

Effectiveness of Citrus lemon juice's waste essential oil and aqueous phase as a preservative against *Salmonella enteritidis* in chicken meat

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Abstract

Through hydro-distillation of industrial lemon juice waste, the study obtained *Citrus lemon's* aqueous phase (AP) and essential oil (EO), with the EO yield at approximately 0.89% and a high monoterpene content (50.77% limonene), while the AP contained high total phenols ($36.96 \pm 1.2 \text{ mg g}^{-1}$). The EO and AP exhibited Strong bactericidal effects with minimal cidal concentrations values (MCC) ranging from 2.5 to 10 g mL^{-1} and 187.5 to 1500 g mL^{-1} , respectively. The inhibitory efficacy of various concentrations of the EO and AP against a foodborne pathogen belonging to *Salmonella enteritidis* inoculated in chicken meat showed that AP demonstrated a bacteriostatic effect of 0.25–0.5 and 1 mg AP g^{-1} meat. However, the bacterial count ($> 10^2 \text{ CFU/25 g meat}$) rendered it inedible for humans. Additionally, at the maximum concentration (1% v/w), the EO exhibited bactericidal action, but even at the minimum concentration (0.5 v/v EO/v box), the EO vapor action was still the most effective. Sensory analysis indicated that meat treated with the highest concentrations of EO and AP had an unpleasant taste and was discarded. Nonetheless, the study suggests that the AP and EO of lemon juice industrial waste could be a viable alternative to chemical preservatives in the food and pharmaceutical industries.

Introduction

Salmonellosis was most problematic in extremities of age. Moreover, most deaths happened in elderly patients with comorbid diseases¹. *Salmonellae* render a wide range of illnesses, including enteric fever and gastroenteritis. There are numerous types of *Salmonella*. However, they are kept under two broad categories: typhoidal, which causes typhoid or enteric fever, and non-typhoidal, which mainly causes gastroenteritis². In 2015, an estimated 680,000 deaths from non-typhoid *Salmonella* occurred worldwide, with more than half of the cases in Africa³. Consumption of contaminated chicken and eggs appeared to be the most closely associated with *S. enteritidis* illness^{4–6}.

Artificial additives are usually utilized to control bacterial contamination during production, distribution, and sale. There is an increased awareness of the potential dangers of these preservatives and their toxic residues, which may be responsible for carcinogenicity and teratogenicity⁷. Consequently, scientists searched for natural alternatives, such as plants, known since ancient times for their ability to treat many diseases, including microbial infections. Recently, plant extracts, whether aqueous or alcohol and essential oil, whether aqueous, alcoholic, or in the form of essential oils, have attracted the interest of researchers in terms of their capability to inhibit bacterial growth, fungi, as well as viruses, in addition to their antioxidant properties^{8,9,10}.

The *C. limon* belongs to the Rutaceae family and is considered among the significant species of the *Citrus* spp., which includes approximately 140 genera and 1300 species¹¹. *Citrus* spp. is among the significant world fruits in production; the Food and Agricultural Organisation (FAO) mentioned that 240,780 million metric tons were yielded¹². The volume of waste generated by this massive amount of global production in one year and its accumulation year after year emphasizes the importance of converting it into valuable products to promote sustainability and minimize waste management expenses. Several researchers have indicated the possibility of pollution resulting from unused parts of lemon fruits, including peels and seeds^{13,14,15}. However, several procedures are used to alter these by-products into valuable products, where citrus peels are used as activated carbon, fish feed, and conventional paper raw materials¹⁶. Moreover, researchers found that the essential oils of citrus are mainly found in the peels of the fruits. Therefore, it is considered a suitable material for manufacturing essential oils^{17,18}.

Several studies discovered that citrus peel essential oils and aqueous or alcohol extracts are rich in phytochemical compounds such as phenols, flavonoids, glycosides, tannins, saponin, and alkaloids^{14,15}. These chemical

compounds extracted from the citrus peel have the capability to inhibit several foodborne bacteria, e.g., *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Citrobacter freundii*, *Yersinia ruckeri*, *Listonella anguillarum* and *Aeromonas hydrophila*^{14,15,18,19}. Furthermore, these extracts have a substantial advantage as they are safe and antioxidant^{15,20}.

This study's objectives were to (i) assess the chemical composition of *C. lemon* waste EO and AP extracted by hydro-distillation, (ii) study their antioxidant and antibacterial activities *in vitro*, (iii) evaluate EO and AP's antibacterial effectiveness against *S. enteritidis*, in chicken breast fillets during refrigerated storage and (iv) assess the effect of EO and AP addition on sensory parameters of chicken breasts after refrigerated storage.

Material and methods

Sample

In this study, we used the EO and AP of *C. lemon* obtained by hydro-distillation with a hydro-distillation unit for 2–3 h of the raw waste of a lemon juice production industry. Extracts of *C. lemon* raw waste were collected in amber-colored bottles and preserved at 4°C until deployment.

The total phenolic content

The total phenolic content of *C. lemon* waste extracts was estimated using the Folin-Ciocalteu reagent using pyrogallol as a standard, as described by Singleton and Rossi²¹. Briefly, 50 µL of an appropriately diluted extract or pyrogallol standard solution was mixed with 250 µL of Folin-Ciocalteu reagent solution and 500 µL of freshly prepared 20% sodium carbonate. After mixing, dilute to 5 mL with distilled water. In the dark, the reaction mixture was left for 30 minutes, and the absorbance was measured at 727 nm against a blank solution prepared by the same procedure as above, except that the extract was replaced by 50 µL of distilled water. TPC is expressed as mg pyrogallol equivalent (mg PE g⁻¹).

Total Flavonoid Content

Using the method described by Zhishen et al.²², The total flavonoid content of the extracts was determined. In brief, 1 mL of appropriately diluted sample solution was mixed with 4 mL of distilled water, followed by 300 µL of 5% NaNO₂ aqueous solution. After 5 minutes, 300 µL of 10% aluminum chloride solution was added and allowed to stand for 6 minutes. Then 2 mL of NaOH solution (1 M) was added, and the volume was adjusted to 10 mL with distilled water. The absorbance of the mixture was measured at 510 nm relative to the prepared water blank. The total flavonoid content was expressed in milligram catechin equivalents per 1 g sample (mg Catechin Eeq g⁻¹ sample).

Flavonol content:

The method²³ described was used to determine the flavonol content. Specifically, 2 mL of the extract (3.5 mg mL⁻¹) was mixed with 2 mL (20 g L⁻¹) of aluminum trichloride and 3 mL (50 g L⁻¹) of sodium acetate solution. After allowing the mixture to stand for 2.5 hours at 20°C, the absorbance was measured at 440 nm. The flavonol content was expressed as milliequivalents of rutin per 1 g of sample (mg REeq g⁻¹ sample) using standard curves obtained from different concentrations of rutin.

GC/MS analysis:

The essential oils were analyzed as described by Yangui et al.²⁴. The components were identified by comparing their mass spectra to data from the Wiley and NIST libraries. As well as standards of the main components^{25,26,27}, the standard external method was utilized to quantify the identified compounds, which involved generating calibration curves by running GC analyses representative of natural compounds. The concentrations of the constituents were determined by integrating the areas of the GC peaks and applying correction factors for semi-quantification.

Radical scavenging assays

ABTS radical scavenging assay

The ABTS (2,2'-azido-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay was conducted with some modifications following the method described by Re et al.²⁸. To prepare the ABTS⁺ solution, 7 mM ABTS salts, and 2.4 mM potassium persulfate were mixed in equal volumes and left to react for 12–24 h at room temperature in the dark. The solution was then diluted with 20 mM sodium acetate (pH 4.5) until an absorbance of around 0.70 ± 0.02 was achieved at 734 nm. Various extracts (0.3 mL) concentrations were added to 1 mL of the ABTS⁺ solution, and the absorbance was measured at 734 nm after 5 minutes. Calibration was performed using a solution of Trolox in ethanol of known concentration. The results were expressed as TEAC or Trolox equivalent antioxidant capacity (mM).

DPPH radical scavenging assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of lemon waste extracts was evaluated using the 24 Yangui et al. 2021 method. In this method, 50 μ L of essential oils in ethanol were mixed with 5 mL of 0.004% DPPH and incubated at room temperature for 30 minutes. The absorbance at 517 nm was read against a blank, and the percentage of DPPH radical scavenging activity was calculated using the formula: $[(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100$. The IC₅₀ value, the concentration of essential oil that provides 50% DPPH inhibition, was calculated from a plot of percent inhibition versus essential oil concentration. The positive control used in this study was the synthetic antioxidant reagent butylated hydroxytoluene (BHT). All experiments were performed in triplicate.

Antimicrobial activity

Microbial cultures

The antimicrobial activity of the samples was evaluated against a broad range of microorganisms, including *S. aureus* ATCC 6538, *E. coli* ATCC 10536, *Enterococcus hirae* ATCC 10541, *Pseudomonas aeruginosa* ATCC 15442, and *S. enteritidis* ATCC 13076, obtained from the American Type Culture Collection (ATCC). The bacteria were cultured in LB broth or LB Agar (Difco Laboratories, USA) at the appropriate temperature (30–37°C) for each strain. The inocula were prepared by adjusting the turbidity of each bacterial culture to match the optical density of a 0.5 McFarland standard, which corresponds to approximately $1-5 \times 10^8$ CFU mL⁻¹.

Minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC)

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the extracts against the test organisms were determined following the guidelines recommended by the National Committee for Clinical Laboratory Standards, as outlined by Yangui et al.²⁴. The assay was performed using sterile 96-well microplates, where sample solutions were serially diluted to obtain two-fold dilutions. Inocula containing 10^4 CFU mL⁻¹ of bacteria were added to each well, and the plates were incubated aerobically at 30°C for 16–20 h. The lowest

concentration at which no growth was observed following incubation indicates the MIC. MBC/MFC values was determined by, 10 μL of the mixture from each well with no visible growth was removed and inoculated in LB plates, and the surviving organisms were counted after a 24 h aerobic incubation period at the proper temperature. Positive control wells contained 10 g L^{-1} of chloramphenicol antibiotics for bacteria, while negative control wells contained only bacteria in their appropriate medium. Additionally, 30 μL of 0.02% resazurin and 12.5 μL of 20% Tween 80 were added, and the wells were observed for a blue to pink color shift after incubation.

Inoculation of the poultry fillets

The *S. enteritidis* stock culture was grown on LB Agar slants and then transferred to LB broth before use. The working culture was prepared by incubating the test strain in LB broth at 37°C for 24 hours and subculturing twice with LB before use.

The fresh poultry breast meat was obtained from a local processing plant and immediately filleted into small pieces. The meat pieces were boiled for 5 minutes and then trimmed with a sterile knife to remove the cooked portions. The fillets were divided into three groups and checked for contamination by *S. enteritidis*. Each group was placed in a polyethylene bag and inoculated with *S. enteritidis* at a concentration of approximately 10³ CFU g⁻¹ of poultry samples. The samples were then massaged for 10 minutes to ensure proper pathogen distribution. After 2 hours, the inoculated and non-inoculated samples were treated with different concentrations of C. lemon EO (0.06, 0.1, and 1% v g⁻¹ of poultry sample) and C. lemon AP (0.25; 0.5 and 1 mg g⁻¹ of poultry sample) or different volumes of C. lemon EO (0.25, 0.5 and 1‰ of EO per volume of the bag) placed in sterile beakers to create micro atmospheres. A control was treated with sterile water. The packages were stored at 4–7°C and analyzed after 3, 6, 9, 12, and 16 days to determine the microbiological characteristics, using three packages from each group on each sampling date.

Microbiological analysis

To prepare samples for microbiological analysis, 25 g of poultry fillets were added to 225 mL of sterile physiological water and homogenized by shaking vigorously for 2 h at 250 rpm min⁻¹. After homogenization, 10-fold serial dilutions were made with sterile 0.9% NaCl solution, and 0.1 mL of the diluted samples were streaked onto *Salmonella Shigella* (SS) Agar. The analysis was performed in triplicate.

Sensory evaluation

In the sensory evaluation, 50 randomly selected panelists evaluated treated samples of chicken breast fillets using a three-point scale (dislike very much 10 to 30%, dislike moderately 30 to 60%, and like very much 60 to 100%) for taste, odor, and texture. The samples were immersed in different concentrations of EO (0.06, 0.1, and 1% v g⁻¹) and AP (0.25, 0.5, and 1 mg g⁻¹) and evaluated after an incubation period of 16 days at 4°C. The samples were covered with aluminum foil and cooked in an oven at 180°C for 15 minutes. The samples were coded with random three-digit numbers and served at room temperature on plastic dishes.

Statistical Analysis

The experimental results were presented as the mean value with standard deviation, and each measurement was performed three times. Statistical analysis was conducted using analysis of variance (ANOVA) with a significance level of $P < 0.05$, and Duncan's test compared means²⁹.

Result and discussion

Hydro distillation is considered an inexpensive and safe method of producing essential oils because it uses water as a solvent and can be easily separated from the oil. This process was carried out to produce EO from *C. lemon* raw waste. The EO and the AP produced were then analyzed and characterized.

EO of *C. lemon* raw waste contents and composition

The EO of *C. lemon* raw waste was obtained in a 0.89% v/w yield (volume / fresh weight). The yield cited by Himed et al.³⁰ of the *C. lemon (Eureka)* peel extracted by steam distillation was 2.04 ± 0.34% wt. Martínez-Abad et al.³¹ reported a 2% wt. Yield for the EO extracted from lemon peel waste by microwave-assisted hydro distillation. The obtained yield for lemon EO from lemon peel waste by microwave-assisted hydro-distillation was around 2% wt. It is worth noting that the yield and chemical composition of EO from the lemon peel will vary depending on the extraction method and the raw material used. In our case, the low yield might be explained by our raw waste being an exhausted lemon peel.

The results of the GC-MS analysis The GC-MS study findings, including the content and composition of the EO, are presented in Table 1. Nine compounds out of 10 were identified with qualitative and quantitative variability. The monoterpene hydrocarbon limonene formed a significant group (50.77%), followed by the oxygenate class of Geranyl acetate (10.96%). Citral, which adds significantly to the richness of citrus taste and aroma³², was detected as its stereoisomers neral and geranial in 6.87 and 6.49% concentrations, respectively.

Table 1
Chemical composition of the EO

No.	Compound name	Retention time (min)	% composition
1	Limonene	21,832	50,77
2	Linalool	17,891	3,73
3	<i>Cis</i> -limonene-oxide	12,011	4,68
4	<i>Trans</i> -limonene-oxide	11,345	5,13
5	Citronellal	34,901	0,75
6	Neral	23,102	6,87
7	Geranial	13,002	6,49
8	Nerol	14,233	5,00
9	Geranyl acetate	12,433	10,96
10	NI	13,300	5,62
NI : Non Identified			

Our results are similar to literature reports on the EO of the lemon peel in which the main compound was D-Limonene. Njoku and Evbuomwan³³ stated that the GC-MS analysis of the EO extracted from Nigerian lemon peel showed approximately 12 principal components, with D-Limonene (64%) being the principal.

Limonene was likewise shown to be the most abundant ingredient in EO of the *C. lemon* fruits peel (43.07%), followed by α-pinene (12.61%), α-terpinene (11.48%), α-terpineol (7.20%), α-pinene (3.39%), myrcene (1.87%), geraniol (1.48%), α-terpinene (1.32%), α-terpinolene (2.37%), linalool (1.08%) and cis-α-bergamotene (1.38%)³⁴. Aslı Gök et al.³⁵

performed a comparative study of the chemical composition of lemon oil after using various extraction techniques: supercritical CO₂ extraction, cold pressing, and hydro-distillation, and discovered elevated concentrations of limonene (71.81, 70.36, and 72.48%) being the significant monoterpene hydrocarbons extracted, respectively.

Genotypic differences, geographical and environmental variables, reaping period, cultural practices, and type of extraction are among the reasons that could explain the differences in composition and proportions ³⁶.

Using a raw waste material can explain our results' limited number of compounds. These compounds are partially released into the juice during the process.

2. Physicochemical characterization of the AP of C. lemon waste

The physicochemical characteristics of the AP are highlighted in Table 2. Overall, the acquired outcomes are close to those found in the previous studies for citrus and other plant extracts. Citrus waste typically has low pH and high water content ³⁷. The total phenolic compounds value found ($36.96 \pm 1,2 \text{ mg EPy g}^{-1}$) is similar to that reported for black tea infusions ($36.28 \text{ mg EPy g}^{-1}$) (Pereira et al. 2014). The total flavonoid content of the AP ($4.81 \pm 0.5 \text{ mg ECa g}^{-1}$) was also close to that reported for orange peels polar fractions ($3.97 \pm 0.27 \text{ mg ECa g}^{-1}$) and below that reported for lime and lemon peels polar fractions (13.61 ± 0.64 and $15.96 \pm 0.24 \text{ mg ECa g}^{-1}$, respectively) ³⁸.

Table 2
Physicochemical characteristics of the AP

Parameters	pH	Dry matter (%)	Volatile matter (%)	total phenolic compounds (mg EPy/g)	Flavonoids (mg ECa/g)	Flavonols (mg ERu/g)
	5,5 ± 0,3	28,9 ± 0,9	18,1 ± 0,5	36,96 ± 1,2	4,81 ± 0,5	3,61 ± 0,2

According to Makni et al. ³⁹, the preponderance of flavonoids in lemon zest consisted of flavonols. The content of flavonols in the AP was $3.61 \pm 0.2 \text{ mg ERu g}^{-1}$. This value indicates that the bulk of flavonoids in the lemon water extract are flavonols.

Biological activities of C. lemon waste's AP and EO

Antioxidant activity:

AP and EO's free radical scavenging activities were examined using DPPH and ABTS assays (Table 3). The AP and EO presented IC₅₀ values of $12.58 \mu\text{g mL}^{-1}$ and $10.95 \mu\text{g mL}^{-1}$, respectively. The synthetic antioxidant BHT obtained a higher antiradical activity against DPPH (IC₅₀ = $8.31 \mu\text{g mL}^{-1}$). According to the ABTS assay, the EO showed the highest antioxidant capacity compared to BHT. Although the AP revealed the lowest activity, the TEAC value is statistically similar to that of the BHT.

Table 3
Radical scavenging activity of the AP and EO

Extract	Antioxidant activity IC ₅₀ (mg L ⁻¹) DPPH	TEAC ABTS
Aqueous phase	12.58 ± 1	3.5 ± 0.33
Essential oils	10.95 ± 0.7	2.1 ± 0.26
Butylated hydroxytoluene (BHT)	8.31 ± 1.07	2.81 ± 0.9

The antioxidant properties of lemon peel extract oil and the aqueous phase resulting from the hydro distillation are related to their chemical composition. For instance, the dominant compound of the EO is limonene, the most popular phenolic found in citrus plants and used as a flavoring agent for foods. Previously, it was demonstrated that limonene possesses antioxidant activity. Himed et al.³⁰ evidenced the antioxidant activity of the oil extracted from lemon peel and obtained a value of 66 µg mL⁻¹ by the DPPH method. They reported that limonene, α-pinene, and α-terpinene are responsible for the neutralization of the DPPH radical. Moreover, Limonene was demonstrated to be the main compound of oils showing high antioxidant activity in celery seeds⁴⁰.

The presence of phenol and flavonoids in the AP might explain its antioxidant properties. The capacity of phenols and flavonoids to act as antioxidants in vitro has been the subject of several studies. Ghasemi et al.⁴¹ reported the significant phenol and flavonoid content of *C. reticulata* var. Ponkan peels and tissue result in this plant's remarkable antioxidant activity. In addition, according to Azman et al.⁴², the antioxidant capacity of fresh and frozen citrus peels was favorably linked with the total phenol and flavonoid content. Other studies considered that only flavonoids with specific structures, precisely specific hydroxyl positions in the molecule, may operate as proton donors and exhibit free radical scavenging ability⁴³.

The antioxidant activities of EO and AP of *C. lemon* waste demonstrated in this study may represent an alternative to synthetic antioxidants for food. EO quenches peroxide radicals and terminates peroxidation chain reactions⁴⁵. In fact, inhibiting lipid peroxidation by adding C.lemon waste EO can enhance food product quality and stability.

Antimicrobial activity of EO and AP

Results of antimicrobial activity of EO and AP against gram-positive (*S. aureus* and *E. hirae*) and Gram-negative (*P. aeruginosa*, *E. coli*, and *S. enteritidis*) bacteria isolates by broth microdilution susceptibility assay are shown on Tables 4 and 5. EO and AP presented antimicrobial activities against all strains tested with varying degrees. For instance, our investigation showed a lower response against Gram-negative than Gram-positive bacteria. In addition, *P. aeruginosa* showed higher resistance among Gram-negative bacteria.

Table 4
Antimicrobial activity of the EO

Microorganism	MIC ($\mu\text{g mL}^{-1}$)	MCC ($\mu\text{g mL}^{-1}$)	MCC/MIC
<i>Pseudomonas aeruginosa</i>	10 – 5	10 – 5	1
<i>Escherichia coli</i>	10 – 5	10 – 5	1
<i>Salmonella enteritidis</i>	10 – 5	10 – 5	1
<i>Staphylococcus aureus</i>	5 – 2,5	5 – 2,5	1
<i>Enterococcus hirae</i>	5 – 2,5	5 – 2,5	1
MIC: Minimal Inhibitory Concentration, MCC: Minimal Cidal Concentration			

Table 5
Antimicrobial activity of the AP

Microorganism	MIC ($\mu\text{g mL}^{-1}$)	MCC ($\mu\text{g mL}^{-1}$)	MCC/MIC
<i>Pseudomonas aeruginosa</i>	1500 – 750	1500 – 750	1
<i>Escherichia coli</i>	750 – 375	750 – 375	1
<i>Salmonella enteritidis</i>	750 – 375	750 – 375	1
<i>Staphylococcus aureus</i>	375 – 187,5	375 – 187,5	1
<i>Enterococcus hirae</i>	375 – 187,5	375 – 187,5	1
MIC: Minimal Inhibitory Concentration, MCC: Minimal Cidal Concentration			

Furthermore, the MIC/MCC ratio validated the EO and AP's bactericidal action. Moreover, EO presented the highest bactericidal activity with MIC values ranging from 10 to 5 $\mu\text{g mL}^{-1}$ and 5 to 2.5 $\mu\text{g mL}^{-1}$ for Gram-negative and Gram-positive bacteria, respectively, compared to AP with a range of 1500 to 375 $\mu\text{g mL}^{-1}$ and 375 to 187,5 $\mu\text{g mL}^{-1}$, respectively.

Antimicrobial properties of the EO and various extracts from the fruit of *C. Lemon* have been studied for phytochemical compounds. In this study, the EO from *C. limon* was high in monoterpenes: limonene (50.77%), and the AP was **enriched in** total phenols ($36.96 \pm 1.2 \mu\text{g g}^{-1}$). Our results are consistent with the findings of Cakir et al.⁴⁶, who observed that monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives, which are the main components of EO, have potential antibacterial properties. Additionally, Keles et al.⁴⁷ showed the antimicrobial properties of EO of lemon because of the presence of active alcohols, terpenes, esters, and aldehydes. Also, Dhanavade et al.⁴⁸ found that different crude extracts of lemon peel have a strong antimicrobial effect on human pathogenic bacteria: *Salmonella typhimurium* and *Micrococcus aureus*. They reported coumarin and Tetrazene as significant antimicrobial substances in the lemon peel extracts.

On the other hand, Hindi and Chabuck⁴⁹ reported that the aqueous extracts from the peel of *C. limon* did not show any antimicrobial reaction against *E. coli* and *P. aeruginosa*.

Most investigations exploring the impact of EO and extracts from many other species have revealed Gram-negative bacteria's low sensitivity to antibacterial action^{19,50,51}. This fact can be attributed to an outer layer around the cell

wall of Gram-negative bacteria, which is barely permeable to lipophilic compounds ⁵².

Anti-Salmonella Efficacy of the EO and AP in chicken meat

Figures 1, 2, and 3 show the results of the inhibitory effect of different concentrations of EO and AO on *S. enteritidis* inoculated into chicken meat (10^3 CFU g⁻¹) and kept at 4–7°C for 16 days by immersion and micro atmosphere treatments. We notice that the examination of the fillets prior to inoculation showed no contamination by *S. enteritidis*.

AP exhibits a bacteriostatic effect against *S. enteritidis* with a reduction of growth by 0.38, 1.36, and 1.69 log CFU g⁻¹ after the treatment with 0.25–0.5 and 1 mg of AP g⁻¹ of meat, respectively (Fig. 1). Nonetheless, the bacterial concentration is still not suitable for human utilization (> 102 CFU/25 g meat)¹².

Similarly, EO showed a bacteriostatic effect against *S. enteritidis* with a reduction of growth by 0.42 and 1.26 log UFC g⁻¹ with 0.06 and 0.1% EO v/g, respectively. At the same time, EO at 1% v/g significantly lowered the *S. enteritidis* count (1.66 log reduction), showing a bactericidal effect (Fig. 2).

On the other hand, the EO vapor (EOV) reaction was most efficient at the lowest concentration (0.25‰ (v EO/v boxes) with a reduction of growth by 1 log UFC g⁻¹. Results are not presented for 1‰ EOV (v/v) and 0.5‰ EOV (v/v) since there were no viable counts showed after and before 3 h of incubation, respectively (Fig. 4).

S. enteritidis was used as a model because it is commonly found in poultry products and has been incriminated in numerous *Salmonella* outbreaks in humans ^{53,54}.

The antimicrobial activity of citrus EO was shown to be effective in vitro against various *Salmonella* strains⁵⁵; however, activity in food varied depending on the nutritional content of the food. The hydrophobic EO constituents and the phenolic compounds of the AP are impaired by interactions with food matrix components such as fats, proteins, and minerals in the case of poultry products, and this can explain the concentration difference between the chicken model and in vitro test.

Enhanced by active alcohols such as linalool, a monoterpene alcohol known to be a powerful antibacterial and antifungal compound. Limonene, shown in this study to be an abundant component of EO, is said to have antimicrobial activity, disrupt bacterial cell membranes, and cause morphological changes^{36,56}. This result is consistent with other authors who found that citrus EO had potent antibacterial activity against this species ⁵⁷.

The use of citrus EO in its vapor form enhanced the antimicrobial efficacy of the EO against *S. enteritidis*. citrus EO vapor is a compounded combination of volatile substances derived from terpenes and oxygenated terpenes, including limonene, α -terpineol, β -myrcene, sabinene, linalool, α -humulene, and α/β -pinene, which are accountable for antimicrobial activity⁵⁶. The modest increase in EO vapor impact might be due to citrus EO components remaining portioned in hydrophobic environments formed by chicken matrix components. Thus, there was little opportunity for citrus EO to contact bacterial cells growing on more hydrophilic chicken surfaces.

Sensory evaluation:

Acceptance ratings of the processed chicken fillets incubated for 16 days at four °C are presented in Fig. 4. Taste, odor, and texture were used to score the fillets' sensory properties. The results obtained from the analytical examination of the data revealed significant differences ($p < 0.05$) among the treatments.

The concentration of EO substantially impacted the ratings of the processed fillets. Increases were noticed with 0.06% EO from an unacceptable level with 1% EO to score 'like very much,' which was the ultimate score regarding the taste, odor, and texture among all processed meat samples. Additionally, it is visible that the treatment with 0.06% EO had increased scores than the control samples; this could result from a strong smell and degradation of the texture due to an alteration of the connective tissue (collagen), the microfibril structure, and the structural interactions between the fibers and the extracellular matrix at high concentrations of EO.

In contrast to the EO-treated meat, the AP-treated meat demonstrated a considerable decrease in taste, odor, and texture scores.

The use of EO as a natural preservative in the food industry is in line with current consumer trends of "green" and "no added chemicals." The European Commission has approved several EO ingredients as flavoring substances in food and identified them as harmless to consumer health, such as limonene, p-cymene, eugenol, and menthol⁵⁸.

However, the sensory quality of each product needs to be evaluated, as consumers may have negative perceptions about the concentration required to achieve the desired antimicrobial activity.

Several relevant sensory tests have shown that adding rosemary oil or essence increases chicken meat acceptance^{59,60}. According to Alfonzo et al.⁶¹, the highest sensory scores for lemon EO addition to salted fish were obtained for flavor and overall acceptance. Moreover, the antioxidant effects of EO have been proven to minimize lipid oxidation and enhance the flavor of beef patties when rosemary or marjoram EO is added at a dose of 200 mg/kg.⁶² Rosemary EO also inhibits rancidity in heat-treated turkey products⁶³. It has been demonstrated that applying rosemary extract to deboned chicken prevents oxidation while cooking⁶⁴. Furthermore, combining different EO at lower concentrations can ensure food safety without affecting its organoleptic properties⁶⁵.

These results significantly confirmed the study's conclusions. The relevant antioxidant activity found with our EO from the raw waste of *C. Lemon* (Table 3) contributed to preserving the sensory quality of the stored chicken breast fillets.

Conclusion

This study disclosed the chemical and biological characteristics of *C. lemon* waste EO and AP extracted by hydro-distillation. The chemical composition of EO revealed that limonene and Geranyl acetate were the main components. According to the chemical analysis of EO, limonene and geranyl acetate were the primary constituents. AP contains natural antioxidants such as flavonoids and phenols. In vitro and situ, we also demonstrated the efficiency of *C. lemon* waste EO and AP as natural antioxidants and antimicrobial agents. We also demonstrated the effectiveness of *C. lemon* waste EO and AP as natural antioxidants and antibacterial agents, both in vitro and in situ.

The findings highlight the potential for efficient utilization of this waste source and pave the way for sustainable practices in the industry. Furthermore, EO applied on chicken breast fillets by immersion and micro atmosphere exhibited bactericidal activity against *S. enteritidis* during refrigerated storage. On the other hand, AP showed bacteriostatic activity. Finally, this EO could be beneficial as a natural preservative because of the flavour improvement shown after adding chicken breast fillets. Ultimately, this study offers a promising alternative for valorizing citrus lemon waste by employing a cost-effective hydro-distillation process to extract valuable molecules.

Abbreviations

Aqueous phase (AP); Essential oil (EO); Minimal Cidal Concentrations (MCC); Food and Agricultural Organisation (FAO); 2,2'-azido-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH); Minimal inhibitory concentration (MIC); minimal bactericidal concentration (MBC)

Declarations

Author contributions

H.C.. T. Y. and A.H.A. conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

I.A. , R.A, A. D. reviewed both the experiments and reviewed drafts of the paper, and approved the final draft.

Data Availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Competing interests

The authors declare no competing interests.

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Figures

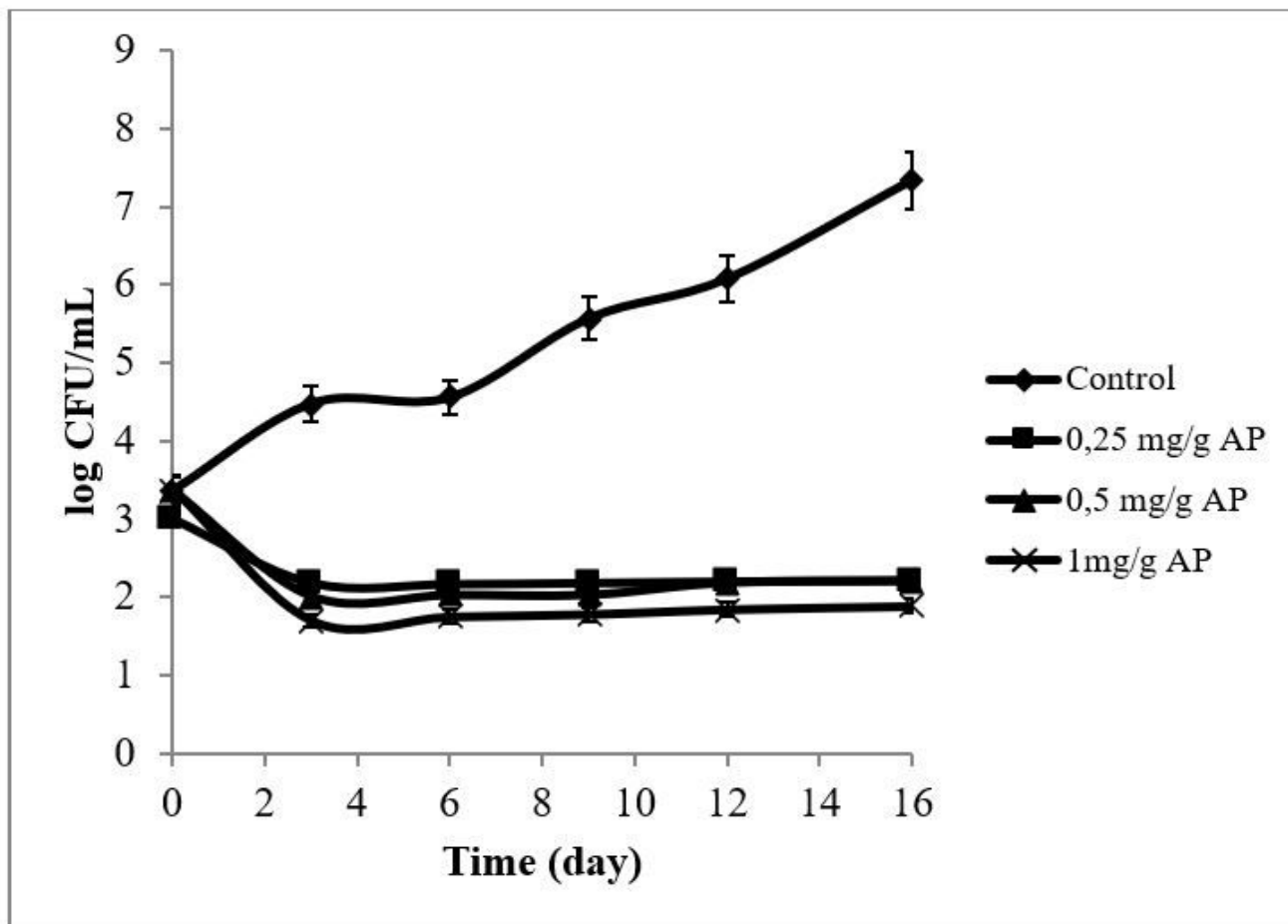


Figure 1

Effect of the AP on the survival of *S. enteritidis*

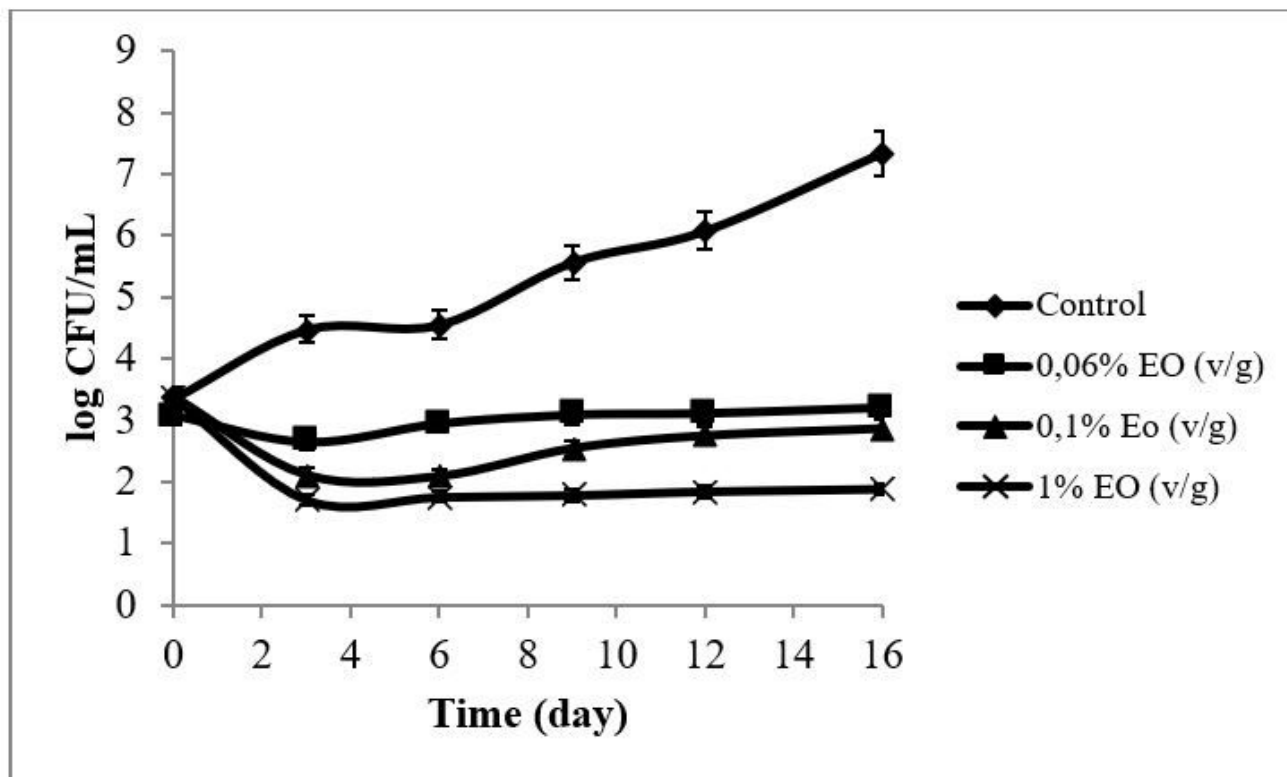


Figure 2

Effect of the EO on the survival of *S. enteritidis*

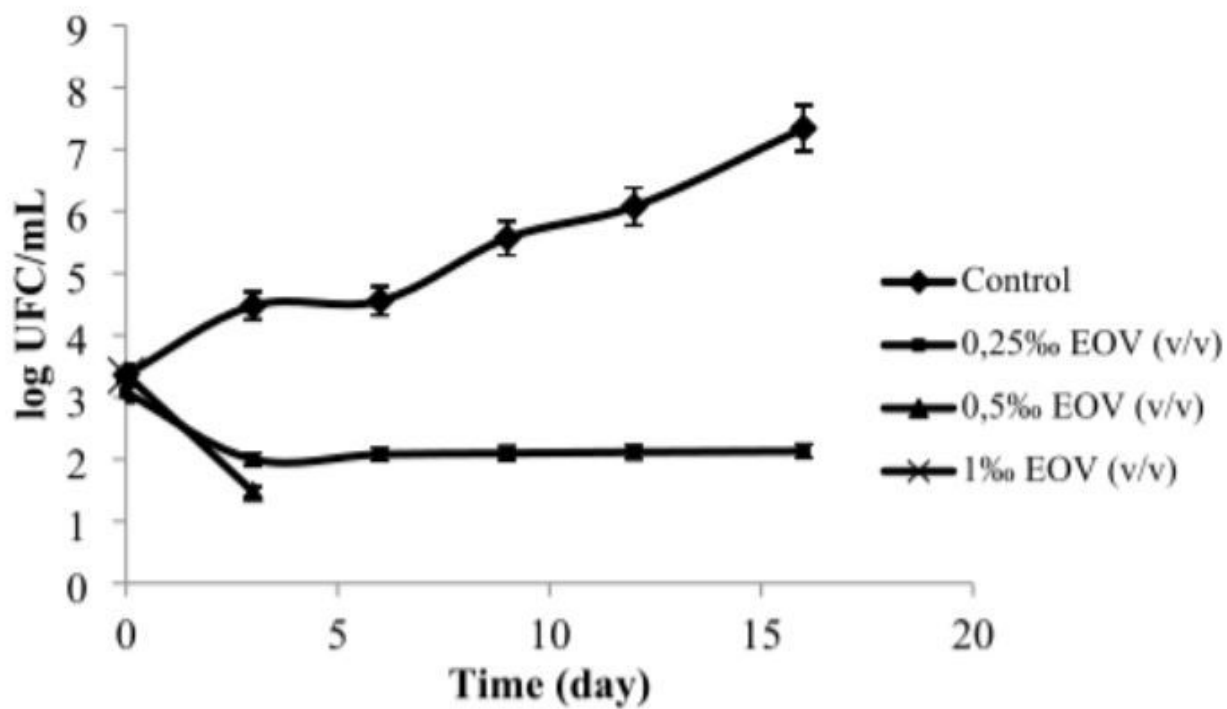


Figure 3

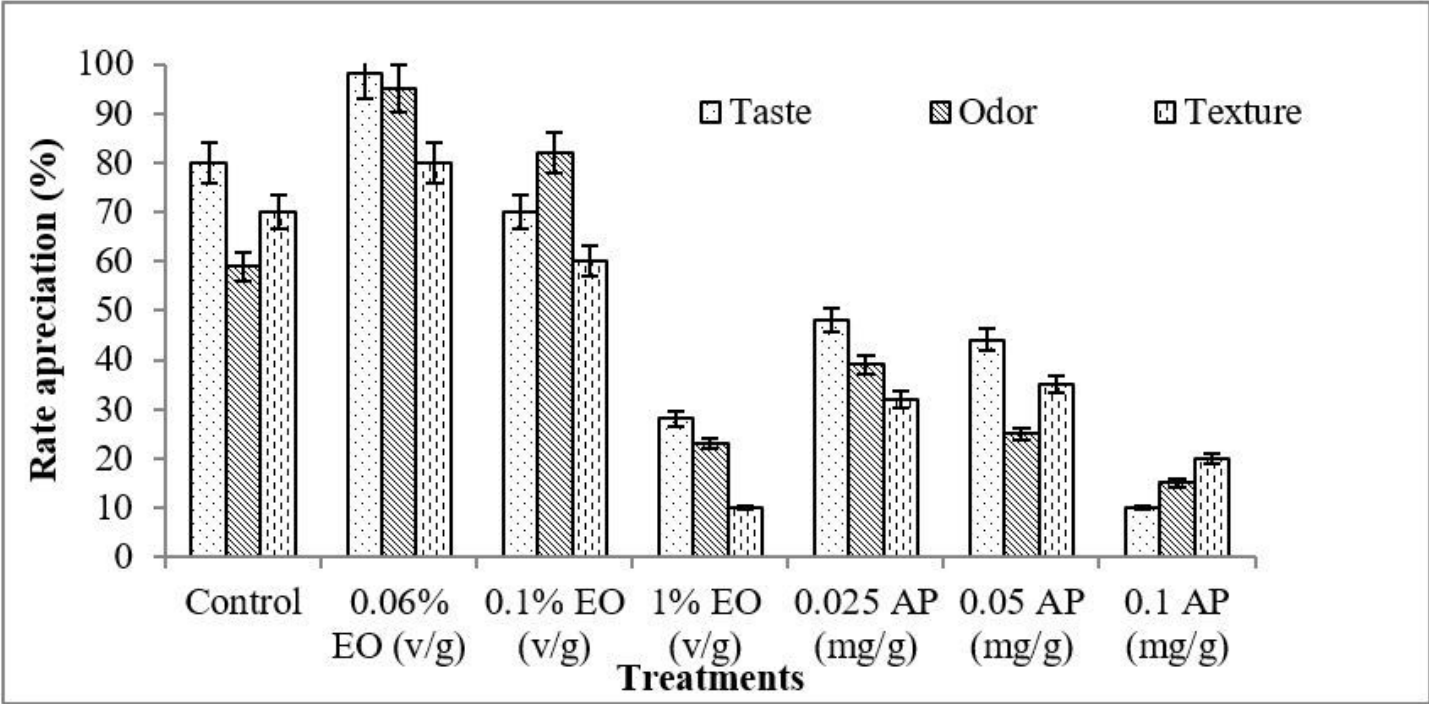


Figure 4

Effect of different treatments EO (0.06%, 0.1% and 1% v/g) and AP (0.25, 0.5 and 1 mg/g) on the taste, odor and texture parameters in chicken breast fillets