{c}	43.89 ± 1.56^{d}	65.29 ± 3.19^{c}	86.26 ± 1.23^{b}
	Pseudomonas aeruginosa	25.30 ± 2.56^{c}	44.20 ± 0.81^{d}	66.14 ± 1.21^{c}	87.29 ± 2.57^{b}
	Yersinia enterocolitica	26.03 ± 2.30^{c}	45.96 ± 1.52^{d}	66.11 ± 1.24^{c}	86.66 ± 2.06^{b}
	Escherichia coli	25.29 ± 2.21^{c}	45.89 ± 2.77^{d}	65.77 ± 3.88^{c}	85.30 ± 3.50^{b}
G-positive bacteria	*Staphylococcus aureus*	65.96 ± 2.79^{a}	77.75 ± 1.25^{a}	85.15 ± 0.51^{a}	97.41 ± 3.48^{a}
	Listeria monocytogenes	53.75 ± 2.81^{b}	66.73 ± 1.93^{bc}	86.41 ± 4.39^{a}	95.56 ± 1.73^{a}
	Bacillus subtilis subsp. *Spizizenii*	65.88 ± 1.71^{a}	73.75 ± 2.17^{ab}	86.69 ± 1.97^{a}	96.00 ± 2.27^{a}
	Streptococcus constellatus	66.77 ± 1.92^{a}	75.59 ± 2.72^{a}	84.82 ± 2.63^{a}	96.22 ± 2.21^{a}
Yeast	*Candida albicans*	-7.32 ± 1.88^{d}	25.63 ± 2.68^{e}	33.93 ± 0.65^{d}	45.34 ± 1.64^{c}
	Candida tropicalis	-6.45 ± 2.59^{d}	23.40 ± 2.10^{e}	35.30 ± 1.70^{d}	46.14 ± 2.63^{c}
	Candida glabrata	-8.21 ± 1.46^{d}	26.33 ± 2.68^{e}	37.44 ± 1.59^{d}	45.78 ± 2.09^{c}
	Candida krusei	-7.15 ± 4.49^{d}	24.67 ± 5.36^{e}	36.37 ± 2.25^{d}	45.40 ± 4.92^{c}
	Candida parapsilosis	-10.89 ± 1.86^{d}	26.06 ± 2.24^{e}	34.93 ± 2.79^{d}	45.98 ± 2.42^{c}
BFB	Biofilm-forming *S. enterica*	54.17 ± 7.18^{b}	65.59 ± 3.80^{c}	73.82 ± 0.64^{b}	87.37 ± 2.59^{b}
Carrot					
G-negative bacteria	*Salmonella enterica*	44.19 ± 1.30^{bc}	55.63 ± 1.22^{b}	67.40 ± 1.81^{b}	78.17 ± 2.22^{b}
	Pseudomonas aeruginosa	47.84 ± 1.79^{b}	57.18 ± 1.64^{b}	65.23 ± 2.35^{b}	74.73 ± 3.25^{b}
	Yersinia enterocolitica	43.24 ± 0.35^{bc}	54.71 ± 2.00^{b}	65.37 ± 3.84^{b}	77.45 ± 2.60^{b}
	Escherichia coli	35.66 ± 1.91^{c}	47.13 ± 1.66^{c}	55.74 ± 1.00^{c}	77.39 ± 1.77^{b}
G-positive bacteria	*Staphylococcus aureus*	66.62 ± 2.78^{a}	76.98 ± 1.80^{a}	85.22 ± 2.18^{a}	95.55 ± 1.23^{a}
	Listeria monocytogenes	68.28 ± 1.22^{a}	77.36 ± 1.61^{a}	84.89 ± 4.34^{a}	96.37 ± 2.14^{a}
	Bacillus subtilis subsp. *Spizizenii*	63.98 ± 4.47^{a}	74.43 ± 3.50^{a}	85.49 ± 1.90^{a}	96.64 ± 1.86^{a}
	Streptococcus constellatus	66.62 ± 1.22^{a}	76.40 ± 2.30^{a}	86.95 ± 2.24^{a}	95.26 ± 1.68^{a}
Yeast	*Candida albicans*	-12.70 ± 4.50^{de}	14.96 ± 2.73^{d}	34.66 ± 0.10^{d}	45.33 ± 2.26^{c}
	Candida tropicalis	-12.93 ± 3.59^{de}	15.30 ± 2.31^{d}	33.19 ± 1.19^{d}	45.45 ± 2.56^{c}
	Candida glabrata	-8.00 ± 1.61^{d}	15.59 ± 3.21^{d}	34.60 ± 2.17^{d}	44.29 ± 1.20^{c}
	Candida krusei	-13.06 ± 4.65^{de}	13.84 ± 2.28^{d}	33.60 ± 1.80^{d}	44.21 ± 1.28^{c}
	Candida parapsilosis	-20.89 ± 3.61^{e}	3.89 ± 1.60^{e}	34.15 ± 0.45^{d}	46.80 ± 1.06^{c}
BFB	Biofilm-forming *S. enterica*	45.52 ± 2.22^{b}	56.34 ± 1.82^{b}	67.18 ± 2.24^{b}	74.07 ± 2.74^{b}

Note: Data are the mean (± SD) of 3 samples. Different letters in each column (for each vegetable model: sweet potato and carrot) refer to significant differences (Tukey, $p \leq 0.05$).

3.7 | Microbiological Quality of Sweet Potato Samples

A thorough microbiological examination of uncooked sweet potatoes was conducted, which confirmed the presence of *S. enterica* on XLD agar. On day zero, no coliforms were detected, and the total bacterial count (TBC) was 2.04 log CFU/g. On the first and seventh days of storage, the vacuum-packed sweet potato's microbiological performance was evaluated (see Figure 8). The control group exhibited bacterial counts ranging from 1.27 to 2.29 log CFU/g, while on day 7, these counts ranged from 1.31 to 2.37 log CFU/g. It was found that the TBC count in the vacuum-packed group varied from 1.18 to 2.13 log CFU/g on the first day and from 1.28 to 2.23 log CFU/g on the seventh day. In the vacuum-packed group treated with ECEO, the log CFU/g varied between 1.08 and 1.69 on the first day and between 1.19 and 1.79 on the seventh. In contrast, the group treated with *S. enterica* exhibited a variation in the log CFU/g for TBC ranging from 1.08

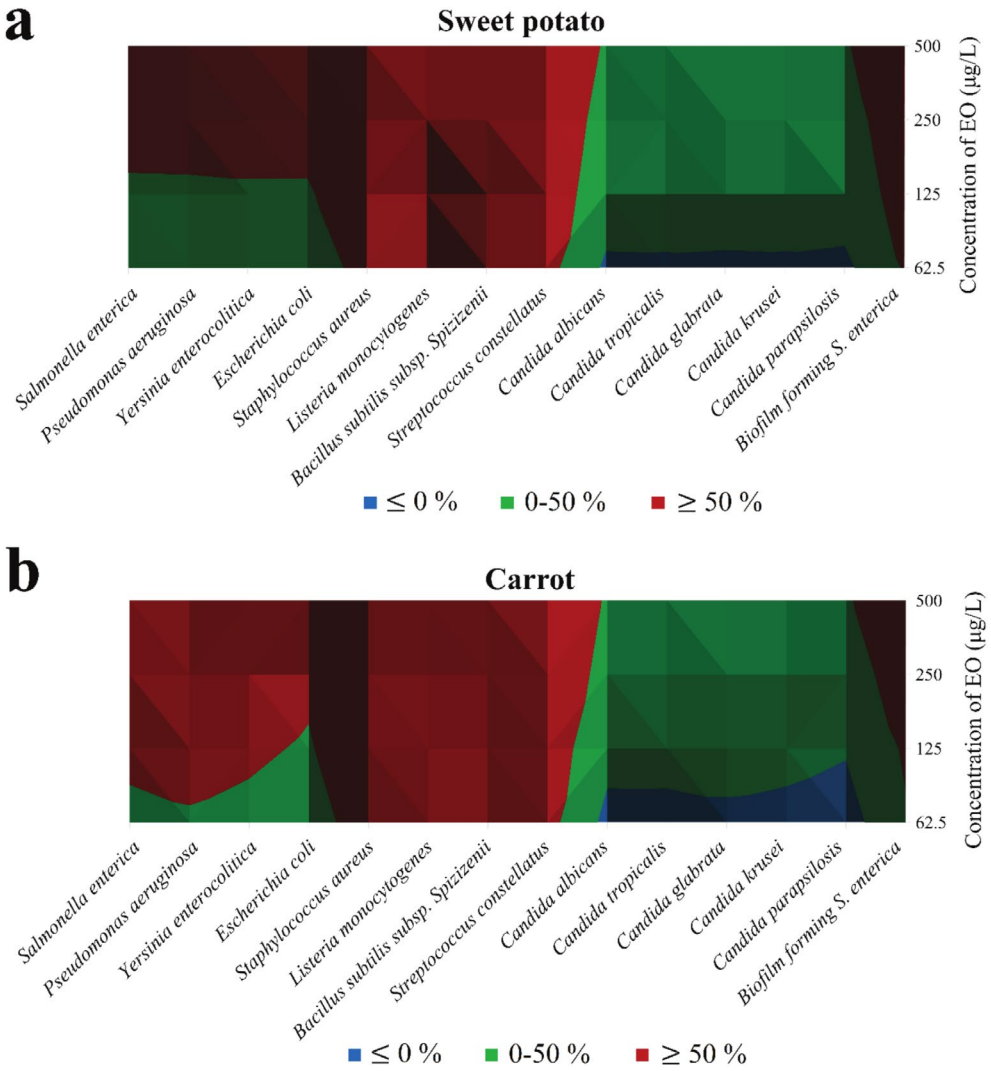


FIGURE 2 | Isometric elaboration of the data of Table 5 (In situ analysis of the antimicrobial activity of the vapour phase of ECEO in vegetable model): (a) Sweet potato; (b) Carrot. Blue ≤0%; Green: 0%–50%; Red: ≥50%.

to 1.16 on day one and from 1.24 to 2.44 on day seven following vacuum packaging and ECEO treatment. When *S. enterica* was present, TBC demonstrated variability, ranging from 1.12 to 2.17 log CFU/g on day 1 and from 1.32 to 2.26 log CFU/g on day 7. This variability was observed following vacuum packaging and ECEO treatment. In summary, it can be concluded that the *S. enterica* and ECEO injection groups exhibited a reduced bacterial burden.

3.8 | Identification of Bacterial Strains From Sweet Potato

As illustrated in Figure 9, the microbial species, genera and families that have been isolated from sweet potatoes since their inception are displayed. Mass spectrometry was used to identify 175 isolates, with scores in all categories potentially reaching 2. The isolates were classified into 9 taxa, 9 families and 17 species. The majority of the species discovered belonged to the Pseudomonadaceae and Bacillaceae families. On the zero day of the experiment, the most frequently isolated species from the samples in the experimental groups were *Enterococcus faecium* (12%), *Bacillus amyloliquefaciens* subsp. *plantarum, Bacillus mojavensis, Bacillus subtilis, Pantoea agglomerans* and *Pseudomonas poae*, all of which had 9% inoculation from sweet potatoes.

The microbial species, genera and families that have been isolated since the cultivation of the sweet potato on day one are displayed in Figure 10. Mass spectrometry was used to identify 270 isolates; scores in all categories might go as high as 2. The isolates were found to belong to 13 genera, 13 families and 21 species. The majority of the species detected were affiliated with the Enterobacteriaceae and Bacillaceae families. On the first day of the experiment, the most prevalent species isolated from the samples in the experimental groups were *S. enterica* (12%), *B. amyloliquefaciens* subsp. *plantarum* (8%), *B. mojavensis* (7%) and *Ralstonia pickettii* (6%).

Since sweet potato day 14, the microbial species, genera and families that have been isolated are displayed in Figure 11. A total of 223 isolates were identified through the utilisation of mass spectrometry, with numerical values in all categories potentially attaining a maximum of 2. The isolates were categorised

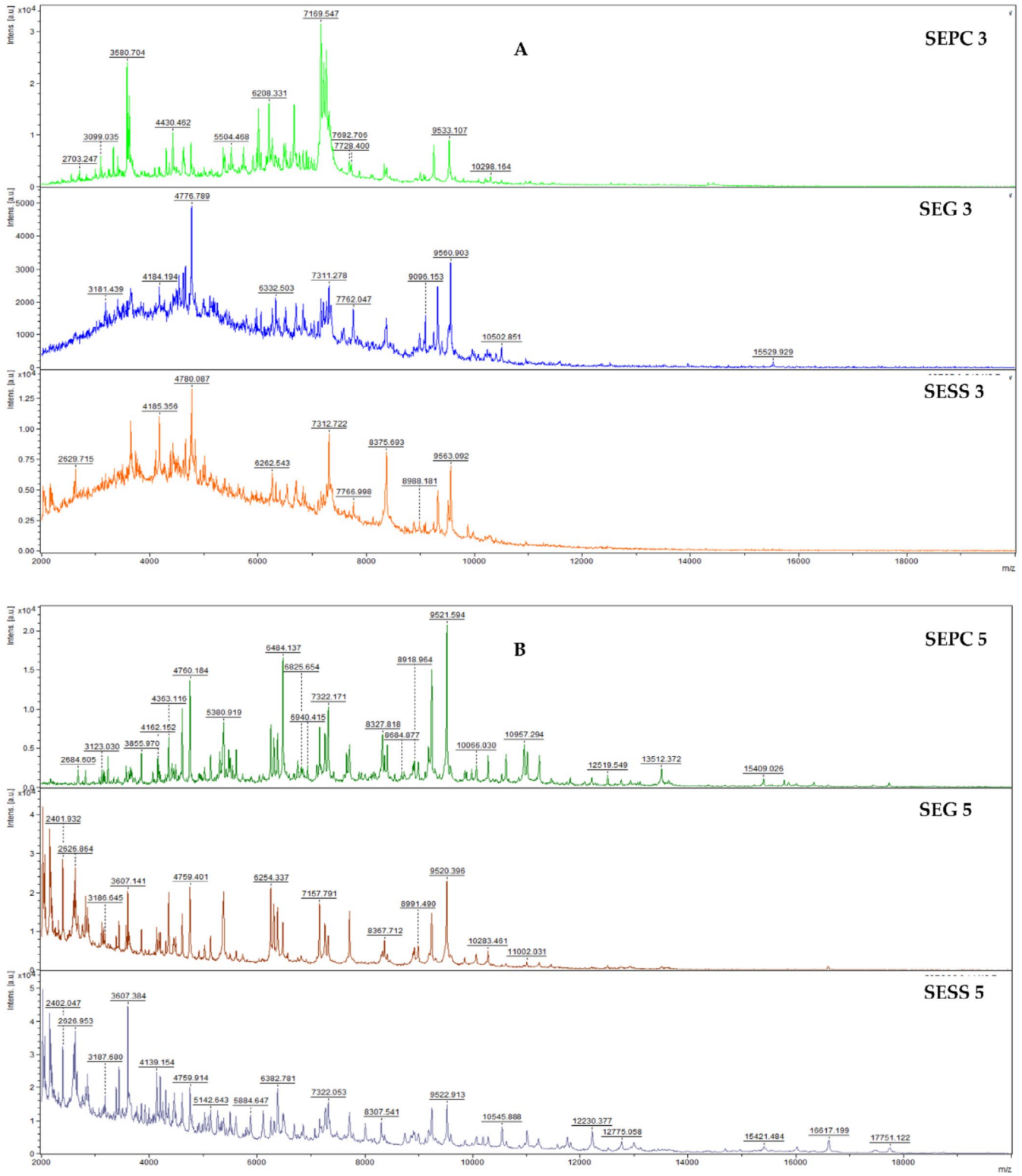


FIGURE 3 | The following examples are provided to illustrate the MALDI-TOF mass spectra of *S. enterica*: (A) 3 day; (B) 5 day; (C) 7 day; (D) 9 day; (E) 12 days; and (F) 14 days. The symbols G, SS, SE and PC denote glass, stainless steel, *S. enterica* and planktonic cells, respectively.

into 16 species, 12 genera and 9 families. The majority of the species identified were affiliated with the Pseudomonadaceae and Bacillaceae families. The most prevalent species isolated from the experimental groups during the 14-day study were *S. enterica* inoculated into the vegetable model (30%), followed by *Pantoea agglomerans* (11%) and *Brevibacillus agri* (8%) inoculated from sweet potatoes.

4 | Discussion

Cardamom EO has been used for centuries in both medicinal and culinary applications [29, 30]. Our analysis revealed that 1,8-cineole and α-terpinyl acetate were the predominant compounds, which aligns with findings from [31], Pavarino et al. [30] and Noumi et al. [32]. Studies have shown that the distinct

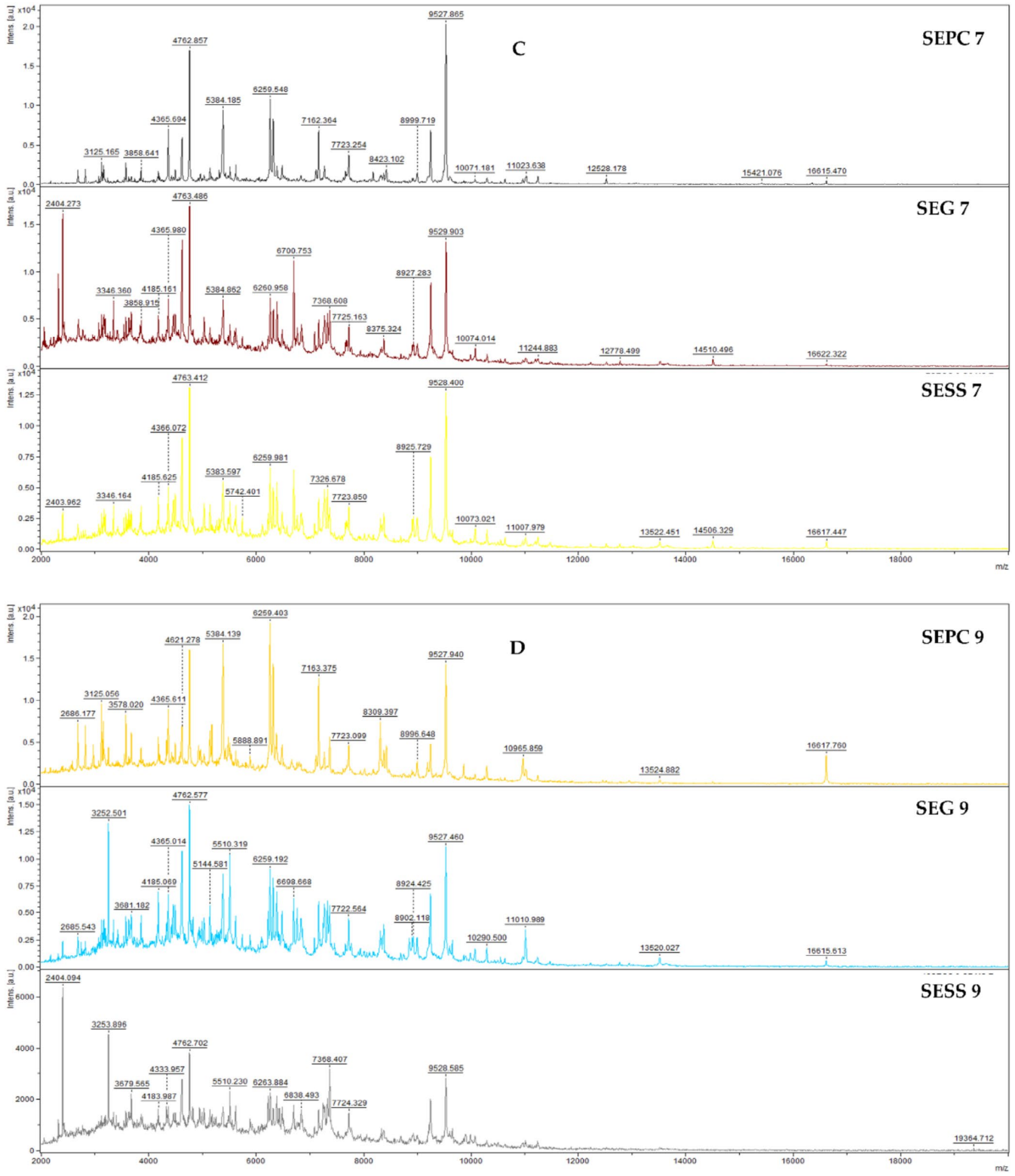


FIGURE 3 | (Continued)

fragrance of green cardamom is a blend of 1,8-cineole and α-terpinyl acetate, with the ratio varying according to the plant's geographical origin and stage of growth [33]. Alqahtani et al. [34] also confirmed that these compounds were the dominant ones in Indian cardamom genotypes at different stages of maturity. Compared to our previous research, the examined sample of *E. cardamomum* essential oil contains almost the same concentration of α-terpinyl acetate (37.3%), while the amount of 1,8-cineole is 8.3% lower [31]. On the other hand, Hymete et al. [35] found that the husk oil was mainly composed of sesquiterpenes, with (*E*)-nerolidol at 17.2%, β-caryophyllene at 9.7% and caryophyllene oxide at 6.9%. In contrast, the ECEO in our study was notably rich in oxygenated monoterpenes (76.2%).

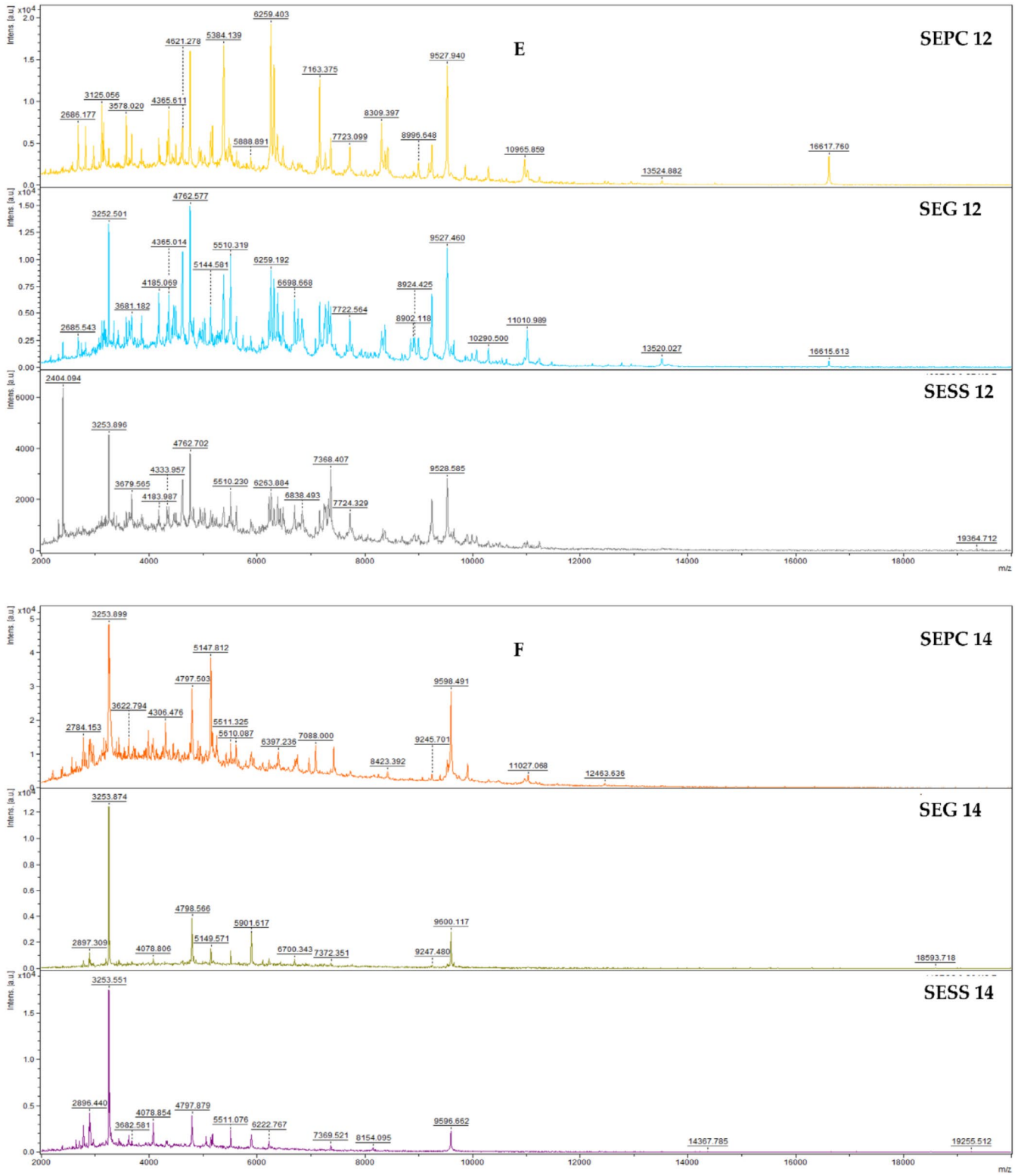


FIGURE 3 | (Continued)

The antioxidant, antibacterial, antifungal, antispasmodic, antidiarrheal and anticarcinogenic properties of ECEO have been extensively studied. In the current research, the focus was on examining the antibiofilm activity of the EO against *S. enterica* using mass spectrometry. Additionally, the impact of ECEO on antimicrobial activity in the vapour phase on fruits and vegetables was explored. The study also assessed the effect of EO on the microbiological quality and activity against *S. enterica* in sous vide sweet potatoes during storage. Notably, such a comprehensive investigation of ECEO remains unprecedented. To evaluate antimicrobial activity, the disc diffusion method was employed to determine the optimal effect against *L. monocytogenes*. The study further compared the antimicrobial results with antibiotic resistance in single-nodule microorganisms. Another

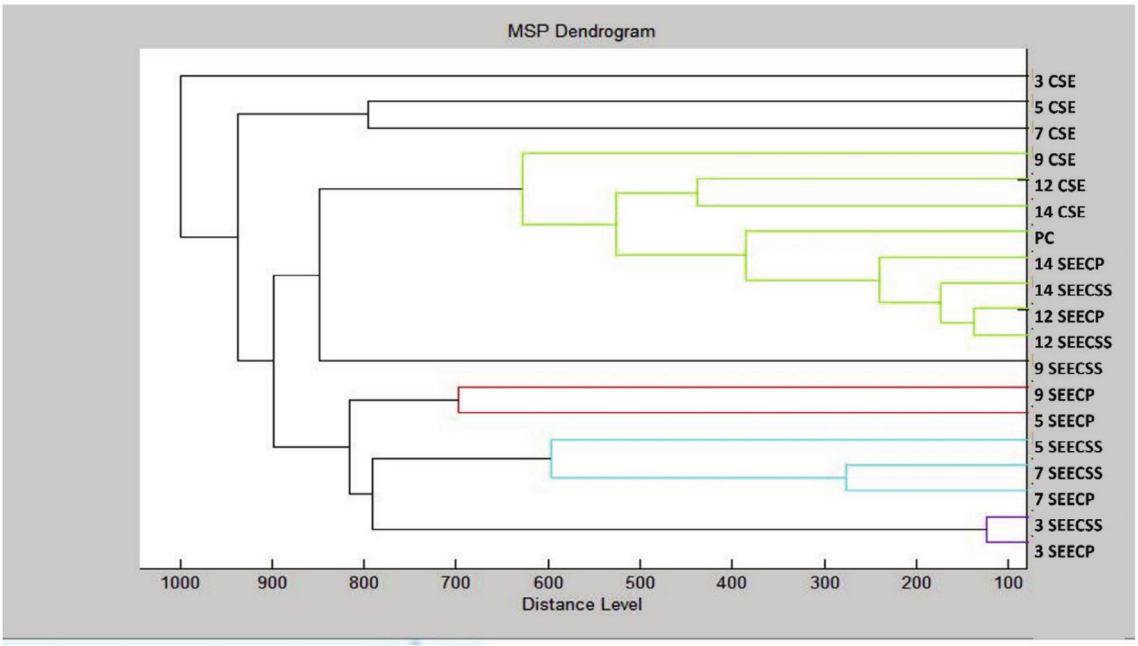


FIGURE 4 | *S. enterica* dendrogram was produced using the control and planktonic cell MSPs. G stands for glass, SS for stainless steel, SE for *S. enterica* and PC for planktonic cells.

experiment investigated the ability of ECEO to inhibit the growth of both gram-positive bacteria (*Bacillus cereus*, ATCC 6629; *S. aureus*, ATCC 6538) and gram-negative bacteria (*E. coli*, ATCC 25922; *P. aeruginosa*, ATCC 27853), along with yeast (*Candida albicans*) [34]. The results showed inhibition zones of 15 mm for *S. aureus* and 11 mm for *E. coli*, indicating strong antibacterial and anti-yeast activity of ECEO. The inhibition zones for all tested microorganisms ranged between 9.0 mm and 15.0 mm. *P. aeruginosa* (9 mm) and *B. cereus* (10 mm) exhibited the greatest resistance to ECEO, while *C. albicans* had an inhibition zone of 15 mm [34]. While antibiotics demonstrated superior antimicrobial activity compared to *E. cardamomum* essential oil (ECEO) in disc diffusion assays, the practical use of antibiotics in food preservation is limited by regulatory restrictions and concerns about antimicrobial resistance. Consequently, ECEO represents a promising natural alternative with antimicrobial properties suitable for application in food systems. The antimicrobial activity of ECEO is believed to be mediated by several mechanisms, including disruption of microbial cell membranes, interference with metabolic pathways, and induction of oxidative stress. Although our study did not directly investigate these mechanisms, future research will focus on employing techniques such as transmission electron microscopy and cellular staining to further elucidate the structural and functional impacts of ECEO on microorganisms [36]. *Candida* spp. consistently showed lower susceptibility to ECEO across all assays. This reduced sensitivity can be attributed to the unique structural features of fungal cells, such as the composition of their cell walls and membranes, which differ significantly from bacteria. The lower responsiveness of Candida spp. highlights the need for further investigation into the optimisation of ECEO use for antifungal applications in food safety. In a study by Younus [37], cardamom was found effective against both gram-positive and gram-negative bacteria, as well as yeast, with stronger antifungal effects against *C. albicans*. The disc diffusion test revealed that the most sensitive microorganisms were *C. albicans* and *Streptococcus mutans*, followed by the sensor strains of *S. aureus*, *L. monocytogenes*, *B. cereus* and *S. typhimurium*. However, *P. aeruginosa* showed the greatest resilience, as ECEO did not inhibit its growth [33]. This contradicts the results of our earlier study, which indicated that gram-positive bacteria were the most susceptible. The MIC test revealed that *S. constellatus* was the most sensitive. As Abdullah et al. [33] demonstrated, cardamom EO is effective against *S. mutans*, *S. typhi*, *C. albicans* and *S. aureus*. Its potent antibacterial properties suggest it could be key in the development of new antibiotics. Additionally, ECEO has been shown to inhibit the growth of *Aspergillus terreus* and demonstrate antifungal activity [38]. The MIC test further confirmed that ECEO exhibited both antibacterial and antifungal effects against nearly all bacterial strains [39].

Cardamom oil's primary constituent, 1,8-cineole, is an oxygenated monoterpene, and both the oil and its compound are commonly used as food flavourings and supplements in dairy, confections and beverages [22]. Given these uses, cardamom oil can also serve as an anti-browning agent. In this study, the antibacterial properties of ECEO were tested in fruit and vegetable models over a 7-day period, as its antimicrobial activity for produce has been underexplored. The highest antibacterial effect was observed against *B. subtilis* in the apple model and *S. aureus* in the pear model during steam exposure. For the vegetable models, *S. aureus* was the most sensitive bacterium on the sweet potato model, and *B. subtilis* was most susceptible on the carrot model. The results regarding bioactive compounds align with findings from Singh et al. [38], who identified 1,8-cineole as the dominant component in cardamom EO. These compounds provide antibacterial and antioxidant benefits, contributing to the protection of food from microbial contamination. The bioactive and aromatic properties of these substances are of notable importance. Additionally, analysis of cardamom oil reveals the presence of natural antioxidants, tocopherols, carotenoids and phenolic compounds [40]. This oil has been shown to be a

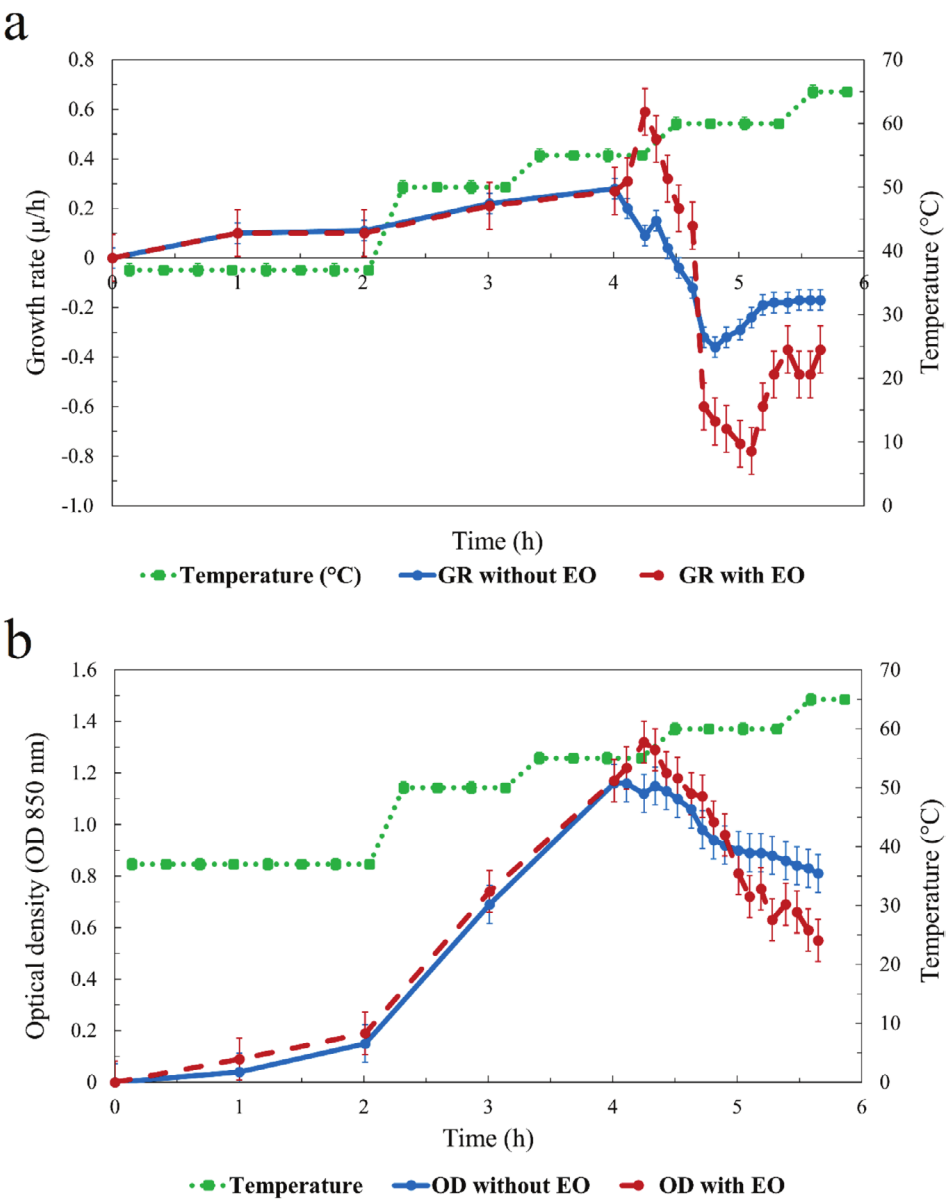


FIGURE 5 | Comparison of optical density at 850 nm (a) and growth rate (b) of *S. enterica* over time at different temperatures between ECEO-treated and untreated samples.

safer and more effective option than artificial preservatives for food preservation, such as in orange juice [41]. Furthermore, it enhances the flavour profile of food [41]. The disc volatilisation method is commonly used to assess the antibacterial properties of EOs [42]. This technique involves applying the oil's vapour phase to a material like filter paper for antimicrobial testing [43]. According to El Khetabi et al. [44], EOs have been shown to extend the shelf life of postharvest fruits and vegetables and prevent spoilage. As natural antibacterial agents, EOs offer an eco-friendly alternative to chemical preservatives in fruit and vegetable preservation [45]. In the manuscript, we observed pro-bacterial and pro-yeast growth at certain concentrations of ECEO, which is evaluated relative to the control; if control samples show less growth than treated samples, this is considered pro-growth. This phenomenon may occur not only at low but also at high concentrations. These results do not contradict the inhibitory effects demonstrated by MIC and disc diffusion assays, which confirm the efficacy of ECEO at higher concentrations. The concentration-dependent effect and exposure conditions are consistent with findings from previous studies by Kačániová et al. [46] and Verešová et al. [47].

In our study, the antibiofilm activity of cardamom EO was assessed using two methods, both of which showed a beneficial effect on *S. enterica* biofilm formation. Identifying safe and effective alternative antimicrobials is crucial for controlling biofilm production by pathogenic bacteria, a challenge that persists in combating antibiotic resistance and recurrent infections [48]. Numerous studies have shown that plant-derived bioactive compounds can interfere with quorum sensing, block peptidoglycan synthesis, disrupt cell membrane integrity or inhibit signal detection, thereby preventing biofilm formation) [49]. For example, cardamom extract has been found to inhibit biofilm formation in *P. aeruginosa* and *S. typhimurium* [49, 50]. Additionally, *E. cardamomum* essential oil has demonstrated antibacterial properties [45]. Phenolic compounds from ginger

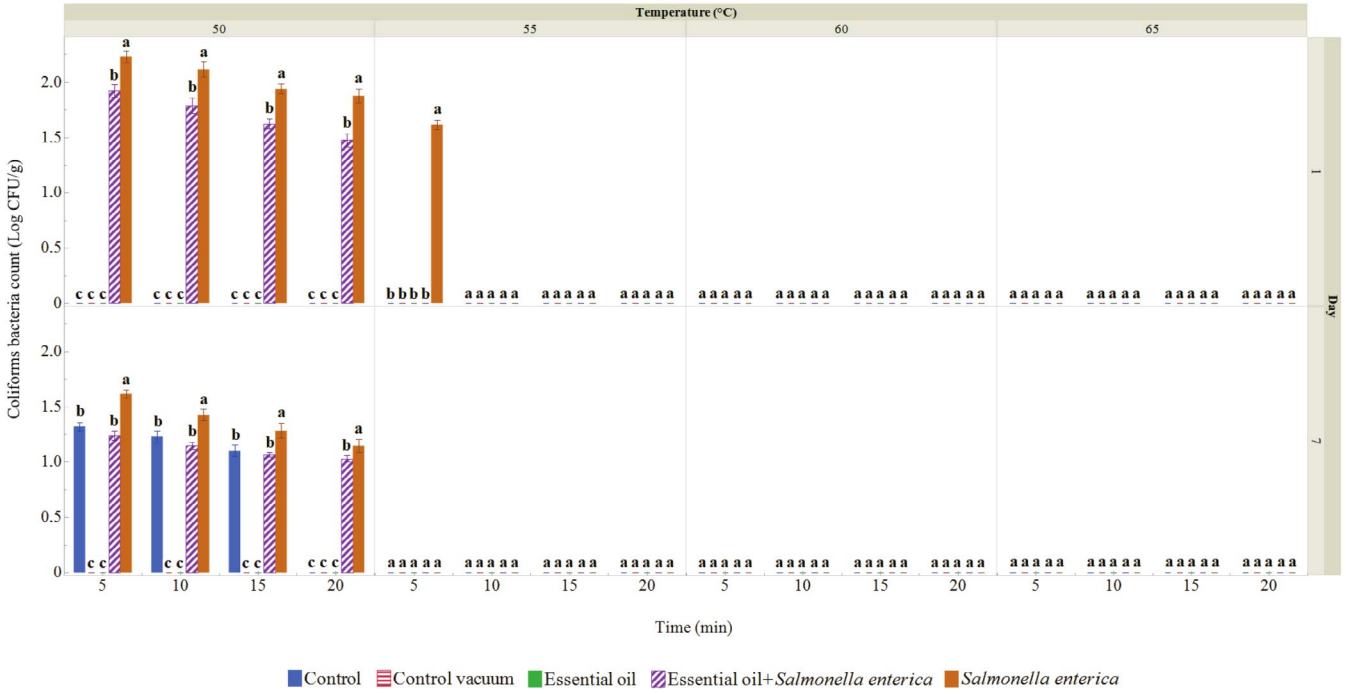


FIGURE 6 | The number of coliform bacteria (log CFU/g) in the samples that were exposed to temperatures between 50°C and 65°C for 5–20 min on the first and seventh days is demonstrated in this study. The data represent the mean (± SD) of the three samples. Significant differences are indicated by different letters in each group (Tukey, $p \leq 0.05$). The control experiment involved the storage of a fresh sample at 4°C in polyethylene bags. The control vacuum experiment entailed the vacuum-packing of a fresh sample in polyethylene bags and its storage at 4°C. The essential oil experiment involved the application of 1% ECEO to a fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C. *S. enterica* was used to treat a fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C. *S. enterica* with essential oil: A fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C was treated with 1% ECEO and *S. enterica*.

have also shown similar inhibitory effects [51]. The antibacterial potential of ECEO is further supported by its bacteriostatic properties against the tested bacterial strains. To deepen the understanding of how cardamom's bioactive components inhibit microbial virulence factors and to develop antibiofilm therapies, further research is required on the safety, purification and isolation of these compounds for use as anti-infective treatments [45].

Building on our expertise, we aimed to examine the growth kinetics of *S. enterica* under different temperature and time conditions, as well as the effect of EOs on its growth. The antibacterial effect of EOs is thought to arise from their combined actions, which include altering the cell membrane's permeability, causing the release of cellular contents, denaturing membrane proteins and disrupting proton gradients [52–55]. Additionally, recent advancements involve the integration of antimicrobials and natural preservatives into polymer matrices.

Our study aimed to evaluate the antimicrobial effects of ECEO on *S. enterica* in sous vide sweet potatoes under different storage conditions, such as varying temperatures and durations. The results indicated that ECEO significantly influenced the growth of microorganisms, coliforms and *S. enterica*. Using mass spectrometry, we identified the microorganisms in the stored sweet potatoes, with *B. amyloliquefaciens* subsp. *plantarum*, *E. faecium* and *P. agglomerans* being the most commonly found, aside from *S. enterica*. Other studies have shown that compounds like thymol and cinnamon aldehyde successfully prevented Salmonella contamination in alfalfa seeds [36]. Similarly, oregano EO was effective in protecting eggplant salad from *E. coli*, and carvacrol helped protect rice seeds from *B. cereus*, while a combination of carvacrol and cinnamon aldehyde reduced bacterial presence on kiwis [36]. The microbial activity that leads to the deterioration and weight loss of fruits and vegetables remains a significant challenge, reducing their shelf life and posing a problem for producers and consumers alike [56]. While low temperatures during storage and transport help alleviate some of these challenges, they are not sufficient to fully address the needs of consumers and businesses [57]. As a result, complementary methods such as vacuum packaging have been implemented to extend shelf life [58]. Although chemical agents have limitations in terms of environmental impact and food safety, they continue to play a key role in controlling infections during the postharvest period [59]. This has led to a growing interest in exploring alternative natural approaches, like EOs from aromatic plants, for controlling postharvest microbial growth [60]. The antibacterial potential of EOs derived from various plant species has proven effective in mitigating microbial activity in fruits and vegetables, both in vitro and in vivo [61]. Natural preservation techniques such as biocontrol agents and antimicrobial compounds are essential in inhibiting microbial growth and extending the shelf life of products like sweet potatoes [62]. Vacuum packaging has also been found to improve shelf life by creating an unfavourable environment for bacteria responsible for spoilage [63]. Heat treatment further helps to reduce microbial load and increase storage time. This study emphasises the importance of understanding the microorganisms involved in sweet potato spoilage and implementing effective measures to prevent

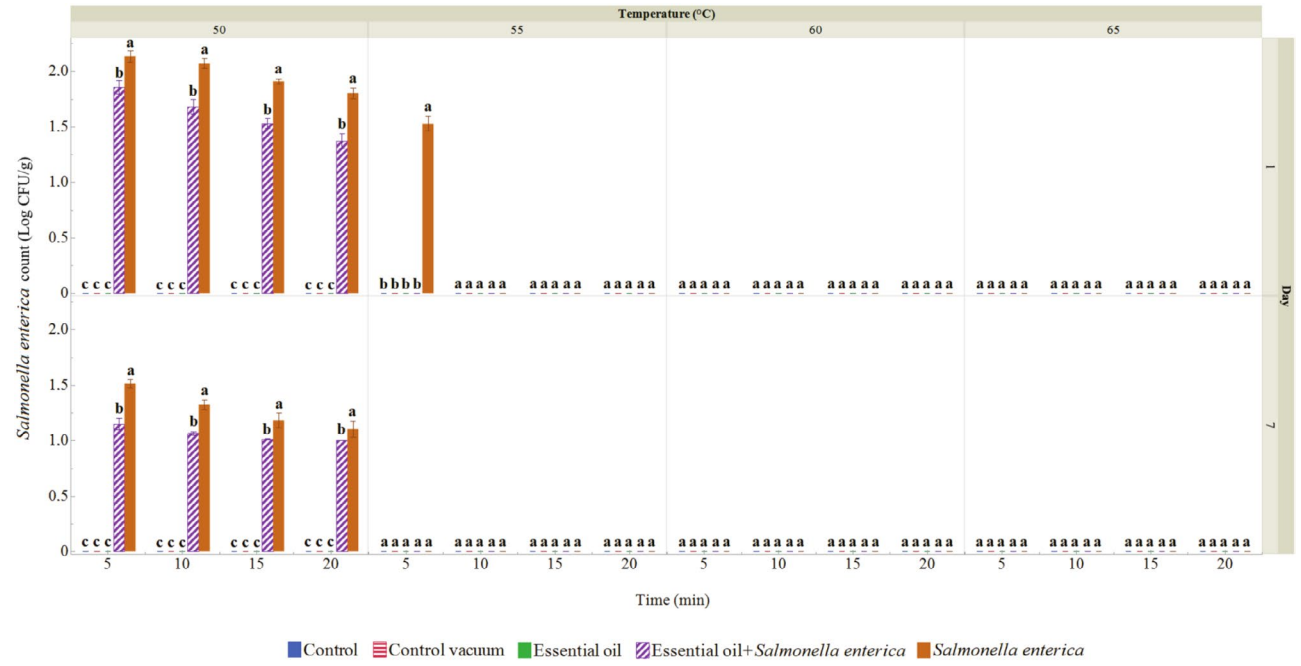


FIGURE 7 | A quantitative analysis was conducted to determine the quantity of *S. enterica* (log CFU/g) in samples that underwent temperature exposure ranging from 50°C to 65°C for durations between 5 and 20 min on the first and seventh days. The data presented hereafter represent the mean values (± SD) of the three samples. Significant differences are indicated by different letters within each group (Tukey, $p \leq 0.05$). The control experiment involved the storage of a fresh sample at 4°C in polyethylene bags. The control vacuum experiment entailed the vacuum-packing of a fresh sample in polyethylene bags and its storage at 4°C. The essential oil experiment involved the application of 1% ECEO to a fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C. *S. enterica* was used to treat a fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C. *S. enterica* with essential oil: A fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C was treated with 1% ECEO and *S. enterica*.

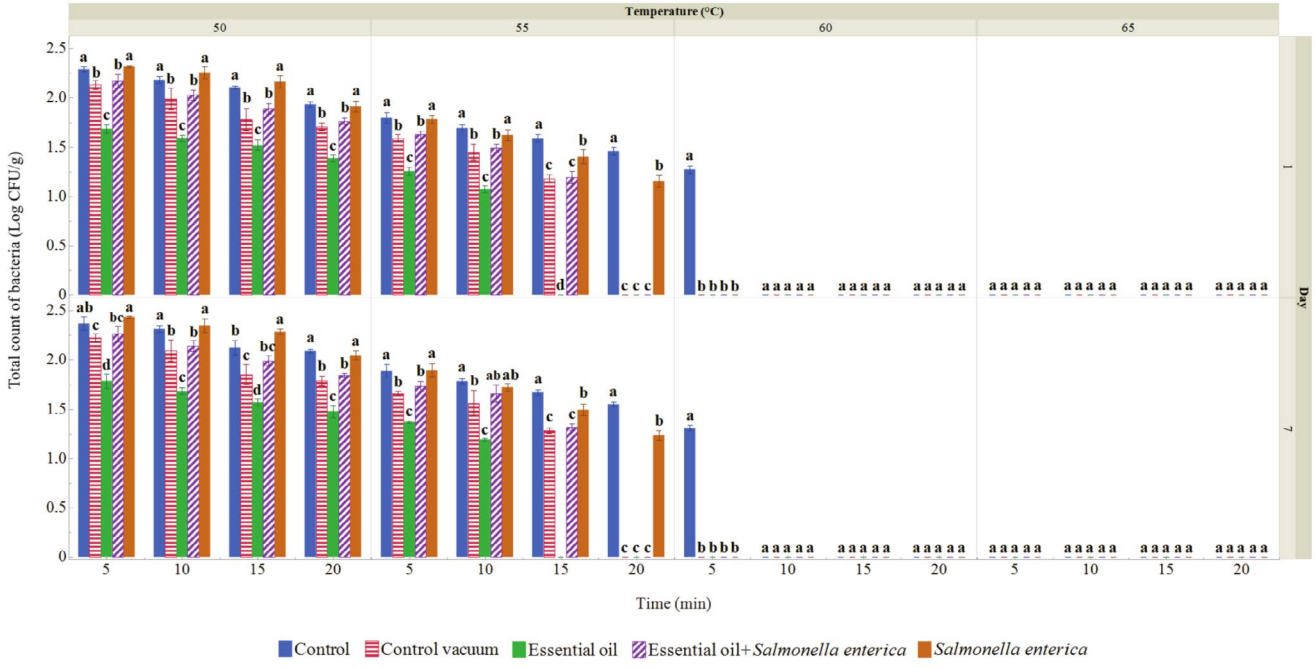


FIGURE 8 | The total bacterial count (log CFU/g) in the samples was assessed after treatment for 5–20 min on the first and seventh days at temperatures ranging from 50°C to 65°C. The data represent the mean (± SD) of the three samples. Significant differences are indicated by different letters in each group (Tukey, $p \leq 0.05$). The control experiment involved the storage of a fresh sample at 4°C in polyethylene bags. The control vacuum experiment entailed the vacuum-packing of a fresh sample in polyethylene bags and its storage at 4°C. The EO experiment involved the application of 1% ECEO to a fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C. *S. enterica* was used to treat a fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C. *S. enterica* with essential oil: A fresh sample that had been vacuum-packed in polyethylene bags and stored at 4°C was treated with 1% ECEO and *S. enterica*.



FIGURE 9 | Microorganisms isolated from sweet potatoes on day 0 expressed by Krona chart.

it [64]. In the context of sustainable development, agriculture must ensure food security while promoting economic stability, social responsibility and minimal environmental impact as the global population continues to grow [64].

5 | Conclusions

E. cardamomum essential oil (ECEO) demonstrates significant potential as a natural antibacterial agent. By further investigating this bioactive compound and providing adequate scientific backing, the prospects of developing effective, eco-friendly and safer food preservatives that can substitute synthetic chemicals may be enhanced. This study aimed to assess the antibacterial effectiveness of ECEO. The results indicated that 1,8-cineole, a well-known antibacterial compound, is the main active ingredient in the essential oil tested. This was confirmed through antimicrobial activity tests, which showed strong antibacterial effects, especially against gram-positive bacteria, across both in vitro and in situ models using fruit and



FIGURE 10 | Microorganisms isolated from sweet potatoes on day 1 expressed by Krona chart.

vegetable samples. These findings point to the promising use of ECEO for prolonging shelf life and improving the preservation of fruits and vegetables. Sous vide technology, a method involving low-temperature vacuum-sealing, is known for preserving the flavour and nutritional content of food. However, careful management of temperature and cooking duration is crucial, as improper conditions can lead to bacterial growth and potential health risks. Our study demonstrated that combining ECEO with sous vide technology effectively halted *S. enterica* growth in sweet potatoes. Furthermore, the crystal violet staining method and MALDI-TOF MS Biotyper showed that ECEO exhibited strong antibacterial and antibiofilm effects against biofilm-forming *S. enterica* on various surfaces. In summary, this study highlights the importance of understanding the microorganisms responsible for the degradation of sweet potatoes and the need for effective preventive measures to avoid spoilage.

Author Contributions

Miroslava Kačániová: methodology, investigation, writing – original draft, formal analyses, supervision, project administration, funding



FIGURE 11 | Microorganisms isolated from sweet potatoes on day 7 expressed by Krona chart.

acquisition and visualisation. **Margarita Terentjeva:** formal analyses, writing – review and editing. **Anis Ben Hsouna:** formal analyses, writing – review and editing. **Rania Ben Saad:** formal analyses, writing – review and editing. **Zhaojun Ban:** formal analyses, writing – review and editing. **Li Li:** formal analyses, writing – review and editing. **Joel Horacio Elizondo-Luevano:** formal analyses, writing – review and editing.**Alessandro Bianchi:** statistics, formal analyses, methodology, writing – original draft. **Stefania Garzoli:** formal analyses, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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