



Chemical and biological characterization and unveiling of anti-*Salmonella* effect of sous vide Hokkaido pumpkin model of *Cananga odorata* (Lam.) hook. F. & Thomson essential oil

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Abstract

This work investigated the chemical composition, antioxidant, antimicrobial and insecticidal properties of *Cananga odorata* essential oil (COEO) in relation to the use of essential oils to extend the shelf life of foods. In addition, microbiological tests were performed on Hokkaido pumpkin sous vide after *Salmonella enterica* challenge. According to GC/MS analysis, linalool (25.1%) was the main component of COEO. COEO showed its superiority to neutralize the ABTS radical cation (TEAC, 0.018) compared to DPPH radical (TEAC, 0.006). The statistically highest ($p \leq 0.05$) antibacterial activity of COEO with disc diffusion method was against *Streptococcus constellatus* (15.67 mm). In assay used for determination of minimum inhibitory concentrations (MIC) COEO showed the best activity against *Escherichia coli* (0.230 mg/mL). In situ investigations activity COEO demonstrated its statistically highest ($p \leq 0.05$) efficiency against biofilm-forming *S. enterica* in the cucumber model (86.63%), *Candida parapsilosis* and in the peach model (85.83%), *C. glabrata* in the radish model (86.99%) and *E. coli* in the strawberry model (93.27%). The results of our analyses show that the application of COEO on the sous vide Hokkaido pumpkin model is effective against *S. enterica* using the highest temperatures and time. The insecticidal activity of COEO was demonstrated at the highest concentrations of EO. Additionally, *Bacillus amyloliquefaciens* was the most often isolated bacteria found in sous vide Hokkaido pumpkin samples with different treatment. Changes in the protein composition of the biofilm-forming bacteria *S. enterica* were detected by MALDI-TOF MS. The results of our study suggest that both as a natural pesticide and as a food additive to extend the shelf life of Hokkaido pumpkin, COEO may be a suitable candidate for use in the food sector.

Keywords Chemical composition · Biological activity · Food spoilage · Ylang-ylang · Antimicrobial activity *in vitro* · Hokkaido pumpkin model

Introduction

Cananga odorata, commonly known as ylang-ylang, is a member of the Annonaceae family, which consists of over 2,050 species and 125 genera. At present, the *Cananga* genus consists of just two plant species: *C. latifolia* and *C. odorata* [1]. The geographical origin of *Cananga odorata* Hook. is tropical Asia, including the Philippines, Malaysia, Indonesia, and certain other Indian Ocean islands, such as the Comoro, Nossi Be, and Madagascar islands. F. & Thomson is the tree that is commonly known as “ylang-ylang”.

This plant was introduced to China, India, Africa and America, and is renowned for its fragrant blossom [2]. It has been documented that *C. odorata* essential oil possesses an initial scent that is characterised as medium-to-strong in intensity, with a delicate, fresh, floral, and slightly fruity quality [2]. The bloom is also said to yield a very pleasant aroma that is reminiscent of jasmine [3]. Chemical point of view, *p*-cresyl methyl ether and methyl salicylate, linalool, α -terpineol, and geraniol, methyl benzoate and eugenol, benzyl acetate, cadinene and α -caryophyllene were identified as several characterizing compounds of this oil [4]. Sesquiterpenoids

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including (*E,E*)-farnesene, humulene, germacrene D, and (*E,E*)-farnesol were also found [4].

Since the therapeutic efficacy of many currently available antibiotics has been weakened over the past decade, mainly due to their overuse, the fight against multidrug-resistant pathogens has become a global concern [5]. This has prompted the search for alternative antimicrobial agents derived from natural sources. Among them, *Cananga odorata* essential oil (COEO) has attracted increasing attention due to its promising antimicrobial properties. Linalool, identified as the main constituent of COEO in our study, has been shown to exert antibacterial and antifungal effects by disrupting microbial cell membranes, increasing membrane permeability, and causing leakage of intracellular contents. Furthermore, linalool interferes with biofilm formation by inhibiting quorum sensing and reducing the production of extracellular polymeric substances. These mechanisms contribute to the broad-spectrum antimicrobial potential of COEO and support its application as a natural preservative in food systems [6–8].

A considerable number of tests have previously been conducted on the antibacterial activity of *Cananga* essential oils, with a view to investigating their efficacy against a variety of Gram-positive and Gram-negative bacteria, as well as against harmful fungi. The results of these tests have demonstrated the beneficial effects of the oils in these contexts [9, 10]. As demonstrated in numerous reports, the bioactive constituents of EOs appear to interact with the cell surface, subsequently penetrating the membrane phospholipid bilayer. The structural integrity of cell membranes is subject to disruption due to their accumulation. This process has the potential to exert a deleterious influence on cell metabolism, ultimately resulting in cell death [11, 12].

The extensive utilisation of *C. odorata* essential oil as a flavouring agent within the food industry is substantiated by the absence of literature evidence indicating adverse outcomes resulting from its incorporation into food products [2]. These findings suggest that *C. odorata* essential oil does not present a health risk to people at the current level of consumption as a food ingredient [13].

Although several foodborne *Salmonella* spp. outbreaks have been linked to raw vegetables, little epidemiological or molecular information is available regarding *Salmonella* spp. contaminants in raw vegetables [14]. In the context of food safety, several bacterial species such as *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Yersinia enterocolitica* are of particular concern due to their occurrence in plant-based foods, including minimally processed vegetables and ready-to-eat products. Notably, *Y. enterocolitica* has been reported in salad mixes and leafy greens in various countries [15–17]. Therefore, these species were selected in this study as target microorganisms for the

assessment of the antimicrobial efficacy of *Cananga odorata* essential oil. Vegetable consumption has increased as a result of consumers' increased understanding of the health advantages of their diet. Scientific study is being directed on the application of natural antimicrobials in vegetable packaging [18]. It has been established that these products have the potential to act as a vector for the transmission of microorganisms that are responsible for foodborne poisoning. This is primarily due to the fact that they are typically consumed raw [19]. Combining several techniques, sous vide is a method for preserving the nutritional value and sensory qualities of raw or partially cooked (grilled or baked) foods [20]. It involves packaging the food in altered environments, sealing it tightly, and, if necessary, pasteurizing it. Numerous research support the advantages of sous vide [21–23]. Plant-derived substances, such essential oils (EOs), are widely used in response to customer requests to reduce or eliminate synthetic preservatives [24].

Sessile cells are those that have become permanently bonded to a substrate and are encased in an extracellular polymer matrix that has been generated by the microorganisms in question. The resulting composition of biofilms is of particular interest [25]. This state of cellular differentiation is hypothesised to facilitate the survival of the microorganisms outside the host organism and to enable further infection of new hosts. *Salmonella* spp., for instance, has been observed to exhibit resistance to external stress factors such as low water activity or the presence of disinfectants [26]. Furthermore, a number of studies have shown that *Salmonella* spp. can produce a biofilm on abiotic surfaces such plastic, rubber, cement, glass, and stainless steel [27, 28].

Controlling pests is important for both medical and economic reasons [29]. In Africa and Asia, the traditional methods of controlling storage pests have involved the use of pesticides derived from plants [30, 31]. A growing number of studies are examining the effectiveness of plant products and the mechanisms underlying their protective action, given their potential as eco-friendly substitutes for insect pest control [32–37].

The present study is motivated by the beneficial biological properties of *Cananga* species. It provides a thorough description of the chemical composition and biological profile of *C. odorata* (Lam.) Hook, F. & Thomson EO obtained from fresh flowers (COEO). The present study is an extensive investigation of the bioactive effects of *C. odorata*, with emphasis on antioxidant, antimicrobial (in vitro and in situ), antibiofilm and insecticidal activities. The medicinal benefits of *C. odorata* have been a significant stimulant in conducting this research. Moreover, the study aimed to provide significant insights into the potential application and further development of COEO as a green preservative, notably in the context of the preservation of products such

as sous vide Hokkaido pumpkin, which had been contaminated with *S. enterica*.

Materials and methods

Essential oil

The *Cananga odorata* essential oil (COEO) used in this study was purchased from Hanus s.r.o. (Nitra, Slovakia). The *Cananga odorata* (Lam.) Hook. F. & Thomson was grown in Madagascar and the EO was obtained by steam distillation of fresh CO flowers. Prior to the examination, the COEO was stored at a temperature of +4 °C.

GC/MS analysis of volatile constituents present in COEO

An Agilent Technologies (Santa Clara, California) 6890 N gas chromatograph coupled to a 5975B quadrupole mass detector was used to analyse the volatile composition of the COEO sample. The EO was dissolved in a 10% solution of hexane before analysis. The oven was configured to operate at 50 °C with a 4 °C/min increase to 70 °C (hold for 2 min), 70 °C with a 5 °C/min increase to 120 °C (hold for 1 min), and 120 °C with a 5 °C/min increase to 290 °C. The injection volume was 1 µL, and the total run duration was 52 min. Data acquisition was scheduled to begin following a solvent delay of 3.2 min. Furthermore, the temperature of the MS quadrupole was 150 °C, and the temperature of the MS ion source was 230 °C. With a split ratio of 40.8:1, the studied sample was injected in the split mode. Helium 5.0 was employed as the carrier gas, flowing at a rate of 1 mL/min. Data from electron-impact mass spectrometry (EI-MS; 70 eV) were collected in scan mode within the 35–550 *m/z* range.

To determine the retention times and mass spectra of individual n-alkanes, 1 µL of a C7-C35 mixture solution was chromatographed under slightly modified conditions. The solvent delay time was set to 2.30 min, and data acquisition continued until the GC oven maintained a final temperature of 290 °C for 8.00 min, resulting in a total run time of 65.33 min.

The compounds present in the COEO sample were identified by comparing the recorded mass spectra with those in the Wiley7 and NIST libraries, as well as by comparing the acquired MS data and experimentally calculated Kovats retention indices with literature data [38]. From their GC peak regions, compound percentages (amounts > 0.1%) were computed. The analyses were performed in duplicate.

Free radical-scavenging activity of COEO

Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Taufkirchen, Germany) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (Sigma-Aldrich, Taufkirchen, Germany) free radical-scavenging activities of COEO sample were performed in accordance with previously described methodologies [39–41].

In order to perform DPPH assay, DPPH• (Sigma-Aldrich, Schnelldorf, Germany) was prepared in methanol (Uvasol® for spectroscopy, Merck, Darmstadt, Germany). Prepared solution of DPPH• was added in the volume of 190 µL in a 96-well microtiter plate, followed by the addition of 10 µL of COEO solution in the final concentrations ranging from 3.0 to 0.1875 mg/mL (in methanol), or 10 µL of the reference compound Trolox (Sigma-Aldrich, Schnelldorf, Germany) in final concentrations in the wells ranging from 10.00 to 2.00 µg/mL (in methanol). Following the addition of the essential oil or reference compound, the reaction mixture was shaken at 1000 rpm for 30 min at room temperature in the dark. Subsequently, absorbance was measured using a microplate reader (Glomax, Promega Inc., Madison, WI, USA) at 515 nm.

For the ABTS assay, corresponding radical cation (ABTS^{•+} obtained from Sigma-Aldrich, Schnelldorf, Germany) was prepared as previously described [42]. Before conducting the analysis, prepared solution of ABTS^{•+} was dissolved using methanol to achieve the absorbance of 0.7 at 744 nm. Similarly as for the DPPH assay, 190 µL of ABTS^{•+} was added in a 96-well microtiter plate, followed by the addition of 10 µL of COEO solution in the final concentrations ranging from 3.0 to 0.01875 mg/mL (in methanol), or 10 µL of the reference compound Trolox (Sigma-Aldrich, Schnelldorf, Germany) in final concentrations in the wells ranging from 3.0 to 0.015 µg/mL (in methanol). Next, the reaction mixture was shaken at 1000 rpm for 5 min at room temperature in the dark. Afterwards, absorbance was measured using a microplate reader (Glomax, Promega Inc., Madison, WI, USA) at 744 nm.

The results of free radical-scavenging activities were obtained from appropriate calibration curves, while the values of Trolox equivalent antioxidant capacity (TEAC) were determined using standard procedures [43, 44]. The mean IC₅₀ and TEAC values are presented as the mean ± standard deviation (SD) from three independent measurements.

Antimicrobial analyses

Microorganisms tested

The assessed COEO antibacterial activity was evaluated against the following strains: Gram-positive bacteria

(G⁺): *Bacillus subtilis* subsp. *Spizizenii* CCM 1999, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* CCM 3953, and *Streptococcus constellatus* CCM 4043; Gram-negative bacteria (G⁻): *Escherichia coli* CCM 3953, *Pseudomonas aeruginosa* CCM 1959, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 7204T, and yeasts include *Candida albicans* CCM 8186, *Candida glabrata* CCM 8270, *Candida krusei* CCM 8271, *Candida parapsilosis* CCM 8260, and *Candida tropicalis* CCM 8223. All G⁺ and G⁻ bacterial species as well as yeasts were obtained for this investigation from the Czech Collection of Microorganisms (CCM), which is housed in Brno, Czech Republic. To assess the antimicrobial and antibiofilm activity, the biofilm-forming G⁻ *Salmonella enterica* was isolated and sequenced from milk production. All tested strains were screened for their ability to form biofilms prior to analysis. While the majority of microorganisms were obtained from established culture collections and represent reference strains, their capacity to form biofilms was verified experimentally. The *S. enterica* strain used in the antibiofilm assays was isolated from a dairy production environment due to its strong biofilm-forming ability. The selected microbial panel included both pathogens and food-related microorganisms of relevance to food safety. The bacterial and yeast inoculums were cultured in Mueller Hinton Broth (MHB, Oxoid, Basingstoke, UK) and Sabouraud Dextrose Broth (SDB, Oxoid, Basingstoke, UK) for a full day at 37 °C and 25 °C, respectively, prior to analysis. The optical density of the bacterial and yeast inoculum was set at 0.5 McFarland standard (1.5×10^8 CFU/mL) on the day of the experiment [45].

Disc diffusion method

The microbial strains previously documented were employed in the disc diffusion susceptibility test. Blank discs soaked in 10 µL COEO (6 mm in diameter) were used for inoculation of bacterial strains in Mueller Hinton Agar (MHA, Oxoid, Basingstoke, UK) and yeast strains in Sabouraud Dextrose Agar (SDA, Oxoid, Basingstoke, UK). The incubation process was conducted over a 24-hour period at temperatures of 37 °C for bacteria and 25 °C for yeast. The antibacterial activity of the EO was evaluated for different levels of intensity (0–5 mm), (5–10 mm), and (> 10 mm), respectively, based on the zone of inhibition. The calculation of inhibition zones was conducted from the edge of the disc. The results were expressed in mm. Clear blank discs and antibiotics/antifungal drugs (cefoxitin for G⁺, gentamicin for G⁻ bacteria; fluconazole for yeasts; Oxoid, Basingstoke, UK) were used to establish the negative and positive controls, respectively. Measurements were taken in three repetitions.

Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration values (MIC₅₀ and MIC₉₀) were previously described by Kačániová et al. [46]. The initial step in the procedure was to fill a 96-well microtiter plate with 50 µL of the microbial inoculum. This was followed by different concentrations of COEO [46] (10 mg/mL to 0.00488 mg/mL in MHB). MHB and SDB with EO (at the appropriate concentration) and MHB and SDB with inoculum were used to create the negative and positive controls, respectively. Following incubation, a spectrophotometer (Glomax, Promega Inc., Madison, WI, USA) was used to detect absorbance at 570 nm. The lowest concentrations of COEO, which inhibit 50% and 90% of bacterial growth, respectively, are represented by the MIC₅₀ and MIC₉₀ values. Three attempts were made at the exam. The MIC value results (concentration that caused 50% and 90% inhibition of microbial growth) were determined by probit analysis.

In situ analyses on the fruit and vegetables

Selected Gram-positive and Gram-negative bacteria along with yeast strains were used on commercially available strawberry, peach, radish and cucumber substrates to evaluate the antimicrobial properties of COEO in situ or in vapor phase [47]. After being cut into 0.5 mm pieces, the substrates were cleaned, dried, and put in 60 mm Petri dishes. Sliced vegetable and fruits were placed on agar. Then, an inoculum was prepared, as previously described. Ethyl acetate (Sigma-Aldrich, Taufkirchen, Germany) was used to dissolve COEO samples at concentrations of 0.5, 0.25, 0.125, and 0.0625 mg/mL. As controls, ethyl acetate filter sheets were used. After being sealed, petri dishes were incubated at 37 °C for seven days. In situ bacterial growth was determined using stereological methods. In this concept, the volume density (V_v) of bacterial colonies was first estimated using ImageJ software counting the points of the stereological grid hitting the colonies (P) and those (p) falling to the reference space (growth substrate used). The volume density of bacterial colonies was consequently calculated as follows: V_v (%) = P/p. The antibacterial activity of EO was defined as the percentage of bacterial growth inhibition (BGI) BGI = [(C – T)/C] × 100, where C and T were bacterial growth (expressed as V_v) in the control group and the treatment group, respectively. Negative results represented growth stimulation [46, 48].

Sous vide antimicrobial activity in Hokkaido pumpkin model

Two and half kg of Hokkaido pumpkin samples were used in this investigation, and they came from an approved dealer

in the Slovak Republic. After refrigeration, the Hokkaido pumpkin were transported to the microbiology laboratory and subsequently cut into 5 g pieces using a sterile knife. The knife was sterilized with ethanol after each slicing. A total of 483 five gram samples were prepared, 3 samples before treatment, 240 samples on day 1, and 240 samples on day 7. Following treatment with a 100 µL solution of 10 mg/mL COEO dissolved in commercial rapeseed oil (Poľnoservis, Slovakia), the chopped Hokkaido pumpkin samples weighing 5 g each were individually vacuum-packed in polyethylene bags using a Concept vacuum packer (Zlín, Czech Republic). Rapeseed oil used in the study was tested for microbial contamination and antimicrobial activity, confirming the absence of both microbial growth and inhibitory effects. The results of our findings showed zero values. Control samples were prepared as both vacuum-packed and unpackaged samples. The samples were then vacuum-packed after stirring for about a minute. For the experiment, *Salmonella enterica* was utilized. Muller–Hinton agar was used to culture the microbial inoculum for a duration of 24 h at 37 °C. After adjusting the inoculum to an optical density of 0.5 McFarland standard, or 1.5×10^8 CFU/mL, 100 µL was added to the samples of Hokkaido pumpkin [49].

While we were analysing it, we had access to the following data (Fig. 1):

- (i) **Control:** Fresh Hokkaido pumpkin samples were placed in polyethylene bags and stored at 4 °C. They were then cooked for five to twenty-five min at 50 to 65 °C.
- (ii) **Control+vacuum:** After being vacuum-packed in polyethylene bags and stored at 4 °C, fresh Hokkaido pumpkin samples were cooked for five to twenty-five min at 50–65 °C.
- (iii) **EO:** Fresh Hokkaido pumpkin samples were vacuum-packed, treated with 10 mg/mL COEO, and stored at 4 °C. They were then cooked to 50–65 °C for five to twenty-five min.
- (iv) ***Salmonella*:** Before being exposed to the bacteria for five to twenty-five min at 50 to 65 °C, fresh Hokkaido pumpkin samples that had been vacuum-packed and inoculated with *S. enterica* were stored at 4 °C.
- (v) ***Salmonella*+EO:** Before being cooked for five to twenty-five min at 50 to 65 °C, vacuum-packed fresh Hokkaido pumpkin samples inoculated with *S. enterica* and containing 10 mg/mL COEO were stored at 4 °C.

The control was made using raw, uncooked Hokkaido pumpkin sample on the first day. Following the application, gentle mixing, and blending of the EO from the first set of samples and the *S. enterica* from the second group of tests, all samples were macerated for an entire day. A business located in Arnsberg, Germany manufactures the CASO SV1000 sous vide apparatus, which was used to prepare the samples. The samples were divided into groups and cooked to a certain temperature and length of time under close observation in order to get them ready for sous vide cooking. The impermeable material used to make the high-barrier polyethylene vacuum packaging bags is impermeable between 40 and 200 microns, protecting the contents from moisture and extreme

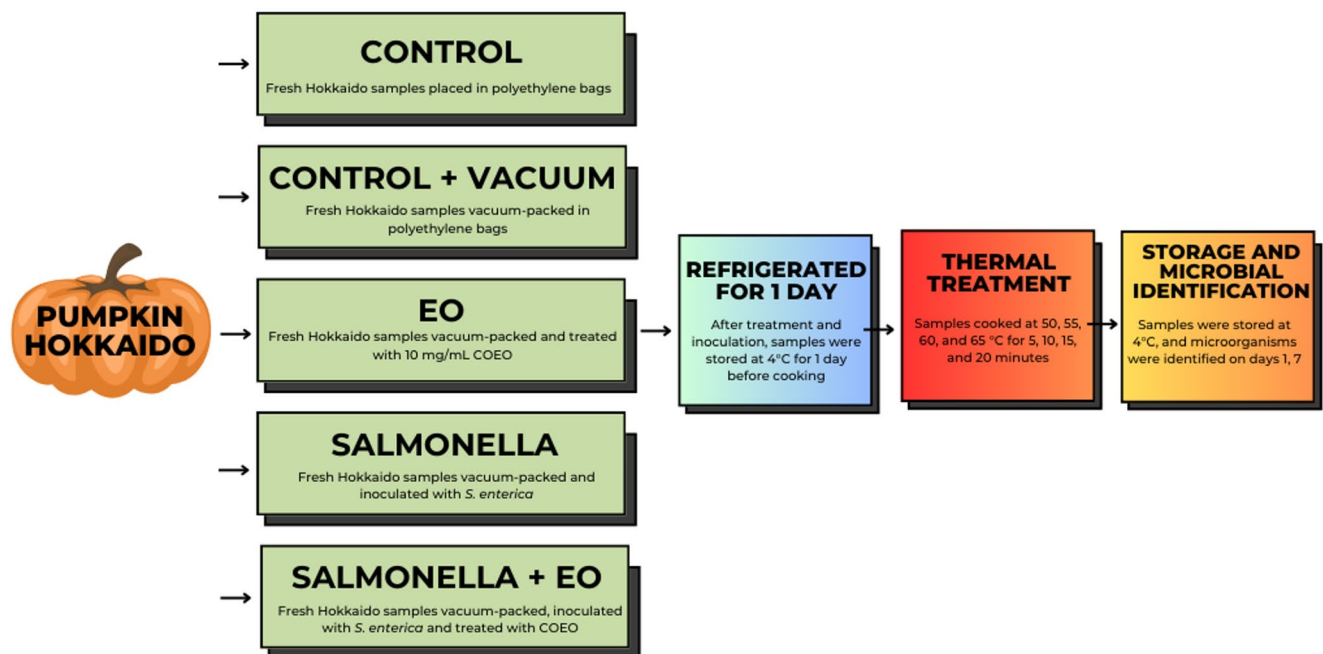


Fig. 1 Scheme of Hokkaido pumpkin samples preparation

heat or cold (-30 °C to 100 °C). Additionally, do not affect flavour, have an exceptionally long shelf life, and are free of microplastics and all plasticizers, including bisphenol A. Stored in the freezer or refrigerator, these packs can be kept for several years.

Microbiological analyses

The samples were evaluated zero day, one and seven-days following heating. Five grams of the Hokkaido pumpkin samples were weighed and then placed in an aseptic stomacher bag. Although 5 g is a relatively small portion for solid matrices, this was compensated by the high number of analyzed replicates, which contributed to result consistency. Future studies should consider testing larger sample sizes when feasible. The materials were homogenized using a stomacher for two min after being diluted to 10^{-1} in 45 mL of peptone water. Next, 0.1 mL of an aliquot pipetted from an appropriate dilution was put over a standard pre-dried plate count agar medium. The samples were homogenized for 30 min in the GFL 3031 shaking incubator (GFL, Burgwedel, Germany). We examined the populations of microorganisms: Violet Red Bile Lactose Agar (VRBL, Oxoid, Basingstoke, UK) was used to cultivate coliform bacteria. It was incubated for 24 to 48 h at 37 °C. Total viable counts (TVCs) were grown on Plate Count Agar (PCA, Oxoid, Basingstoke, UK), which was incubated at 30 °C for 48 to 72 h. For the confirmation of *S. enterica*, Xylose Lysine Deoxycholate medium (XLD, Oxoid, Basingstoke, UK) was used which was incubated at 37 °C for 24 to 48 h. Then the agars were evaluated for bacterial growth and 8 colonies or more (depending on the morphological characteristics of colonies) per plate were selected for further confirmation with MALDI-TOF MS Biotyper (Bruker Daltonics, Germany).

Identification of microbiota

Microorganisms isolated from sous vide Hokkaido pumpkin samples were identified using the MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time of Flight) MS Biotyper (Bruker, Daltonics, Bremen, Germany) and reference libraries. Altogether, 161 bacterial colonies were selected for further testing after storage. The isolates were transferred onto Tryptone soya agar at 37 °C for 24 h and a loopful of bacterial colonies was transferred into 300 µL of distilled water. After the addition of 900 µL of ethanol, the samples were centrifuged for 2 min at 14,000 rpm. The supernatant was discarded, and the same spin was repeated on the pellet. Remains of ethanol were removed, and the pellet was allowed to dry. Ten µL of 70% formic acid were added to the pellet by pipetting and vortexing. Then, 10 µL of acetonitrile were added. The suspension was centrifuged

at 14,000 rpm for 2 min and 1 µL of the supernatant was transferred to the MALDI target. Each sample was overlaid with 1 µL of MALDI matrix (a saturated solution of α -cyano-4-hydroxycinnamic acid, HCCA, Bruker Daltonik, Germany) in 50% acetonitrile and 2.5% trifluoroacetic acid (SigmaAldrich, USA). Mass spectra were automatically generated using the microflex LT MALDI-TOF mass spectrometer (Bruker Daltonik, Germany) operated in the linear positive mode within a mass range of 2000–20,000 Da. The instrument was calibrated using the Bruker bacterial test standard. Results of mass spectra were processed with the MALDI Biotyper 3.0 software (Bruker Daltonik, Germany). The identification criteria used were: a score of 2.300 to 3.000 indicated highly probable identification on species level; a score of 2.000 to 2.299 secure genus identification with probable species identification; a score of 1.700 to 1.999 probable identification to the genus level; The spectra were generated by MALDI-TOF and analyzed with Microflex LT (Bruker Daltonics, Germany) instrument using Flex Control 3.4 software and Biotyper Realtime Classification 3.1 with BC specific software [49].

Antibiofilm assay

Crystal violet study

The minimal biofilm inhibitory concentration (MBIC) by incubating bacterial suspensions in Mueller Hinton Broth at 37 °C were examined by previous study [50]. Two-fold dilutions of EO (100 mg/mL to 0.049 mg/mL) were added to a microtiter plate with an inoculum. After 24 h, wells were washed, stained, and absorbance at 570 nm was measured. MBIC₅₀, and MBIC₉₀ were determined as concentrations inhibiting 100%, 50%, and 90% of biofilm growth, respectively. Three attempts were made at the exam. The MIC value results (concentration that caused 50% and 90% inhibition of microbial growth) were determined by probit analysis.

MALDI-TOF MS biotyper for the detection of biofilm formation

The breakdown of proteins during biofilm development was assessed using the Bruker Daltonics MALDI-TOF MicroFlex device. In 50 mL polypropylene tubes filled with 20 mL of MHB and 100 µL of *S. enterica* biofilm-forming bacterial inoculum, stainless steel and glass slides were introduced. Control tubes remained untreated, while EO was added to experimental tubes to achieve a 10 mg/mL concentration. After shaking at 170×g for 3 days and incubating at 37 °C for 5, 7, 9, 12, and 14 days, biofilms were extracted from glass and stainless-steel surfaces in

triplicate. Planktonic cells from control samples lacking COEO were also examined. Biofilm samples from glass and stainless-steel were taken with a sterile cotton swab and transferred directly on the MALDI-TOF MS Biotyper plate. Planktonic cells were collected by centrifugation of 300 μ L of the medium previously collected to an Eppendorf tube at 3000 g for 3 min. The supernatant was resuspended in 25 μ L of ultrapure water and the washing procedure was performed three times. Next, 1 μ L of the suspension was applied to the MALDI-TOF MS Biotyper plate (Bruker Daltonics, Bremen, Germany). The samples were covered with 1 μ L of an acyano-4-hydroxy-cinnamic acid matrix (10 mg/mL) and dried at room temperature. After crystallization, the samples were processed with MALDI-TOF MicroFlex (Bruker Daltonics) linear and positive mode for the range of m/z 200–2000. The spectra were obtained by an automatic analysis and the same sample similarities were used to generate the standard global spectrum (MSP), 40 spectra of MALDI Biotyper 3.0 software were used for the demonstration of all the phases of biofilm development (Bruker Daltonics). MSP spectra were processed by a standard procedure using the MALDI Biotyper dendrogram method and the most representative sample of each spectrum was selected based on common characteristics. The spectra obtained in the present study were compared with the Flex-Analysis 3.0 database (Bruker Daltonics). From the spectra generated by MALDI Biotyper 3.0, 11 MSP were grouped into dendrograms using Euclidean distances. Protein spectra were obtained using MALDI-TOF in linear positive mode, and dendrograms were generated based on Euclidean distance calculation using 19 standard global spectra [50].

Insecticidal activity

The insecticidal efficacy of COEO was assessed using *Megabruchidius dorsalis* as the model organism. Groups of fifty *M. dorsalis* insects were arranged in petri plates, each sealed with sterile filter paper. COEO was diluted with 0.1% polysorbate to create concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. Sterile filter paper discs were saturated with 100 μ L of each COEO concentration and placed in the plates, which were covered with parafilm and left at room temperature for 24 h. The control group received 100 μ L of 0.1% polysorbate solution. Both live and deceased insect counts were conducted after 24 h. This experimental setup was replicated in three independent studies [50].

Statistical analyses

The data are presented as mean values $\pm$ standard deviation (SD), and each evaluation was carried out in triplicate.

A one-way ANOVA was conducted, and then the 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 program (SAS Institute, Cary, NC, USA) was used for the graphic elaborations. The ability of the strains to grow in the presence of EO was coded to binomial scale (1– growth observed, 0–without growth). Such data were processed by Probit analysis in Statgraphics Centurion XV (Statgraphics) software. Doses that inhibit the growth in 50% respectively 90% of cases (MIC_{50} and MIC_{90}) were reversely predicted from regression equation.

Results

Chemical composition of COEO

The GC/MS technique was utilized to investigate the chemical composition of the COEO volatile constituents. The results are presented in Table 1. This table shows the percentage values of the volatiles that were detected, as well as the number and percentage of compounds that fall into each class. Twenty-two compounds were identified, representing 99.9% of the total essential oil (EO) content. The analyzed sample contained esters, aromatic compounds, monoterpenes, sesquiterpenes, and phenylpropanoids, with a high abundance of oxygenated monoterpenes and sesquiterpene hydrocarbons (38.8% and 34.4%, respectively). Linalool (25.1%), germacrene D (16.1%), geranyl acetate (11.5%), *p*-methyl-anisole (11.2%) and (*E*)-caryophyllene (10.2%) were the most abundant compounds in the examined essential oil sample. These five compounds constitute 74.1% of the total essential oil composition. Additionally, methyl benzoate (6.6%), benzyl acetate (5.5%), (*E,E*)- α -farnesene (4.4%), α -humulene (2.2%), and geraniol (2.2%) were detected in amounts greater than 2%.

COEO free radical-scavenging activity properties

The antioxidant capacity of COEO was assessed based on its ability to neutralize DPPH and ABTS radical cations. The results presented in Table 2 (as IC_{50} and TEAC values) indicate that the tested COEO exhibits potential for scavenging both stable-coloured radicals, DPPH $^{\bullet}$ and ABTS $^{+\bullet}$. Notably, the COEO sample showed superior in neutralizing the ABTS radical cation compared to the DPPH radical cation.

Table 1 Chemical composition and classes of identified compounds in COEO sample

No	Compound ^a	% ^b	KI ^c	
			(lit.)	(calc.)
1	Prenyl acetate	0.7	919	922
2	α -Pinene	0.7	939	937
3	β -Pinene	tr ^d	979	981
4	β -Myrcene	tr	990	991
5	<i>p</i> -Methyl-anisole	11.2	1018	1024
6	Limonene	tr	1029	1033
7	1,8-Cineole	tr	1031	1037
8	Methyl benzoate	6.6	1090	1095
9	Linalool	25.1	1096	1101
10	Benzyl acetate	5.5	1162	1165
11	α -Terpineol	tr	1188	1196
12	Geraniol	2.2	1252	1250
13	Eugenol	tr	1359	1353
14	Geranyl acetate	11.5	1381	1379
15	β -Elemene	tr	1390	1390
16	(<i>E</i>)-Caryophyllene	10.2	1419	1422
17	(<i>E</i>)-Cinnamyl acetate	tr	1446	1447
18	α -Humulene	2.7	1454	1459
19	Germacrene D	16.1	1481	1484
20	(<i>E,E</i>)- α -Farnesene	4.4	1505	1504
21	δ -Cadinene	1.0	1523	1519
22	Benzyl benzoate	2.0	1760	1770
Total		99.9		
Class of compounds		Number of compounds	%	
Nonterpenic compounds		5	26.0	
Esters		1	0.7	
Aromatic compounds		4	25.3	
Monoterpenes		9	39.5	
Monoterpene hydrocarbons		4	0.7	
Monoterpene alcohols		3	27.3	
Monoterpene epoxides		1	tr	
Monoterpene esters		1	11.5	
Phenylpropanoids		2	tr	
Sesquiterpenes		6	34.4	
Sesquiterpene hydrocarbons		6	34.4	

^a Identified compounds; ^b Percentage composition; ^c Literature and calculated values of retention indices; ^d Percentage amounts less than 0.1% (tr)

Table 2 Antioxidant activity of COEO essential oil

Assay	IC ₅₀ (mg/mL)	TEAC
DPPH	0.710±0.019	0.006±0.000
ABTS	0.091±0.001	0.018±0.001

Antimicrobial activity

Disc diffusion assay

Using the disc diffusion method, we investigated five yeast strains, four Gram-positive (G⁺), and four Gram-negative (G⁻) bacteria in addition to *S. enterica* biofilm-forming

Table 3 Inhibitory zone of *Cananga odorata* essential oil against bacterial and fungal species in disc diffusion method (mm)

Microorganism	Inhibition zone	ATB
G ⁺ bacteria		
<i>Bacillus subtilis</i> subsp. Spizizenii CCM 1999	10.67±0.58 ^{de}	31.67±0.57 ^a
<i>Listeria monocytogenes</i> CCM 4699	14.67±0.58 ^{ab}	31.33±0.58 ^{ab}
<i>Staphylococcus aureus</i> CCM 4423	13.33±0.58 ^{bc}	29.33±0.59 ^{cde}
<i>Streptococcus constellatus</i> CCM 4043	15.67±0.57 ^a	31.33±0.58 ^{ab}
G ⁻ bacteria		
<i>Escherichia coli</i> CCM 3953	11.67±0.58 ^{cde}	30.33±0.57 ^{abc}
<i>Pseudomonas aeruginosa</i> CCM 1959	12.33±0.59 ^{cd}	29.67±0.58 ^{bcd}
<i>Salmonella enterica</i> subsp. <i>enterica</i> CCM 3807	10.33±0.56 ^{ef}	30.33±0.57 ^{abc}
<i>Yersinia enterocolitica</i> CCM 7204T	8.67±0.58 ^{fg}	27.67±0.58 ^{efg}
Yeast		
<i>Candida albicans</i> CCM 8186	7.67±0.58 ^g	25.67±0.60 ^h
<i>Candida glabrata</i> CCM 8270	7.33±0.57 ^g	26.67±0.58 ^{gh}
<i>Candida krusei</i> CCM 8271	7.67±0.59 ^g	28.33±0.59 ^{defg}
<i>Candida parapsilosis</i> CCM 8260	8.33±0.58 ^g	27.67±0.58 ^{efg}
<i>Candida tropicalis</i> CCM 8223	8.67±0.59 ^{fg}	27.33±0.59 ^{fgh}
Biofilm forming bacteria (BFB)		
<i>Salmonella enterica</i>	11.67±0.58 ^{cde}	28.67±0.58 ^{cdef}

Data are the mean (±SD) of 3 samples. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$). ATB=Antibiotics (cefoxitin for G⁺, gentamicin for G⁻ bacteria; fluconazole for yeasts)

bacteria (BFB) as part of our inquiry into the antibacterial characteristics of COEO. The results showed that among the Gram-positive species, COEO had the statistically ($p \leq 0.05$) highest antibacterial activity against *S. constellatus* (15.67 mm), Gram-negative species, *P. aeruginosa* (12.33 mm), and yeasts, *C. tropicalis* (8.67 mm). It was shown that COEO has 11.67 mm of antibacterial activity against *S. enterica*, which forms biofilms. When compared to COEO, antibiotics showed higher antibacterial efficiency. However, when treated with cefoxitin, gentamycin and fluconazole, *B. subtilis*, *E. coli*, *S. enterica* and *C. krusei* showed the strongest resistance to antibiotics (Table 3).

Minimal inhibitory concentration

Using the broth microdilution method, MIC₅₀ and MIC₉₀, or minimum inhibitory concentrations, were found in order to give a more thorough understanding of the antibacterial activity of COEO. All things considered, the COEO had the strongest impact on the inhibition of G⁻ species. Particularly, the statistically ($p \leq 0.05$) lowest MIC₉₀ values (0.16

and 0.18 mg/mL) and MIC₅₀ values (0.13 and 0.14 mg/mL, respectively) were shown by *E. coli* and *S. enterica*. For G⁺ *B. subtilis* and *S. constellatus*, MIC₅₀ (0.17 and 0.184 mg/mL, respectively) and MIC₉₀ (0.230 and 0.208 mg/mL, respectively) were determined. The results of COEO's suppression of *S. enterica* biofilm-forming exhibited MIC₅₀ values of 0.33 mg/mL and MIC₉₀ values of 0.38 mg/mL. The yeast strains *C. parapsilosis* and *C. krusei* showed the lowest MIC results; their respective MIC₅₀ and MIC₉₀ values were 0.14 and 0.19 mg/mL and 0.15 and 0.18 mg/mL, respectively. The detailed results of the minimal inhibitory concentration assay are shown in Table 4.

In situ antimicrobial activity

Using fruits and vegetables as food models, we performed an in situ antimicrobial investigation in the following experiment. The same bacterial and yeasts strains that were used in the in vitro evaluation were employed. This action was taken to assess the antimicrobial qualities of the EO obtained from *C. odorata*. Table 5; Fig. 2a, b displays the vapor phase data for the fruit model, while Table 6; Fig. 3a, b presents the results for the vegetable model. Overall, the vapor phase of COEO demonstrated higher efficacy in

suppressing G⁻ strains, following G⁺, and yeasts in the fruits model as compared to the in vitro testing. When COEO was used to assess the development of *E. coli* on strawberry in situ, the results indicated that the highest concentration utilized (0.5 mg/mL) had the statistically ($p \leq 0.05$) strongest inhibitory effect (93.27%). The maximum dose of COEO tested (86.12% and 85.52%, respectively) considerably inhibited the growth of *Y. enterocolitica* and *B. subtilis* in the strawberry. When applied at a dose of 0.5 mg/mL, COEO had the largest inhibitory impact against biofilm-forming *S. enterica* (86.41%); however, even at lower concentrations (0.25, 0.125, and 0.0625 mg/mL), a significant ($p \leq 0.05$) degree of inhibition was maintained against this microorganism in comparison to the other examined. Additionally, EO demonstrated significant antibacterial activity against *S. aureus* (76.21%) at the highest tested dose of 0.5 mg/mL. The statistically maximum ($p \leq 0.05$) antibacterial activity against *C. krusei* was seen at 0.5 mg/mL (74.58%) concentration. COEO showed modest inhibitory effects in the in situ evaluation of G⁺ bacteria and yeast development on peach. At 0.5 mg/mL, COEO showed the highest inhibitory activity among the G⁺ strains against *L. monocytogenes* (85.00%). Similarly, at the highest concentrations examined, COEO showed the maximum inhibitory effect against *C. parapsilosis* (87.00%) among the *Candida* strains growing on peach. According to the results, among the G⁻ bacteria growing on peaches, COEO exhibited the most significant efficiency when applied at the maximum concentration in suppressing the growth of *Y. enterocolitica* (74.59%) and *E. coli* (74.52%). Furthermore, at the highest tested concentration, COEO had a potent inhibitory action against *S. enterica*.

In a subsequent section of our experiment, we report on the antibacterial activity results on vegetables against all microorganisms under observation in the vapor phase. On the radish model, the best antimicrobial results were found against *C. glabrata* (86.99%) in the highest concentration, followed by the G⁺ bacterium *B. subtilis* (86.36%), *S. aureus* (85.89%) and the G⁻ bacteria biofilm-producing *S. enterica* (85.83%) also in the highest concentration of 0.5 mg/mL. Conversely, the statistically ($p \leq 0.05$) lowest applied EO concentration of 0.0625 µg/L showed the highest antimicrobial activity in the cucumber model. The best results were achieved on the cucumber model against *S. enterica* (86.63%), followed by *E. coli* with 78.43% inhibition and *L. monocytogenes* with 78.36% inhibition. Quite satisfactory results were achieved against other G⁺ and G⁻ bacteria, while the highest antimicrobial activity on the cucumber model was achieved with yeast against *C. glabrata* (76.73%) at the lowest COEO concentration.

In conclusion, as shown in Fig. 2a, b and a for the 3 food models used (Strawberry, Peach and Radish), by increasing

Table 4 Minimal inhibitory concentration and minimal biofilm inhibitory concentration of COEO in mg/ml

Microorganism	MIC ₅₀	MIC ₉₀
G ⁺ bacteria		
<i>Bacillus subtilis</i> subsp. Spizizenii CCM 1999	0.184 ± 0.02 ^d	0.208 ± 0.02 ^e
<i>Listeria monocytogenes</i> CCM 4699	0.748 ± 0.01 ^b	0.900 ± 0.05 ^b
<i>Staphylococcus aureus</i> CCM 4423	1.510 ± 0.07 ^a	1.647 ± 0.06 ^a
<i>Streptococcus constellatus</i> CCM 4043	0.166 ± 0.06 ^d	0.226 ± 0.04 ^e
G ⁻ bacteria		
<i>Escherichia coli</i> CCM 3953	0.133 ± 0.01 ^d	0.177 ± 0.03 ^e
<i>Pseudomonas aeruginosa</i> CCM 1959	0.341 ± 0.02 ^c	0.384 ± 0.01 ^d
<i>Salmonella enterica</i> subsp. <i>enterica</i> CCM 3807	0.142 ± 0.01 ^d	0.164 ± 0.01 ^e
<i>Yersinia enterocolitica</i> CCM 7204T	0.145 ± 0.01 ^d	0.184 ± 0.02 ^e
Yeast		
<i>Candida albicans</i> CCM 8186	0.723 ± 0.01 ^b	0.785 ± 0.02 ^c
<i>Candida glabrata</i> CCM 8270	0.344 ± 0.02 ^c	0.382 ± 0.01 ^d
<i>Candida krusei</i> CCM 8271	0.152 ± 0.02 ^d	0.184 ± 0.01 ^e
<i>Candida parapsilosis</i> CCM 8260	0.144 ± 0.02 ^d	0.188 ± 0.02 ^e
<i>Candida tropicalis</i> CCM 8223	0.339 ± 0.02 ^c	0.367 ± 0.01 ^d
Biofilm forming bacteria (BFB)		
<i>Salmonella enterica</i>	0.331 ± 0.01 ^c	0.379 ± 0.01 ^d

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

Table 5 In situ analysis of the COEO antimicrobial activity (%) in fruits model

Food model	Microorganisms	Concentration of COEO in mg/mL			
		0.625	0.125	0.25	0.5
Strawberry					
G ⁺	<i>Bacillus subtilis</i>	27.77±3.21 ^c	44.29±2.71 ^c	64.93±2.56 ^b	85.52±2.00 ^{ab}
	<i>Listeria monocytogenes</i>	33.22±1.78 ^c	44.93±1.45 ^c	54.58±3.31 ^d	73.56±2.95 ^{de}
	<i>Staphylococcus aureus</i>	14.66±1.06 ^d	33.86±2.78 ^d	54.22±2.98 ^d	76.21±4.22 ^{bcd}
	<i>Streptococcus constellatus</i>	42.92±2.39 ^b	54.81±1.37 ^b	64.73±1.22 ^b	72.30±2.06 ^{de}
G [−]	<i>Escherichia coli</i>	34.47±0.16 ^c	53.53±1.06 ^b	74.06±1.48 ^a	93.27±5.10 ^a
	<i>Pseudomonas aeruginosa</i>	44.67±2.53 ^b	53.14±3.93 ^b	64.43±1.10 ^b	74.67±0.11 ^{cde}
	<i>Salmonella enterica</i>	32.66±0.18 ^c	44.76±1.00 ^c	54.44±2.11 ^d	66.48±2.63 ^{de}
	<i>Yersinia enterocolitica</i>	54.66±2.93 ^a	64.26±1.22 ^a	75.81±2.66 ^a	84.41±0.63 ^{abc}
Yeast	<i>Candida albicans</i>	33.15±0.53 ^c	42.89±1.43 ^c	55.36±3.34 ^{cd}	72.35±6.04 ^{de}
	<i>Candida glabrata</i>	44.06±1.56 ^b	55.14±1.40 ^b	63.66±2.18 ^b	74.55±3.99 ^{cde}
	<i>Candida krusei</i>	43.19±2.23 ^b	54.47±2.21 ^b	62.66±2.61 ^{bc}	74.58±3.95 ^{cde}
	<i>Candida parapsilosis</i>	44.14±0.46 ^b	53.84±2.19 ^b	64.12±1.60 ^b	73.45±2.11 ^{de}
	<i>Candida tropicalis</i>	33.66±2.22 ^c	42.48±1.61 ^c	53.59±1.63 ^d	64.91±3.54 ^e
BFB	<i>Salmonella enterica</i>	54.52±3.28 ^a	66.25±1.65 ^a	74.17±2.60 ^a	86.41±1.70 ^{ab}
Peach					
G ⁺	<i>Bacillus subtilis</i>	34.33±2.13 ^{cd}	45.27±1.73 ^c	54.27±2.19 ^c	66.39±2.55 ^c
	<i>Listeria monocytogenes</i>	27.59±6.07 ^{de}	45.00±1.25 ^c	64.25±2.34 ^b	86.00±1.97 ^a
	<i>Staphylococcus aureus</i>	23.93±2.68 ^{ef}	35.96±1.56 ^d	55.26±1.61 ^c	77.95±1.22 ^b
	<i>Streptococcus constellatus</i>	17.88±1.10 ^f	34.27±1.57 ^d	54.33±1.39 ^c	74.21±1.74 ^b
G [−]	<i>Escherichia coli</i>	17.87±1.10 ^f	33.97±1.22 ^d	46.40±2.20 ^d	74.52±2.16 ^b
	<i>Pseudomonas aeruginosa</i>	25.96±0.50 ^e	34.93±1.90 ^d	43.74±2.01 ^d	55.51±4.24 ^d
	<i>Salmonella enterica</i>	34.71±2.11 ^c	45.37±1.78 ^c	56.03±2.30 ^c	66.11±1.60 ^c
	<i>Yersinia enterocolitica</i>	43.73±0.96 ^b	54.63±2.02 ^b	65.41±1.59 ^b	74.59±2.66 ^b
Yeast	<i>Candida albicans</i>	44.77±1.72 ^b	57.00±1.66 ^b	63.74±2.56 ^b	74.56±1.93 ^b
	<i>Candida glabrata</i>	44.07±2.03 ^b	55.11±2.88 ^b	64.06±1.21 ^b	75.10±1.56 ^b
	<i>Candida krusei</i>	44.08±1.45 ^b	54.82±3.62 ^b	66.54±2.32 ^b	74.59±1.80 ^b
	<i>Candida parapsilosis</i>	43.46±0.69 ^b	55.99±1.09 ^b	65.25±1.50 ^b	74.79±1.59 ^b
	<i>Candida tropicalis</i>	33.40±1.10 ^{cd}	46.74±2.05 ^c	54.56±1.94 ^c	66.70±1.68 ^c
BFB	<i>Salmonella enterica</i>	55.79±0.95 ^a	66.62±2.77 ^a	76.66±2.81 ^a	85.83±1.67 ^a

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

the concentration of COEO used, the % inhibition tends to increase. In all of these models, concentrations of 0.25 mg/mL and 0.5 mg/mL led to a % inhibition greater than 50% for all the microorganisms tested. Interesting to note that as regards *S. enterica* BFB, for all 3 models even at COEO concentrations of 0.0625 mg/mL the % inhibition against this pathogen remains above 50% up to the maximum concentration (0.5 mg/mL) at percentages greater than 85%.

On the contrary, in the cucumber model (Fig. 3b) we see an inverse trend. In fact, in this case, by increasing the COEO concentration the inhibition percentage tends to decrease, indicating that a greater effect occurs at low concentrations (0.0625 mg/mL).

As for others, only in the case of *S. enterica* BFB, in all 4 concentrations tested the inhibition percentage remains greater than 50%, and reaches its maximum (86.63%) at the concentration of 0.0625 µg/L.

Sous vide microbiological analyses of Hokkaido pumpkin

Microbiological investigation was performed on raw Hokkaido pumpkin to verify the presence of *S. enterica* on XLD agar. No coliform bacteria were evaluated on day 0., and the total viable count (TVC) was 2.16 log CFU/g. The TVC of the first and seventh days of storage was used to evaluate the microbiological quality of the vacuum-packed Hokkaido pumpkin (Fig. 4).

Within the control group, the range of bacterial counts was 1.31 (60 °C at 5 min) to 2.38 log CFU/g (50 °C at 5 min) on day first and 1.06 (60 °C at 20 min) to 2.45 log CFU/g (50 °C at 5 min) on day seven. TVC ranged between 1.19 (55 °C at 15 min) and 2.22 log CFU/g (50 °C at 5 min) on day 1 and between 1.04 (55 °C at 20 min) and 2.36 log CFU/g (50 °C at 5 min) on day 7 within the vacuum-packed group. Within the group that was vacuum-packed and subjected to COEO treatment, log CFU/g varied between 1.04

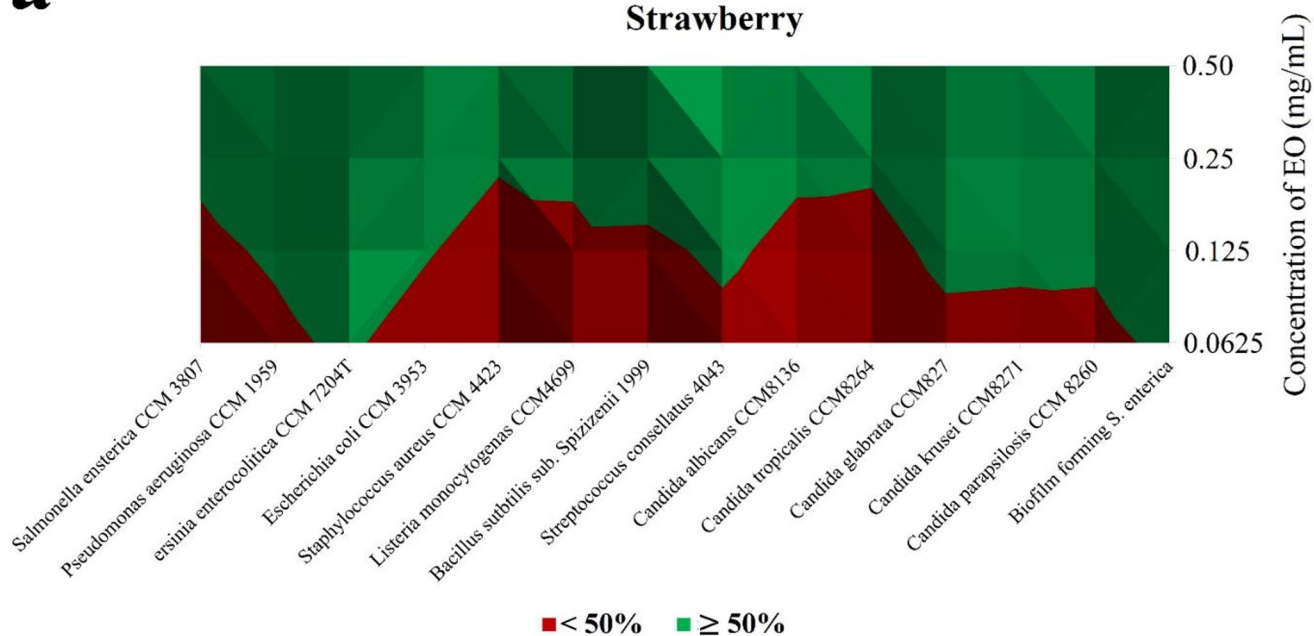
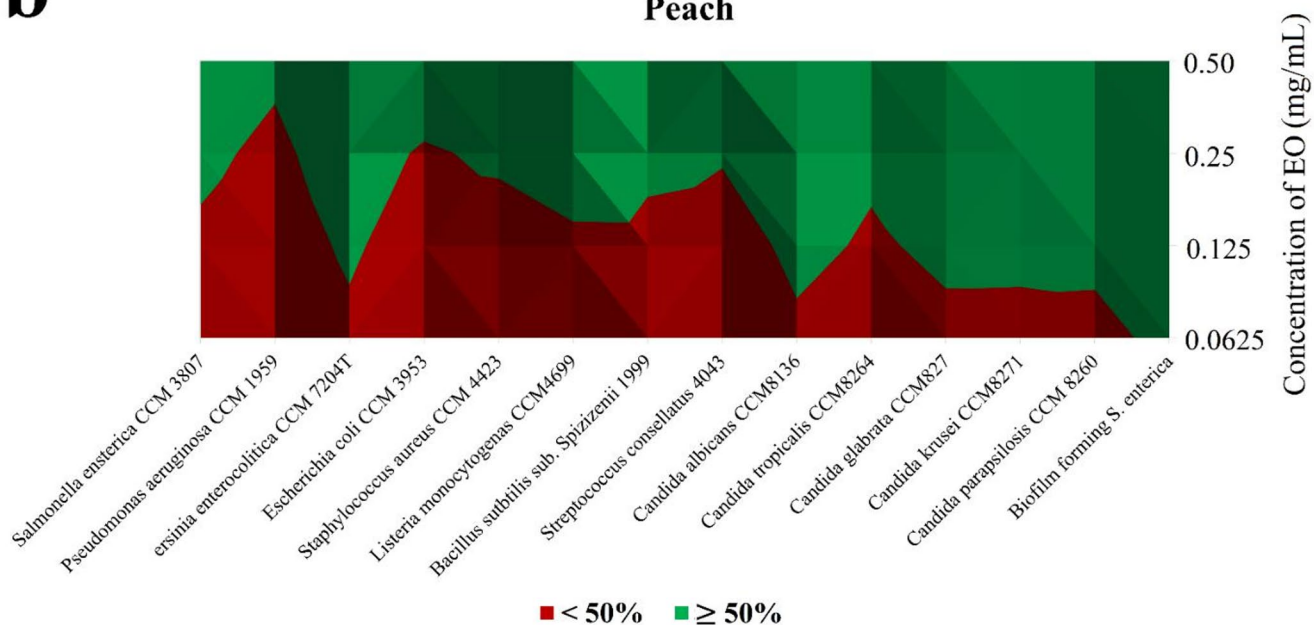
a**Strawberry****b****Peach**

Fig. 2 Isometric elaboration of the data of Table 5 (In situ analysis of the antimicrobial activity of the vapor phase of COEO in fruits model): **a** Strawberries; **b** Peach. Red: < 50% of inhibition; Green: ≥ 50% of inhibition

(55 °C at 10 min) and 1.76 (50 °C at 5 min) on the first day and 1.06 (50 °C at 20 min) and 1.45 (50 °C at 5 min) on the seventh. TVC ranged from 1.22 (55 °C at 20 min) to 2.35 log CFU/g (50 °C at 5 min) on day 1 and from 1.37 (55 °C at 5 min) to 2.43 log CFU/g (50 °C at 5 min) on day 7 for the *S. enterica* treatment group. In the presence of *S.*

enterica, TVC varied from 1.08 (55 °C at 15 min) to 2.23 log CFU/g (50 °C at 5 min) on day 1 and from 1.12 (50 °C at 20 min) to 1.57 log CFU/g (50 °C at 5 min) on day 7 following vacuum-packing and COEO treatment. In general, there were less bacteria in the groups who received COEO and *S. enterica* treatments (Fig. 4).

Table 6 In situ analysis of the COEO antimicrobial activity (%) in vegetable model

Food model	Microorganisms	Concentration of COEO in mg/mL			
		0.0625	0.125	0.25	0.5
Radish					
G ⁺	<i>Bacillus subtilis</i>	56.54±1.46 ^a	66.32±1.15 ^a	75.85±1.67 ^a	86.36±1.39 ^a
	<i>Listeria monocytogenes</i>	44.97±0.61 ^b	56.23±2.76 ^b	64.97±1.14 ^b	84.61±3.91 ^a
	<i>Staphylococcus aureus</i>	54.81±4.66 ^a	64.63±1.05 ^a	78.05±0.82 ^a	85.89±0.77 ^a
	<i>Streptococcus constellatus</i>	34.23±0.58 ^c	44.57±1.21 ^c	57.17±1.22 ^c	67.87±0.02 ^{cde}
G ⁻	<i>Escherichia coli</i>	55.48±2.78 ^a	64.22±2.71 ^a	76.63±1.79 ^a	84.08±2.62 ^a
	<i>Pseudomonas aeruginosa</i>	36.51±1.19 ^c	44.90±1.82 ^c	55.74±2.26 ^c	66.61±2.03 ^c
	<i>Salmonella enterica</i>	43.48±0.85 ^b	55.69±2.21 ^b	63.35±0.17 ^b	74.60±1.17 ^b
	<i>Yersinia enterocolitica</i>	45.63±2.09 ^b	56.40±2.56 ^b	65.15±1.46 ^b	73.74±2.22 ^{bcd}
Yeast	<i>Candida albicans</i>	34.23±0.67 ^c	44.63±1.86 ^c	55.03±1.72 ^c	67.16±1.20 ^{de}
	<i>Candida glabrata</i>	54.56±2.01 ^a	65.14±1.10 ^a	76.77±1.16 ^a	86.99±2.34 ^a
	<i>Candida krusei</i>	45.77±2.06 ^b	56.33±1.78 ^b	67.32±1.68 ^b	74.49±2.11 ^b
	<i>Candida parapsilosis</i>	46.06±2.23 ^b	54.86±1.72 ^b	65.23±2.33 ^b	74.38±2.77 ^{bc}
BFB	<i>Candida tropicalis</i>	36.78±1.11 ^c	44.41±1.09 ^c	54.14±2.18 ^c	66.91±1.67 ^c
	<i>Salmonella enterica</i>	56.63±2.94 ^a	66.95±1.62 ^a	75.66±2.91 ^a	85.83±3.39 ^a
Cucumber					
G ⁺	<i>Bacillus subtilis</i>	76.63±1.99 ^b	64.78±5.05 ^{bc}	56.77±2.93 ^b	45.14±2.39 ^b
	<i>Listeria monocytogenes</i>	78.36±0.43 ^b	66.43±2.79 ^b	56.96±2.31 ^b	45.10±1.24 ^b
	<i>Staphylococcus aureus</i>	76.63±1.22 ^b	67.73±1.22 ^{ab}	56.45±0.58 ^b	46.48±0.92 ^b
	<i>Streptococcus constellatus</i>	76.44±2.26 ^b	65.15±1.57 ^{bc}	54.80±1.75 ^{bcd}	34.63±1.12 ^d
G ⁻	<i>Escherichia coli</i>	78.43±0.52 ^b	56.78±1.11 ^d	46.39±2.28 ^{def}	36.77±1.92 ^{cd}
	<i>Pseudomonas aeruginosa</i>	67.59±0.53 ^c	57.88±1.10 ^{cd}	46.14±2.67 ^{ef}	36.84±2.21 ^{cd}
	<i>Salmonella enterica</i>	76.28±2.41 ^b	67.58±1.51 ^{ab}	54.48±2.21 ^{bcde}	45.74±2.10 ^b
	<i>Yersinia enterocolitica</i>	75.61±2.92 ^b	66.55±2.15 ^b	55.11±2.08 ^{bc}	44.03±1.46 ^{bc}
Yeast	<i>Candida albicans</i>	67.76±0.84 ^c	56.54±0.51 ^d	46.06±1.68 ^{ef}	33.92±1.28 ^{de}
	<i>Candida glabrata</i>	76.73±2.21 ^b	71.03±4.90 ^{ab}	59.67±7.23 ^{ab}	49.10±7.65 ^{ab}
	<i>Candida krusei</i>	57.05±1.78 ^d	46.14±2.67 ^e	35.66±3.10 ^g	26.04±2.31 ^e
	<i>Candida parapsilosis</i>	66.83±2.20 ^c	57.86±1.09 ^{cd}	47.86±1.09 ^{cdef}	35.74±2.84 ^d
BFB	<i>Candida tropicalis</i>	68.80±0.84 ^c	55.33±2.28 ^d	45.08±2.06 ^f	34.26±1.58 ^d
	<i>Salmonella enterica</i>	86.63±3.10 ^a	75.16±3.56 ^a	65.71±1.89 ^a	56.48±1.58 ^a

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

Coliform bacteria (CB) counts were not found in the COEO group, vacuum-packed control group, and control group on the first day (Fig. 5). On the seventh day, the CB in the control group varied between 1.06 (50 °C at 15 min) and 1.32 log CFU/g (50 °C at 5 min). Following a seven-day period, neither the COEO-using group nor the vacuum-packed group contained any coliform bacteria. CB ranged from 1.73 (55 °C at 5 min) to 2.34 log CFU/g (50 °C at 5 min) in the group to which *S. enterica* was added on the first day, and from 1.33 (50 °C at 20 min) to 1.68 log CFU/g (50 °C at 5 min) on the seventh. Within the treatment group that received COEO and *S. enterica*, CB ranged between 1.53 (50 °C at 20 min) and 2.09 log CFU/g (50 °C at 5 min) on day one and between 1.00 (50 °C at 20 min) and 1.36 log CFU/g (50 °C at 5 min) on day seven.

Data are the mean (±SD) of 3 samples. Control: Fresh Hokkaido pumpkin samples were treated at 50–65 °C for 5 to 25 min after being vacuum-packed in polyethylene bags and kept at 4 °C. Control vacuum: Fresh Hokkaido pumpkin

samples were treated at 50–65 °C for 5 to 25 min after being vacuum-packed in polyethylene bags and kept at 4 °C. Ess. oil: vacuum-packed Hokkaido pumpkin fresh sample were treated with 10 mg/mL COEO and were kept at 4 °C and treated for 5–25 min at 50–65 °C. *Salmonella*: vacuum-packed Hokkaido pumpkin fresh samples inoculated with *S. enterica* were kept at 4 °C and treated for 5–25 min at 50–65 °C. *Salmonella*+EO: vacuum-packed Hokkaido pumpkin fresh sample treated with *S. enterica* and 10 mg/mL COEO were kept at 4 °C and treated for 5–25 min at 50–65 °C.

Figure 6 shows the species of microorganisms isolated from all tested samples on day 1 of the experiment. Mass spectrometry was used to identify 255 isolates in total. On the first day of monitoring, a total of 26 species, 15 genera, and 13 families were identified from the vacuum-packed Hokkaido samples. *S. enterica* was the most isolated species (21%), followed by *Bacillus amyloliquefaciens* (13%) and *Enterococcus faecium* (7%).

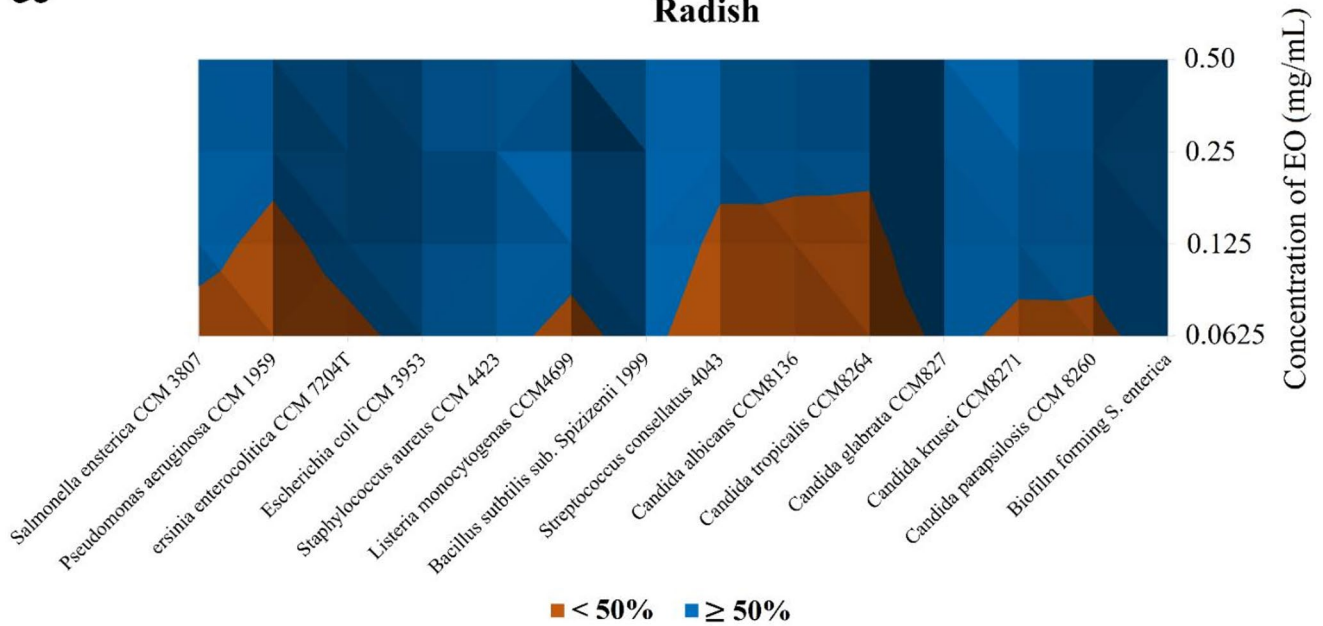
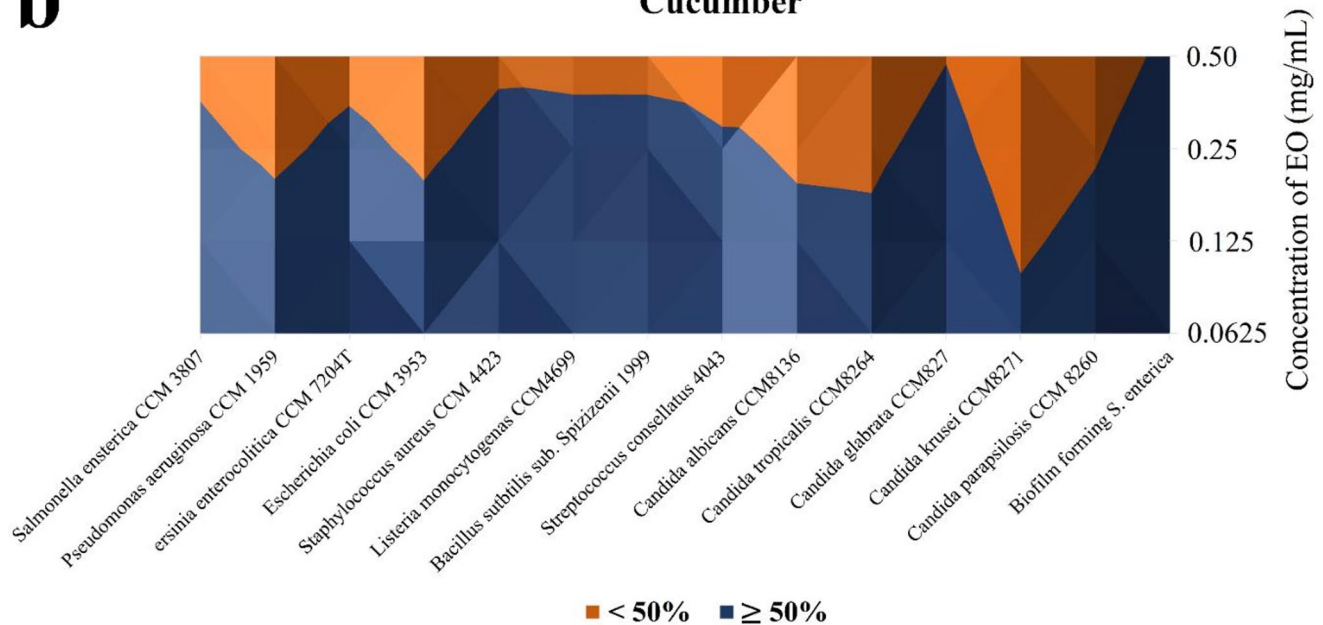
a**Radish****b****Cucumber**

Fig. 3 Isometric elaboration of the data of Table 6 (in situ analysis of the antimicrobial activity of the vapor phase of COEO in vegetable model): (a) Radish; (b) Cucumber. Orange: < 50% of inhibition; Blue: ≥ 50% of inhibition

Figure 7 shows the species of microorganisms isolated from all tested samples on day 7 of the experiment. Using mass spectrometry, 205 isolates comprising 14 families, 17 genera, and 21 species were identified from the sous vide Hokkaido pumpkin samples that were identified on the first observation day. *S. enterica* (30%), *Brevibacillus agri* (7%)

and *Enterococcus faecium* (7%) were the most isolated species.

Although the microbial identification did not represent a full microbial community profile, it served as a qualitative supplementary analysis to illustrate the predominant surviving species across all groups. The isolates displayed in

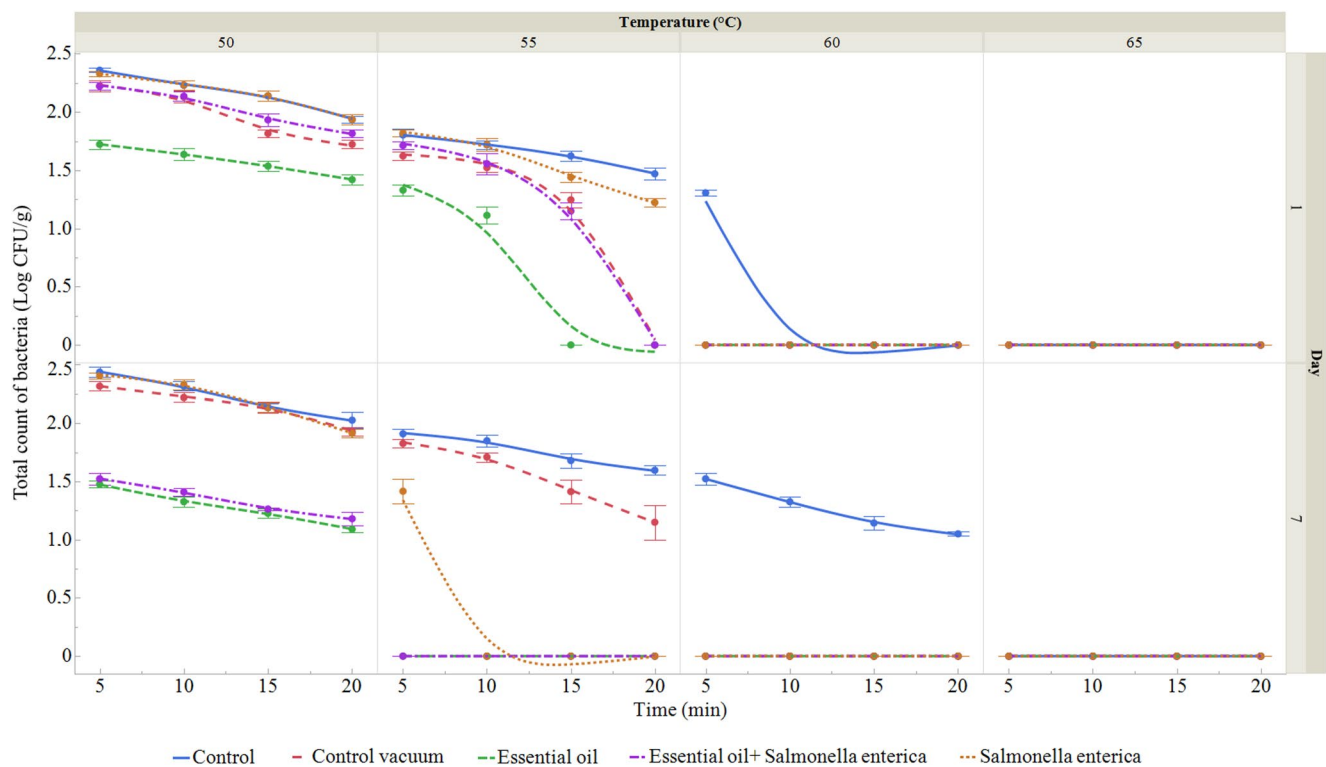


Fig. 4 Total viable count of Hokkaido pumpkin samples treated at temperatures ranging between 50 and 65 °C for durations of 5 to 20 min. (expressed in log CFU/g) on the first day and seventh day. Data are the mean ($\pm$ SD) of 3 samples. Control: Fresh Hokkaido pumpkin samples were treated at 50–65 °C for 5 to 25 min after being vacuum-packed in polyethylene bags and kept at 4 °C. Control vacuum: Fresh Hokkaido pumpkin samples were treated at 50–65 °C for 5 to 25 min after being vacuum-packed in polyethylene bags and kept at 4 °C. Ess. oil:

vacuum-packed Hokkaido pumpkin fresh samples were treated with 10 mg/mL COEO and were kept at 4 °C and treated for 5–25 min at 50–65 °C. *Salmonella*: vacuum-packed Hokkaido pumpkin fresh samples inoculated with *S. enterica* were kept at 4 °C and treated for 5–25 min at 50–65 °C. *Salmonella*+EO: vacuum-packed Hokkaido pumpkin fresh sample treated with *S. enterica* and 10 mg/mL COEO were kept at 4 °C and treated for 5–25 min at 50–65 °C

Figs. 6 and 7 were pooled from all samples collected at the respective time points (day 1 and day 7). The aim was to provide general insight into microbial survival post-treatment. This analysis supports the quantitative microbiological data and is not intended as a standalone outcome. Including data from all sample groups and time points individually would have greatly increased the manuscript's length without adding proportional scientific value.

Antibiofilm activity of COEO

Antibiofilm activity was monitored using the crystal violet method and MALDI-TOF MS Biotyper. Antibiofilm activity against biofilm-forming *S. enterica* bacteria was at MBIC₅₀ 0.330 $\pm$ 0.01 mg/mL and at MBIC₉₀ 0.380 $\pm$ 0.00 mg/mL (Table 4). Utilizing MALDI-TOF MS Biotyper mass spectrometry, the study examined the impact of COEO on bacteria that produce biofilms, particularly *S. enterica*. Planktonic cells without any biofilm treatment were used as control groups, and their spectra were created under the same conditions to compare molecular changes in the

biofilm. Comparable patterns were seen in the development of control planktonic cell spectra and those obtained from model surfaces. In order to identify molecular differences between the biofilm and the experimental group on different days, the control planktonic spectrum was especially chosen. Early biofilms (Fig. 8A–B) and planktonic spectra showed a clear change in mass spectra by days 3 and 5 of the culture, suggesting different protein synthesis in the control and experimental groups for young biofilms.

The biofilms' protein profile underwent notable alterations, as demonstrated by the analysis of spectrum evolution. The experimental group's spectra on day 7 showed denser peaks than the planktonic spectra, which suggested a change in the protein profiles. On days 9 and 12, the effects of COEO on glass and stainless-steel surfaces were observed in the experimental group based on the spectra shown (Figs. 6D and E). The produced dendrogram (Fig. 9) shows that the late-stage biofilms (7 SEOCs and 7 SEOCG) had the shortest of main spectrum profile (MSP) distances, which were comparable to the control and planktonic spectra. An illustration of the visual representation of similarity

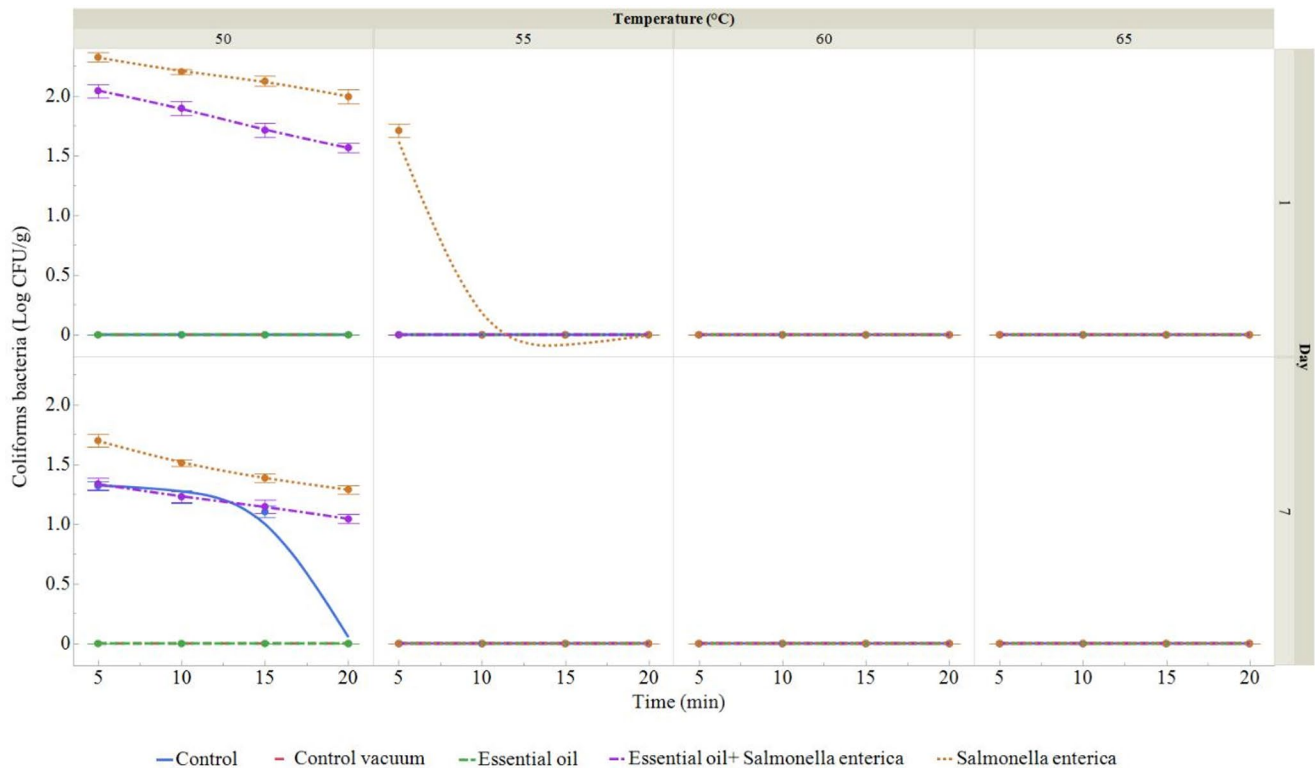


Fig. 5 Coliform bacteria count of Hokkaido pumpkin samples treated at temperatures ranging between 50 and 65 °C for durations of 5 to 20 min (expressed in log CFU/g) on the first and seventh day

in biofilm structure was provided by a dendrogram made with MSP distances. The comparable protein profiles within the control groups are demonstrated by the tight MSP distances between these entities. The early biofilm forms of planktonic cells on day 3 showed modest MSP distances like the control, as confirmed by the mass spectra analysis results. The experimental group's MSP distance increased over the course of the investigation, reaching a high on days 12 and 14. These results demonstrate how COEO affects the *S. enterica* biofilm's homeostasis.

Insecticidal activity of COEO

The evaluation of COEO's insecticidal activity against *M. dorsalis* is presented in Table 7. The data shows that applying dosages of 50% and 100% of the tested EO resulted in the highest insecticidal activity. Administered at 6.25% and 3.125%, COEO did not, however, show any appreciable repellent properties against *M. dorsalis*. Remarkably, the *M. dorsalis* population was affected by a concentration of 12.5%, whilst a concentration of 25% was effective against 50% and 70% of the insects, respectively.

Discussion

On the basis of previous findings, the aim of our study was to investigate its beneficial properties and further industrial applications. Several investigations of the chemical composition of the essential oils found in various *C. odorata* parts have been carried out. Our COEO sample was characterized by a high concentration of the monoterpene alcohol linalool (25.1%), followed by significant amounts of the sesquiterpene hydrocarbons germacrene D (16.1%) and (*E*)-caryophyllene (10.2%), the monoterpene ester geranyl acetate (11.5%), and the aromatic compound *p*-methyl-anisole (11.2%). In a different study, the compounds present in amounts greater than 10% were benzyl acetate and linalool (18.21% and 15.23%, respectively) [42]. A study on the essential oil of *C. odorata* from Malaysia reported a high concentration of linalool (21.8%), along with notable amounts of acetic acid (15.8%), β -ocimene (13.5%), and piperonal (10.0%) [51]. The essential oil obtained by hydrodistillation from *C. odorata* flowers from Brazil was characterized by high quantities of the sesquiterpene hydrocarbons germacrene D and α -farnesene (32.8% and 30.0%, respectively) [52]. Contrary, literature data on the essential oil obtained by distillation of *C. odorata* flowers from Madagascar revealed germacrene D (12.3%), linalool (10.2%), and benzyl acetate (9.9%) as the main constituents [53].



Fig. 6 Krona chart: Isolated species of microorganisms from sous vide Hokkaido pumpkin after 1 day. Note: The identified species represent isolates pooled from all experimental groups at the respective time point and serve as a qualitative indicator of surviving microorganisms

The major compounds in the distilled flower bud oil from an Indian sample were linalool (29.2%) and germacrene D (11.8%) [54]. In contrast, the main volatiles in the oil sample from China were linalool (21.1%), linalool acetate (16.1%), and α -pinene (12.7%) [55]. All these results of the mentioned authors are in contradiction with our results. It

is evident that the chemical composition of essential oils is subject to various factors, including geographic conditions, climate and seasonal variations, flower condition, plant genome, harvesting period, and extraction technique. Consequently, it is anticipated that the presence and amounts of

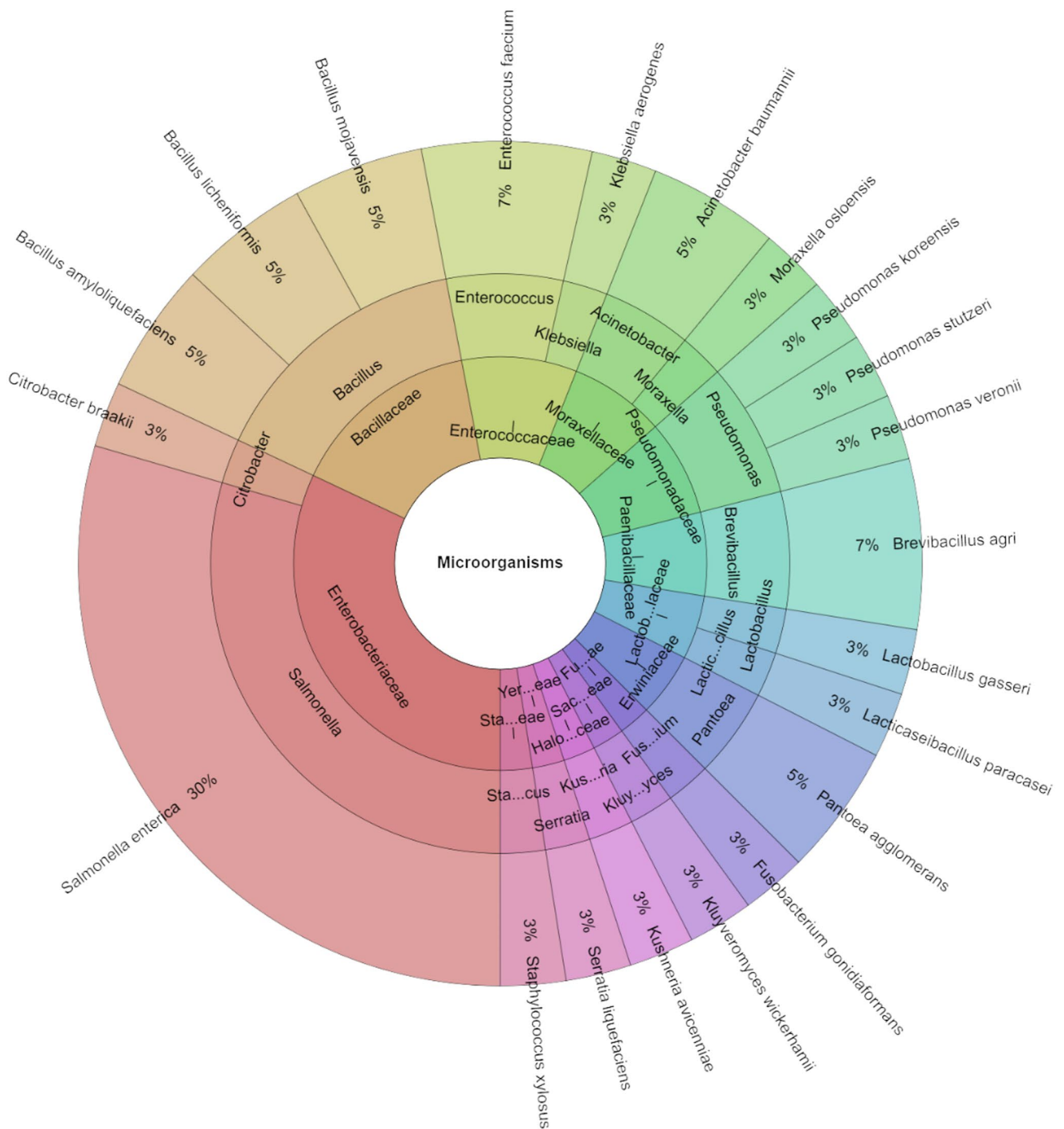


Fig. 7 Krona chart: Isolated species of microorganisms from sous vide Hokkaido pumpkin after 7 days. Note: The identified species represent isolates pooled from all experimental groups at the respective time point and serve as a qualitative indicator of surviving microorganisms

individual volatiles will exhibit variability across different oil samples [56–58].

Plant products, such as EOs, are widely known for their beneficial biological properties (antioxidant, antibacterial, antifungal, antiviral, etc.) which has enabled them to be widely used in various branches of industry [59]. An

efficient food preservative should be able to prevent microbial growth in foods, as well as to retard the autoxidation of pigments, flavors, lipids, and vitamins to reduce or postpone food degradation. The obtained results in this study indicated superiority of the tested EO to scavenge ABTS radical cation over DPPH radical. The present findings are

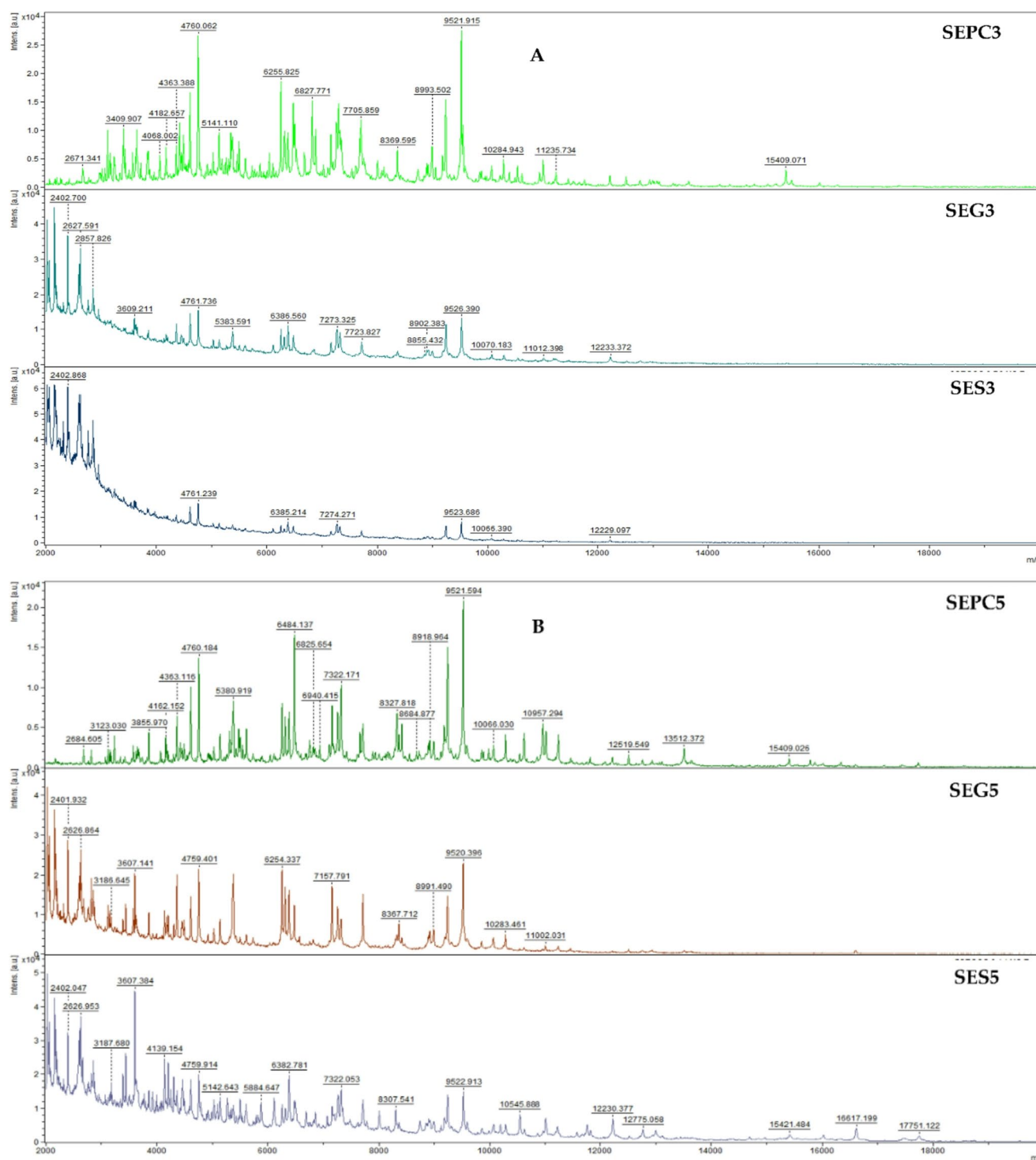


Fig. 8 Representative MALDI-TOF mass spectra of *S. enterica*: (A) 3rd day; (B) 5th day; (C) 7th day; (D) 9th day; (E) 12th day; (F) 14th day. SE = *S. enterica*; G = glass; S = stainless-steel; and PC = planktonic cells

consistent with the earlier conclusion that the ABTS assay is preferable to the DPPH assay when employed in the analysis of a range of plant foods comprising hydrophilic, lipophilic and high-pigmented antioxidant compounds [60]. Similar to the results obtained in our study, Kuspardini et

al. [61] COEO shows really low potential of scavenging the DPPH radical. However, contrary to our results, COEO characterized by the high amount of linalool, benzyl benzoate and benzyl salicylate (around 73% of total oil composition) showed significant effects in DPPH radical cation

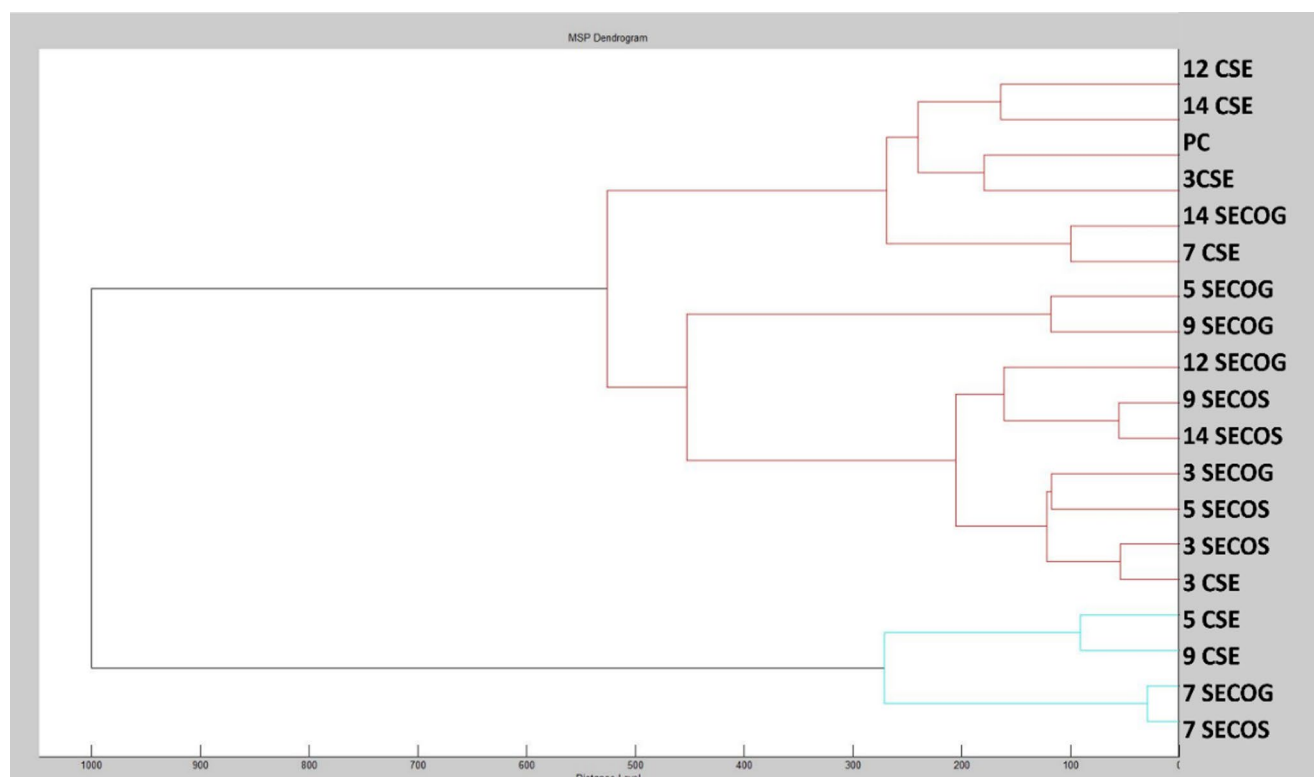


Fig. 9 Dendrogram of *S. enterica* generated using MSPs of the planktonic cells and the control. SE = *S. enterica*; C = control, CO = *Cananga odorata*, G = glass; S = stainless-steel; and PC = planktonic cells

Table 7 Insecticidal activity of *Cananga odorata* EO against *Mega-bruchidius dorsalis*

Concentration (%)	Number of Living Individuals	Number of Dead Individuals	Insecticidal Activity (%)
100	0	100	100.00±0.00
50	20	80	80.00±0.00
25	30	70	70.00±0.00
12.5	50	50	50.00±0.00
6.25	80	20	20.00±0.00
3.125	100	0	0.00±0.00
Control group	100	0	0.00±0.00

neutralization [62]. The differences observed in the results can be attributed to the chemical profile of the tested COEO samples and the diversity of the experimental settings [59].

Next, the focus of this study was directed towards investigating the antimicrobial properties of COEO. Concerning EO obtained from *C. odorata*, a number of authors has previously reported results on its antimicrobial effects towards a range of bacterial and fungi species with different outcomes [59]. Our study shows that the most sensitive bacteria to COEO was *S. constellatus*. Study by Kuspradini et al. [61] examined COEO antimicrobial effectiveness by using disc diffusion method against *S. aureus* and *C. albicans*. Three distinct antibacterial assays, including the zone of clearance assay, turbidometric assay and disc diffusion

assay, were carried out against *E. coli* in an attempt to determine the possible application of COEO as an alternative treatment for irritable bowel syndrome [63]. The authors have also shown a weak antibacterial activity of COEO against *E. coli*. A more recent study also showed low activity of the COEO on the growth inhibition of *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Penicillium chrysogenum*, *Aspergillus niger*, and *Aspergillus flavus* [64]. In another study it was discovered that different *Propionibacterium acnes* strains were not significantly resistant to the EO of *C. odorata* that was produced using steam distillation [65]. The EO of *C. odorata* demonstrated inhibitory zones ranging only from 8.8±0.7 mm to 9.5±0.7 mm against five strains of *P. acnes* [65]. In contrast to previously published results, COEO tested in this study showed higher activity against *E. coli* strain, as well as against different *Candida* strains. Also, previously conclusion was made that COEO showed better antibacterial effectiveness against G^+ bacteria, while our study showed that the COEO had stronger impact on the inhibition of G^- species [2]. However, it is important to note that the biological efficacy of the EOs tested depends on many factors, including the conditions under which the EOs were prepared, as well as the strains used to test and the experimental setting [66].

In this context, the food industries are becoming more interested in application of EOs, which have been used in

pharmaceutical preparations and traditional medicine, to develop environmentally friendly food preservatives with functional qualities [67–69]. With that in mind, the next course of this investigation consisted in exploration of COEO effectiveness in vapor phase. The basis of this study was established on the previous findings of Galovičová et al. [70] which demonstrated that vapor phase of COEO expressed significantly higher efficiency compared to direct contact (disc diffusion assay) application. Moreover, Jiang et al. [71] discovered that application of essential oils in vapor phase require lower quantities of EO compared to its liquid phase or direct contact effect, making them a potential substitute for antimicrobials in the food business. The findings of the present study are in accordance with those of previous research. It is noteworthy that this study represents the initial investigation in which COEO was evaluated for its efficacy against a range of microorganisms in the vapor phase, utilising strawberry, peach, radish, and cucumber as food models. The findings of this study suggest that COEO exhibited the most efficacious effect in regulating the proliferation of microbial species at the highest concentrations applied.

Fresh-cut goods offer the food business a strong opportunity to boost market sales since they fully satisfy the need of contemporary customers for convenient, healthful eating. Due to their great added value and convenience of use, both at home and in the food service business, fresh-cut Hokkaido pumpkins are becoming a more popular choice among consumers in the fresh-cut vegetable market. Considering this, we used *S. enterica* inoculation and COEO application followed by sous vide processing of Hokkaido pumpkin as a food model. In some cases, sous vide is a step that reduces the microbial load, but this is not sufficient to prevent spoilage throughout the shelf life when stored in ambient conditions [20]. According to our findings, the development of *S. enterica*, the quantity of coliform bacteria, and the overall number of microorganisms were all inhibited by the sous vide approach, which involved increasing temperature and duration in addition to COEO. The results of the study by Rizzo et al. [72] demonstrated that minimally processed potatoes meant to be cooked sous vide could benefit from the synergistic use of vacuum packing and rosemary EO in conjunction with chilled storage. Particularly, although the effect varied among cultivars, adding EO to the fresh-cut product promoted the growth of mesophilic bacteria and Enterobacteriaceae throughout the storage period. Kačániová et al. [46] also revealed that EO had a beneficial antibacterial impact on *S. enterica*. In vacuum-packed sous vide carrot samples, EO anti-*Salmonella* activity was marginally higher than in control subjects. These findings demonstrate the benefits of EO antibacterial and antibiofilm qualities and point to possible uses in food preservation.

Several vegetable products have been found to contain of *Salmonella* spp. Studies by various authors have revealed that the prevalence of *Salmonella* spp. in vegetables is 8% [19, 73, 74]. In our study was evaluated also microbiological quality of Hokkaido pumpkin samples treated with different temperature, time, then application of EO and inoculation with *S. enterica*. In a different study the efficacy of EOs against *Salmonella* spp. was evaluated in tomato model. The findings of this study demonstrated that the volatile components of essential oil (EO) exhibited a substantial reduction in the microbial load of target pathogens on tomato samples, with an approximate limit of 6.0 log 10 CFU/mL [75]. The issue of microbial foodborne diseases represents a significant concern for public health. It is important to note that one of the major risk factors in this respect is related to consumer preferences for “ready-to-eat” or minimally processed (MP) fruits and vegetables [76]. Essential oils (EOs) are a viable alternative. They are used to reduce pathogenic bacteria and extend the shelf-life of MP foods due to the health risks associated with food chlorine [77]. There has been an increased interest in the use of ethylene oxide (EO) in the preservation of fresh produce. However, further research is required to ascertain the extent to which EO is present in MP foods. For instance, although in vitro studies have demonstrated that EO is a valuable antimicrobial agent, its practical application in MP foods may be constrained by unrealistic concentrations. This is because the majority of studies concentrate on growth inhibition rather than bactericidal activity [77].

According to our findings, the most isolated bacteria discovered in sous vide Hokkaido pumpkin were *Bacillus amyloliquefaciens*, *Brevibacillus agri*, and *Enterococcus faecium*. Two *Bacillus* species and one *Clavibacter* species were gram positive, although most of the isolates from vegetables in other studies were gram-negative bacteria. *Pseudomonas fluorescens* constituted the majority of the diverse *Pseudomonas* species that were isolated [16].

The number of chemical preservatives in food or food items that are preserved has been a source of worry. Because of their potential uses in controlling dangerous infections in food model systems, EOs have recently drawn attention from researchers [78]. The emergence of foodborne illnesses has been observed in numerous regions globally, attributable to the presence of pathogenic bacteria in food storage or food items that undergo deterioration or compromise their quality [79]. In this context, using EOs, strong antimicrobials with low toxicity, can be an effective tactic to reduce or eliminate foodborne pathogenic bacteria in processed foods or marketable food products that have a higher acceptability rate among consumers [80, 81].

The use of EOs obtained from plants to prevent the formation of biofilms has been well studied. Considering, the

next step in our work, was to monitor the antibiofilm activity of COEO against *S. enterica*. *Cananga* EO's antibiofilm activity has recently been examined in a number of investigations [82]. The antibacterial and antibiofilm properties of 15 essential oils were assessed in a separate investigation on *Salmonella enterica* serovars Typhimurium 14,028 and Enteritidis ATCC 13,076. The chemical composition of EO, the presence of functional substances in its active components, and the synergistic interactions between the compounds have all been demonstrated to contribute to their antimicrobial effectiveness [83]. EOs with a stronger antibacterial activity were more likely to contain phenolic compounds such as carvacrol and thymol. EOs also prevented *S. enteritidis* and *S. typhimurium* biofilms from forming in the research [84]. Additionally, we use the MALDI TOF MS Biotyper technique to report the COEO's antibiofilm activity against *S. enterica* for the first time. Therefore, an understanding of the behaviour of biofilms with respect to different biocides can be a valuable insight into the effective control of biofilm development on different surfaces. Biofilm formation can occur on a variety of equipment and contact surfaces (stainless steel, glass, rubber, plastic, wood) [85]. *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* are reported to be the most important biofilm forming pathogens in the food industry [86].

In our study, COEO demonstrated a high mortality rate and an insecticidal efficacy against *M. dorsalis* at increasing concentrations. It has been determined that EOs from a variety of plant species, including *Cananga odorata*, exhibit larvicidal, ovicidal, and repellent qualities against mosquito vectors. The research has shown that *C. odorata* EO has ovicidal and oviposition-deterrent effects on a variety of mosquito species in addition to repellent qualities [87]. It was discovered that the *C. odorata* flower EO, when applied at 1%, 5%, and 10% (w/v), showed minimal insecticidal efficacy and a low rate of knockout against the adult mosquito species *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles dirus* [88]. In addition, it has been shown that *C. odorata* leaf EO has anti-pest qualities as well, and it may be conceivable to develop this EO as a natural fumigant or insecticide to control insects linked to storage products [55]. Without substantially reducing crop yields, our study examined the potential of COEO, which is useful to increase the effectiveness and dependability of EO, and promoted the use of substitute products to replace synthetic pesticides in the control of weeds and plant diseases [89].

Conclusions

The present study investigates the potential of *Cananga odorata* essential oil (EO) as a natural food preservative. The present study constitutes the first investigation into the efficacy of *C. odorata* EO in combating a range of Gram-positive, Gram-negative bacteria and yeasts. The objective of the study was to determine the EO's efficacy in preserving sous-vide vacuum-packed Hokkaido pumpkin inoculated with pathogenic bacteria *S. enterica*. The findings of this study demonstrated that EOs have the ability to impede the formation of *S. enterica* biofilms. Chemical agents that are able to penetrate the biofilm matrix are rendered less effective by the fact that the majority of chemicals are only active against unattached microorganisms. In order to penetrate biofilms and cause their breakdown, it is necessary to hydrolyse the matrix of the biofilm. A range of essential oils derived from a variety of medicinal plants have been the focus of considerable research interest. It has been demonstrated that essential oil derived from *C. odorata* displays antibacterial properties, effective against a range of microorganisms. In order to evaluate the inhibitory potential of COEO against cell adhesion at the MBIC value concentration, tests were performed to determine the level of anti-adhesion. These were carried out using the crystal violet assay. The results indicated that COEO could inhibit the adhesion of bacterial cells of the species *S. enterica*. The findings of this study demonstrated the efficacy of COEO in the inhibition of biofilm growth and formation. In summary, COEO can be regarded as a prospective antimicrobial preservative. These results support the application of *Cananga odorata* essential oil as a natural antimicrobial agent in food systems, especially in minimally processed vegetables treated by sous vide technology.

Author contributions “A. V.: Methodology, Investigation, Formal analyses, Visualization, Writing-review & editing. M.D. V.: Conceptualization, Writing-review editing, Supervision. N. L. V.: Conceptualization, Writing-review & editing, Supervision. J. B.: Formal analyses, Writing-review & editing. Z. B.: Formal analyses, Writing-review & editing. A. B.: Formal analyses, Methodology, Writing-original draft. R. B. S.: Formal analyses, Writing-review & editing. A. B. H.: Formal analyses, Writing-review & editing. J. H. E.-L.: Formal analyses, Writing-review & editing. S. G.: Formal analyses, Supervision, Writing-review & editing. M. K.: Conceptualization, Methodology, Investigation, Writing-original draft, Formal analyses, Supervision, Project administration, Funding acquisition, Visualization, Writing-review & editing. All authors reviewed the manuscript.”

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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