LWT - Food Science and Technology 215 (2025) 117270

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Antimicrobial effect of essential oils and terpenes coupled with supercritical carbon dioxide for chicken meat preservation

Santi Fabio [a], Lincetti Elisa [a], Alberghini Giulia [b], Giaccone Valerio [b], Zambon Alessandro [c,*], Spilimbergo Sara [a,**]

[a] Department of Industrial Engineering, University of Padova, Via Marzolo 9, 35131, Padova, Italy
[b] Department of Animal Medicine, Production and Health (MAPS), School of Agricultural Sciences and Veterinary Medicine, University of Padua, 35020, Legnaro, Italy
[c] Department of Civil, Chemical, Environmental, and Materials Engineering (DICAM), University of Bologna, Via Terracini 28, 40131, Bologna, Italy

ARTICLE INFO

Keywords:
High pressure carbon dioxide
Natural antimicrobial substances
Poultry meat
Food treatment
Microbial inactivation

ABSTRACT

This study was focused on technological strategies to increase the shelf life of chicken breast meat by using supercritical carbon dioxide (sc-CO_2) and antimicrobial natural substances, i.e. lemon, coriander and basil essential oil and their relative terpenes (linalool and limonene). The synergism was demonstrated on the inactivation of Gram-positive (*Listeria innocua* and *Enterococcus faecium)* and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas fluorescens*). A higher inactivation was observed when 1% terpenes were used in combination with sc-CO_2 (14 MPa and 40 °C for 15 min) achieving 3.05 and 2.91 log cfu/g reduction for *E. coli* using limonene and linalool respectively. Similar results with the same sc-CO_2 treatment were obtained also for *Pseudomonas fluorescens* (2.51 and 3.18 log cfu/g inactivation) and *Enterococcus faecium* (1.47 and 2.06 log cfu/g inactivation) using limonene and linalool, respectively. The mesophilic load of samples treated with 1% terpenes and sc-CO_2 after 9 days of storage at 4 °C was below 7 log cfu/g (6.55 and 5.63 cfu/g for limonene and linalool, respectively). These results promise interesting developments through the preservation of fresh chicken meat.

1. Introduction

Chicken meat is highly valued for its nutritional content and its substantial impact on the food market. However, 'broiler meat and products thereof' are considered the top food vehicles causing well-documented outbreaks of campylobacteriosis and salmonellosis. In recent years, campylobacteriosis and salmonellosis have been the two most commonly reported foodborne gastrointestinal infections in humans in Europe (European Food Safety Authority, & European Centre for Disease Prevention and Control, 2022; EFSA, 2022)). Consequently, there is a significant need for transformation in the food industry to reduce the risk of food-borne illness caused by pathogens. In this contest, innovative technologies are good candidates to achieve this goal. Recently, various alternatives have been explored, including the use of antimicrobial substances (Sweet et al., 2022) and new processing technologies (Allai et al., 2023). Several research focused on the potential of essential oils (EOs) as effective antimicrobial agents in food processing (Ekonomou et al., 2023; Scollard et al., 2016) and in active food packaging (Bibow and Oleszek, 2024). Essential oils, extracted from various plants, are gaining recognition for their ability to enhance food safety and prolong shelf life. Their antimicrobial properties have been recognized since the 1950s, and they are known also for their antiviral, antimycotic and antitoxigenic qualities (Burt, 2004).

The antimicrobial efficacy of essential oils does not rely on a single mechanism, but it varies depending on the specific constituents of the matrix and the targeted microorganisms (Pateiro et al., 2021). A prevalent mechanism responsible for EOs antimicrobial activity is the disruption of cellular membranes, which is due to the accumulation of bioactive compounds within the phospholipid bilayer of the cytoplasmic membrane, leading to damage and leakage of intracellular contents, disruption of embedded proteins, and ultimately, cell death (Ji et al., 2021). Although it is believed that Gram-positive bacteria, with their thick peptidoglycan layer in the cell wall, are more resistant than Gram-negative bacteria. However, conflicting opinions exist in literature (Nabrdalik & Grata, 2016) and a deeper investigation in this aspect is highly desirable. To enhance the antimicrobial effect, the active

* Corresponding author.
** Corresponding author.
E-mail addresses: alessandro.zambon2@unibo.it (Z. Alessandro), sara.spilimbergo@unipd.it (S. Sara).

https://doi.org/10.1016/j.lwt.2024.117270
Received 29 August 2024; Received in revised form 8 December 2024; Accepted 22 December 2024
Available online 24 December 2024

substances can be also encapsulated, as described by (Javadian, Shahosseini, & Ariaii, 2017). This approach ensures a slow and consistent release of bioactive compounds and maintains the stability of them over prolonged periods of time as reported for minced animal food products by Bagheri et al. (2016). An innovative approach involves using a bacterial cellulose/essential oils emulsion, as demonstrated by Xu et al. (2024). Their study explored the application of a bacterial cellulose/-thyme oil emulsion coating to extend the shelf life of chilled chicken.

Considering the chicken meat as a case product, Chouliara et al. (2007) investigated the effect of oregano essential oil and modified atmosphere packaging (MAP) on the shelf-life extension of chicken meat, managing to prolong it by up to 5–6 days. Similarly, Fratianni et al. (2010) experimented with thyme and balm essential oils, finding balm essential oil effective against *Salmonella* spp., and thyme essential oil efficient against *E. coli*. The simultaneous use of essential oils and low temperature processing were also investigated. Recently rosemary essential oil was coupled with supercritical CO_2 used in two different processes showing interesting results in the inactivation of *E. coli, Listeria innocua* and natural mesophilic bacteria (Santi et al., 2023). However, the study was limited to one essential oil, few microorganisms with no study on the growth of spoilage microorganisms during storage. Similar limitations appear also in other works that studied the synergism between supercritical carbon dioxide and essential oils on honey (Dacal-Gutiérrez et al., 2022) and almonds (Chen et al., 2022).

Furthermore, not only essential oils, but also terpenes, major constituents, have been studied as natural preservatives due to their antimicrobial activity. For example, Hussein et al. (2021) discovered that α-terpineol exhibits high antimicrobial activity against various bacteria (aerobic mesophilic counts, *P. lundensis*, *L. monocytogenes*, and *Salmonella enterica ser.* Typhimurium) when applied to chicken meat under refrigerated conditions. Another example of a successful use of terpenes is eugenol for the inactivation of *Campylobacter jejuni* in a chicken breast meat model. Gürbüz et al., (2024) studied the effect, observing that eugenol reduced *C. jejuni* for up to 7 days of storage. Similarly,Ekonomou et al., (2023) observed an antioxidant effect of linalool and eugenol on chicken breast meat.

To the best of the authors' knowledge, the antimicrobial properties of essential oils and their primary components typically occurred in controlled *in-vitro* settings (Mangalagiri et al., 2021) and a systematic comparison of the antimicrobial effects of essential oils, their constituent terpenes on food products is missing.

In this context, the primary objectives of this research were (i) to evaluate, compare and optimize the antimicrobial properties of three essential oils, lemon (LEO), basil (BEO) and coriander (CEO), and their constituent terpenes, linalool (LIN) and limonene (LIM), at different concentration alone, and in combination with sc-CO_2; (ii) to extend the shelf-life of chicken breast using the optimized concentration and components. This is the first study investigating the synergism between supercritical carbon dioxide (sc-CO_2) and terpenes to increase storage and safety of chicken breast meat. The optimization was achieved using four different surrogates of foodborne pathogenic bacteria: *E. coli* and *Pseudomonas fluorescens* (Gram-negative) were used as surrogates of *E. coli* O157:H7 and *Pseudomonas aeruginosa*, while *L. innocua* and *E. faecium* (Gram-positive) were used as surrogate for *L. monocytogenes* and *Campylobacter jejuni/Salmonella*, respectively.

2. Materials and methods

2.1. Experimental strategy

This work investigated the efficacy of the sc-CO2 treatment coupled with essential oils or their respective terpenes at a small scale, treating 5 g of chicken meat in each trial. The experimental plan was divided into three phases. In the first phase, the concentrations of essential oils (LEO, BEO, CEO) and terpenes (LIM, LIN) were optimized in combination with sc-CO2, studying their inactivation effect against surrogate pathogens, *E. coli* and *L. Innocua*, Gram negative and Gram positive, respectively. The substances that showed the highest degree of inactivation were used to inactivate other species, including *Pseudomonas fluorescens* and *Enterococcus faecium*, to demonstrate their different behavior against Gram-positive and Gram-negative bacteria. The substance-concentration combination that showed the highest degree of inactivation was selected for testing in phase three during the storage test at 4 °C. The processing conditions and natural substance concentrations were determined based on previous studies, using Santi et al. (2023), Chouliara (2007), and González-Alonso et al. (2020) as references. All experiments were conducted in at least triplicate.

2.2. Culture and cell suspensions

Escherichia coli NCTC 9001, *Listeria innocua* NCTC 11288, *Pseudomonas fluorescens*, *Enterococcus faecium* ATCC 6057 were used in this study. In order to carry out the artificial inoculation, 4 bacterial suspensions (one for each microorganism) with a final concentration of 10^8 cfu/mL were prepared. The suspensions were prepared according to Santi et al. (2023). Specifically, *Escherichia coli* was incubated overnight in Luria–Bertani (LB) medium Broth (Lennox, Sacco System, Como, Italy) at 37 °C ± 1 °C, while *Listeria innocua*, *Pseudomonas fluorescens* and *Enterococcus faecium* were incubated in Brain Heart Infusion Broth (BHI) (Microbiol diagnostici, Cagliari, Italy) at 37 °C ± 1 °C.

2.3. Test samples preparation and microbial inoculation

The samples were prepared as previously described by Santi et al. (2023), with some modifications regarding the essential oils and antimicrobial substances that were used. Briefly, in this work, the chicken cubes (5 ± 0.05 g) were sprinkled with lemon (Citrus limon) essential oil (LEO) (Erbamea, Perusa, IT), coriander (Coriandrum sativum) essential oil (CEO) (Erbamea, Perugia, IT), basil (Ocimum basilicum) essential oil (BEO) (HP Italia s. r.l., Padova, Italia), linalool (LIN) (Ansce Bio Generic s. r.l., Milano, IT) and L-limonene (LIM) (Ansce Bio Generic s. r.l., Milano, IT). For the inactivation of the inoculated bacteria strains, 100 μL of the microbial suspension was applied onto chicken cubes, followed by a 15-min incubation at room temperature in a laminar flow cabinet. For the antimicrobial substances, varying percentages (0.1, 0.5, and 1.0% vol/wt) were sprinkled onto the chicken cubes, which were then left for 15 min at room temperature within the laminar flow cabinet. The samples were subsequently either directly examined as controls or underwent processing prior to analysis.

2.4. SC-CO_2 treatment

Samples were treated at 14 MPa and 40 °C for 15 min using a multi batch apparatus, as previously described by Santi et al. (2023). In brief, the samples were put into a stainless-steel vessel with a volume of 15 mL. Each reactor was filled with liquid carbon dioxide (Nippon gasses, carbon dioxide 4.0, Milan, Italy) and pressurized at 14 MPa through a volumetric pump (LEWA, mod. LCD1/M910s, Germany).

Some samples were treated without the addition of antimicrobial natural substances, while others were treated after being sprinkled with these substances at the following percentages: 0.1,0.5 and 1% (vol/wt).

The process conditions were determined through preliminary tests (data not shown), ensuring the potential to observe any synergistic effects between the application of antimicrobial substances and the sc-CO2 treatment. These conditions were employed by Santi et al. (2023).

2.5. Microbial enumeration after inoculation and treatment

Each sample was analyzed to quantify the degree of post-treatment reduction of the inoculated bacteria. To study the synergistic effect of sc-CO_2 treatment and application of antimicrobial natural substances, it was necessary to analyze the control samples, the inoculated samples

with antimicrobial natural substances at different percentages (0.1%, 0.5% and 1%) and the same samples treated with sc-CO_2 (sc-CO_2 + 0.1%, sc-CO_2 + 0.5 %, and sc-CO_2 + 1%).

The standard plate count technique was used in order to quantify the bacteria, as reported in Santi et al. (2023). Each sample was homogenized in sterile buffered solution, then the decimal serial dilutions were obtained. 100 μL of solution were spread on the following media plates: MacConkey agar with crystal violet (Microbiol diagnostici, Cagliari, Italy) for *Escherichia coli*, BHI Agar for *Listeria innocua* (Microbiol diagnostici, Cagliari, Italy), *Pseudomonas* Agar for *Pseudomonas* (Microbiol diagnostici, Cagliari, Italy) and KF-*Streptococcus* agar for *Enterococcus faecium* (Microbiol diagnostici, Cagliari, Italy). Subsequently, they were incubated respectively at 37 °C ± 1 °C for 24 h, 37 °C ± 1 °C for 24 h, 20 °C ± 1 °C for 72 h and 37 °C ± 1 °C for 48 h into a thermostat (Memmert, Schwaback, Germany). *Enterococcus faecium* and *Pseudomonas fluorescens* inactivation were tested exclusively in presence of linalool and limonene because the antimicrobial effect was higher with respect to essential oils on *E. coli* (see Experimental Strategy 2.1).

2.6. Microbial storage test

A microbial storage test was conducted to evaluate the degree of post-treatment reduction of natural microbial communities, considering in detail: total viable count (TVC), *Pseudomonas* spp., total coliforms, lactic acid bacteria (LAB), yeasts and molds. Six different theses were compared: control, 1% limonene, 1% linalool, sc-CO_2, sc-CO_2 + 1% limonene and sc-CO_2 +1% linalool.

The storage test was carried out for 9 days. The temperature of storage was pointed at 4 °C as outlined also by EU Regulation 853/2004 for the conservation of fresh meat. On days 0, 4, 8 and 9 of storage, three test samples for each thesis were subjected to microbiological analysis using the following methods: UNI EN ISO 6887–1: 2017, UNI EN ISO 7218: 2013 and UNI EN ISO 4833–1: 2013. Furthermore, the method described by Ben Mhenni et al. (2023) was used for *Pseudomonas* spp. enumeration and confirmatory tests. The media used for microbial analysis were: Plate Count Agar (Merck®) for TVC, *Pseudomonas* agar base supplemented with CFC Supplement (Liofilchem®) for *Pseudomonas* spp., MacConkey agar (Scharlab Italia s. r.l) to isolate and quantify total coliforms, De Man–Rogosa–Sharpe agar (MRS) for LAB and Yeast Glucose Chloramphenicol agar (Merck®) to detect yeasts and molds. On the PCA the inclusion technique was used, whereas in the other media we employed the spread plate method. Petri dishes were then incubated in thermostats and fridge-thermostats respectively at these conditions: 30 °C ± 1 °C for 72 h, 25 °C ± 1 °C for 72 h, 37 °C ± 1 °C for 24 h, 35 °C ± 1 °C for 48 h and 25 °C ± 1 °C for 48 h. The results were expressed in colony-forming units per gram (cfu/g) and then subjected to the determination of arithmetic mean, standard deviation and logarithmic transformation.

2.7. Statistical analysis

Statistical analyses were conducted using Minitab®. Mean values were compared to assess differences between treatments. Significant differences ($\alpha = 0.05$) among treatments were evaluated using ANOVA, followed by post hoc analysis pairwise comparisons with Fisher's test. All the experiments were performed at least in triplicated and the statistical analysis were based on data from at least three independent experiments.

3. Results and discussion

3.1. Application of sc-CO_2 with essential oils and their terpenes

In the first phase of this study, the use of supercritical carbon dioxide (sc-CO_2) along with three essential oils (LEO, BEO and CEO) and their constituent terpenes (LIM and LIN) was evaluated to enhance microbial inactivation of inoculated spoilage bacteria and surrogate strains of foodborne pathogens on chicken breast samples. The results obtained for, *E. coli* and *L. innocua* are reported in Tables 1 and 2.

According to the statistical analysis, the application of LEO, CEO and LIM alone did not result in any significant difference in microbial inactivation compared to the initial microbial load. On the other hand, BEO and LIN demonstrated statistically significant differences at concentrations 1 % of BEO and 0.5%–1% of LIN, although the reductions were less than 1 log cfu/g. The minimal inactivation observed may be attributed to the binding of antimicrobial volatile compounds to fats and proteins present in meat as reported by Ji et al. (2021). Based on this evidence, essential oils and terpenes alone were not effective to inactivate high amounts of surrogate strains of foodborne pathogens, making their standalone use insufficient to enhance food safety. Sheerzad et al. (2024) addressed this limitation by using a coating method to enhance the effectiveness of cinnamon essential oil (0.5%, 1%, and 1.5%) on chicken breast meat. The study tested several coating types, including whey protein isolate, nanochitosan, and bacterial nanocellulose. The best formulation was whey protein isolate combined with nanochitosan (WPI) + 1.5% cinnamon essential oil. These findings suggest that essential oils require relatively high concentrations and additional methods, such as coatings, to achieve significant bacterial inactivation.

The application of sc-CO_2 alone produced a statistically significant difference in microbial load compared to the control.

The inactivation achieved with sc-CO_2 was 0.81 log cfu/g for *E. coli* and 1.26 log cfu/g for *L. innocua* starting from a load 6.74 and 7.25 log cfu/g respectively. Our degree of inactivation was similar to that reported by Wei et al. (1991), where approximately 2 log cfu/g with a treatment at 13.7 MPa and 35 °C for 2 h were achieved for *L. monocytogenes*. The lower inactivation observed in our study can be attributed to the shorter process time, as the extended duration used by Wei et al. (1991) contributed to greater microbial inactivation. The antimicrobial effect of sc-CO_2 has also been studied on cooked ham (Ferrentino et al., 2013) and raw pork meat (Cappelletti et al., 2015) demonstrating that inactivation kinetics depend on both the process and the food matrix.

The highest microbial inactivation was observed when sc-CO_2 was applied in combination with natural antimicrobial substances. The synergism between the three essential oils tested (LEO, CEO, BEO) and sc-CO_2 was significant when essential oils were combined at concentrations of 1%, resulting in reductions of about 1 and 1.5 log cfu/g for *E. coli* and *L. innocua,* respectively, from the initial average load. Compared to sc-CO_2 alone, the synergistic effects led to an additional reduction of 0.5 log cfu/g in *E. coli* for all the essential oils tested, while a significant reduction on the *L. innocua* was achieved only with LEO and CEO. At low concentrations (0.1%) of essential oils combined with sc-

Table 1
Microbial loads of *E. coli* and *L. innocua* inoculated on chicken breast samples, with and without the application of lemon essential oil (LEO), limonene (LIM), supercritical carbon dioxide (sc-CO_2) and their combination at various concentrations.

	(%)	LEO		LIM	
		E. coli (log CFU/g)	*L. innocua* (log CFU/g)	*E. coli* (log CFU/g)	*L. innocua* (log CFU/g)
Control	–	6.74 ± 0.26^{a}	7.25 ± 0.09^{a}	7.03 ± 0.06^{a}	7.36 ± 0.21^{a}
	0.1	6.84 ± 0.14^{a}	7.24 ± 0.06^{a}	6.73 ± 0.05^{a}	7.42 ± 0.11^{a}
	0.5	6.92 ± 0.10^{a}	7.39 ± 0.04^{a}	7.13 ± 0.07^{a}	7.42 ± 0.13^{a}
	1.0	6.63 ± 0.16^{a}	7.14 ± 0.11^{a}	7.05 ± 0.14^{a}	7.41 ± 0.04^{a}
sc-CO_2	–	5.93 ± 0.54^{b}	5.99 ± 0.11^{b}	5.93 ± 0.54^{b}	6.13 ± 0.29^{b}
	0.1	5.01 ± 0.46^{c}	5.80 ± 0.18^{bc}	6.62 ± 0.17^{a}	6.39 ± 0.02^{b}
	0.5	5.45 ± 0.45^{bc}	5.91 ± 0.08^{bc}	5.23 ± 0.33^{c}	6.23 ± 0.54^{b}
	1.0	5.16 ± 0.32^{c}	5.68 ± 0.40^{c}	3.98 ± 0.60^{d}	5.39 ± 0.10^{c}

Values are mean ± standard deviation population recovered (log cfu/g) (n = 3). Values in the same columns sharing a common letter are not significantly different ($P < 0.05$).

Table 2
Microbial loads of *E. coli* and *L. innocua* inoculated on chicken breast samples, with and without the application of basil essential oil (BEO), coriander essential oil (CEO), linalool (LIN), supercritical carbon dioxide (sc-CO_2) and their combination at various concentrations.

	(%)	BEO		CEO		LIN	
		E. coli (log CFU/g)	*L. innocua* (log CFU/g)	*E. coli* (log CFU/g)	*L. innocua* (log CFU/g)	*E. coli* (log CFU/g)	*L. innocua* (log CFU/g)
Control	–	6.93 ± 0.05^{a}	7.17 ± 0.11^{ab}	6.93 ± 0.07^{a}	7.63 ± 0.35^{a}	6.66 ± 0.40^{a}	7.17 ± 0.05^{a}
	0.1	6.93 ± 0.12^{a}	7.47 ± 0.30^{a}	6.93 ± 0.06^{a}	7.27 ± 0.06^{a}	6.60 ± 0.32^{a}	7.11 ± 0.07^{ab}
	0.5	6.87 ± 0.10^{a}	7.51 ± 0.20^{a}	6.94 ± 0.17^{a}	7.31 ± 0.23^{a}	5.87 ± 0.22^{b}	6.80 ± 0.08^{bc}
	1.0	6.35 ± 0.18^{b}	7.49 ± 0.03^{a}	6.57 ± 0.16^{a}	7.24 ± 0.01^{a}	5.93 ± 0.39^{b}	6.72 ± 0.03^{cd}
sc-CO_2	–	5.93 ± 0.54^{c}	6.49 ± 0.19^{cd}	5.93 ± 0.54^{b}	6.05 ± 0.42^{b}	5.50 ± 0.26^{b}	6.36 ± 0.17^{de}
	0.1	5.72 ± 0.09^{cd}	6.91 ± 0.43^{bc}	5.62 ± 0.20^{bc}	6.35 ± 0.20^{b}	4.64 ± 0.66^{c}	6.27 ± 0.23^{ef}
	0.5	4.86 ± 0.06^{e}	6.37 ± 0.50^{d}	5.41 ± 0.26^{c}	5.96 ± 0.23^{b}	5.30 ± 0.23^{b}	5.92 ± 0.49^{f}
	1.0	5.36 ± 0.07^{d}	6.53 ± 0.18^{cd}	5.36 ± 0.28^{c}	5.55 ± 0.09^{c}	3.75 ± 0.27^{d}	5.33 ± 0.19^{g}

Values are mean ± standard deviation population recovered (log cfu/g) (n = 3).
Values in the same columns sharing a common letter are not significantly different ($P < 0.05$).

CO_2 no significant synergism except for LEO in the case of *E. coli*. However, increasing the concentration of LEO did not further enhance the degree of inactivation. For BEO, the maximum inactivation for *E. coli* was achieved at 0.5%, while no additional reduction was observed for *L. innocua*.

Considering the bacterial inactivation achieved by coupling terpenes, it was higher compared with essential oils, and it was also different between LIM and LIN. When LIM was used in combination with sc-CO_2, the synergism was observed only at a concentration of 1% for both *E. coli* and *L. innocua* achieving almost 2 log cfu/g reduction for both microorganisms compared to sc-CO_2 alone. In contrast, when LIN was used, synergism was evident even at 0.1% but only for *E. coli*. Similar to LIM, the highest inactivation with LIN was achieved at a concentration of 1%, achieving about 1–1.5 log cfu/g inactivation for both microorganisms.

In general, the synergism on microbial inactivation was higher for the *E. coli* (Gram-negative) compared to *L. innocua* (Gram-positive) across all the natural substances tested. This behavior could be explained by the differences in the thickness of the peptidoglycan layer of the cell walls between the two types of microorganism. This hypothesis aligns with findings from previous studies where sc-CO_2 treatment demonstrated a higher inactivation for Gram-negative than Gram-positive bacteria. Furthermore, the antibacterial mechanisms of LIM and LIN against both *E. coli* and *L. monocytogenes* have been documented in the literature, although not in combination with sc-CO_2. Specifically, He et al. (2022) found that linalool acts on the metabolism of *L. monocytogenes* cells, ultimately causing their death, while Herman et al. (2016) reported that the action of linalool alone was more effective against *E. coli* than essential oils containing linalool as a component. On the other hand, Espina et al. (2013) showed that limonene is effective against *E. coli,* particularly when used in combination with other bactericidal treatments, supporting our results combining sc-CO_2. Additionally, using Scanning Electron Microscope (SEM), Han et al. (2020) observed that limonene alters the normal morphology of *L. monocytogenes*, causing disruption of the cell wall and membrane. Considering the overall results obtained in previous analyses, higher microbial inactivation was consistently achieved with terpenes. Therefore, only the two terpenes were included in subsequent experiments.

3.2. Application of sc-CO_2 in combination with terpenes for the inactivation of *P. fluorescens* and *E. faecium*

Since a different behavior was observed between the two classes of microorganisms, Gram-positive and Gram-negative, additional bacteria from these two genera were investigated. Specifically, *P. fluorescens* (Gram-negative) and *E. faecium* (Gram-positive) were treated with LIM and LIN both individually and in combination with sc-CO_2. The results are shown in Table 3.

LIM alone did not significantly reduce *P. fluorescens* and *E. faecium*, neither at 1% concentration, similar to *E. coli* and *L. innocua*. LIN alone was able to slightly reduce both microorganisms and the reduction was significant for *E. faecium* compared to the control achieving 0.56 log cfu/g reduction. Gürbüz et al., (2024) investigated the antimicrobial effects of eugenol against various *Campylobacter jejuni* strains inoculated on a raw chicken breast meat model, including isolates from chicken and reference strains. The highest inactivation was achieved at concentrations eight times higher than the MIC. Additionally, the study highlighted differences in sensitivity among the tested strains, with the strain isolated from chicken meat exhibiting greater resistance to eugenol compared to the reference strain. In our study, as previously mentioned, *Enterococcus faecium*, used as a surrogate for *C. jejuni*, did not show significant inactivation when treated with LIN and LIM at the tested concentrations. However, treatment with eugenol resulted in reductions of 1.5 log cfu/g for the isolated strain and 4.5 log cfu/g for the reference strain. These findings suggest that antimicrobial efficacy is influenced not only by the specific substance used but also by the strain being tested.

The application of sc-CO_2 alone was able to reduce almost 2 log cfu/g

Table 3
Microbial loads of *Pseudomonas fluorescens* and *Enterococcus faecium* inoculated on chicken breast samples, with and without the application of limonene (LIM), linalool (LIN), supercritical carbon dioxide (sc-CO_2) and their combination at various concentrations.

	(%)	LIM		LIN	
		Pseudomonas fluorescens (log CFU/g)	*Enterococcus faecium* (log CFU/g)	*Pseudomonas fluorescens* (log CFU/g)	*Enterococcus faecium* (log CFU/g)
Control	–	6.56 ± 0.07^{a}	6.63 ± 0.19^{a}	6.41 ± 0.10^{ab}	6.73 ± 0.26^{a}
	0.1	6.49 ± 0.18^{a}	6.66 ± 0.10^{a}	6.49 ± 0.27^{ab}	6.49 ± 0.09^{ab}
	0.5	6.36 ± 0.09^{a}	6.32 ± 0.03^{b}	6.74 ± 0.74^{a}	6.46 ± 0.17^{ab}
	1.0	6.12 ± 0.20^{a}	6.39 ± 0.09^{ab}	5.91 ± 0.22^{b}	6.17 ± 0.31^{b}
sc-CO_2	–	4.67 ± 0.16^{c}	5.39 ± 0.10^{de}	4.96 ± 0.24^{c}	5.72 ± 0.26^{c}
	0.1	5.20 ± 0.13^{b}	5.46 ± 0.25^{cd}	4.65 ± 0.46^{c}	5.70 ± 0.17^{c}
	0.5	4.41 ± 0.53^{cd}	5.71 ± 0.29^{c}	3.79 ± 0.41^{d}	5.25 ± 0.14^{d}
	1.0	4.05 ± 0.37^{d}	5.16 ± 0.06^{e}	3.23 ± 0.24^{d}	4.67 ± 0.32^{e}

Values are mean ± standard deviation population recovered (log cfu/g) (n = 3).
Values in the same columns sharing a common letter are not significantly different ($P < 0.05$).

and 1.5 log cfu/g for *P. fluorescens* and *Enterococcus faecium*, respectively. The inactivation was higher compared to *E. coli* and *L. innocua*. Regarding *P. fluorescens*, this difference in inactivation may be due to the sensitivity to high-pressure treatments, as demonstrated by Carlez et al. (1993) and to CO_2 (Chouliara et al., 2007).

The microbial reduction was increased when LIN and LIM were combined with sc-CO_2. Synergism was observed against *P. fluorescens* with both LIN and LIM, while against *E. faecium*, it was only evident with LIN. For LIM, synergism was significant starting at 0.5%, whereas 1% was required for LIM. Specifically, when sc-CO_2 + 0.5% LIN was used against *P. fluorescens* and *E. faecium,* increments of about 1.17 and 0.47 log cfu/g were achieved, respectively. Where sc-CO_2 + 1% LIN was applied, reductions of 1.73 log cfu/g for *P. fluorescens* and of 1.05 log cfu/g for *E. faecium* were observed, respectively. In case of LIM, only *P. fluorescens* showed a synergism, obtaining an increment of 0.62 log cfu/g compared to sc-CO_2 alone when used at 1%. For *E. faecium,* no synergism was achieved at any of the concentrations of LIM used in combination with sc-CO_2.

These results are consistent with those obtained for *E. coli* and *L. innocua*, within the same Gram category. They confirm the differing inactivation behaviors between Gram-negative and Gram-positive bacteria, with Gram-negative bacteria exhibiting greater sensitivity to the process and a higher degree of synergism. However, within the Gram-positive category, different behaviors between *L. innocua* and *E. faecium*. The antimicrobial action of LIM is attributed to its lipophilic properties, which can lead to protein denaturation and disruption of lipid layer, ultimately resulting in microbial death (Hernandes et al., 2014). According to Schneider et al. (2023) these differences could be due to variations in composition of cell wall and cell membrane. Additionally, differences in energy metabolism and genetic material may also play a significant role, warranting further investigation.

3.3. Microbial storage test

In the last phase of the work, the synergism between LIM, LIN and sc-CO_2 was investigated on the naturally present microorganisms on the samples. The previously most effective concentration (1%) was used for this study. A 9 days-long storage test at constant refrigeration temperature (4 °C) was performed. The test was conducted on six different theses: raw untreated chicken (control), 1% LIM, 1% LIN, sc-CO_2, sc-CO_2 with 1% LIM (sc-CO_2 + 1% LIM), and sc-CO_2 with 1% LIN (sc-CO_2 + 1% LIN). The microorganisms identified for the enumeration during the storage test were Total Viable Count, *Pseudomonas* spp., total coliforms, lactic acid bacteria, yeasts and molds.

3.3.1. Total viable count (TVC)

TVC is an important parameter for the acceptability of food consumption. Specifically, for chicken it was established by ICMSF (1986) 7 log cfu/g as the upper microbiological limit to ensure a good quality of fresh poultry meat (Chouliara et al., 2007). In several studies present in the literature, the microbiological acceptability criterion (total viable counts reaching 7 log cfu/g) has been used to define spoilage (Rouger et al., 2017).

The results are shown in Fig. 1. The initial load of the raw chicken was 4.95 log cfu/g. After 9 days of storage at 4 °C, the control (7.75 log cfu/g), 1% LIM (7.62 log cfu/g), and 1% LIN (7.25 log cfu/g) were found to be above the ICMSF limit. These results are similar for the control with the ones reported by Chouliara et al., (2007), where the combined effect of several concentrations of oregano essential oil and modified atmosphere packaging were investigated on chicken breast meat stored at 4 °C. In our study, LIM and LIN used alone demonstrated to be unaffected on controlling the growth of spoilage microorganisms. On the other hand, samples treated with sc-CO_2, sc-CO_2 + 1% LIM, and sc-CO_2 + 1% LIN, resulted below the limit of 7 log cfu/g (6.49 log cfu/g, 6.55 log cfu/g, 5.63 log cfu/g respectively) at 9 days of storage and statistically different from the other three theses. Sc-CO_2 treatments were able to reduce about 1.5 log cfu/g, however there were no significant differences between samples treated with sc-CO_2 alone or with the addition of LIM and LIN at time 0. The synergistic effect of LIM and LIN combined with sc-CO_2 treatment was evident during the storage, beginning on day 4. By the end of the shelf life, significant synergism was observed only for the sample treated with sc-CO_2 + 1% LIN, demonstrating it to be a more effective bacteriostatic agent. Specifically, sc-CO_2 + 1% LIN samples on 9 days showed a microbial load of 5.63 log cfu/g, nearly 1 log lower than the sc-CO_2 treatment alone. sc-CO_2 + 1% LIN maintained a more consistent bacterial load throughout the storage period. The results obtained with sc-CO_2 + LIM were similar to those reported by Chouliara et al., (2007) using 1% oregano essential oil. After 9 days they

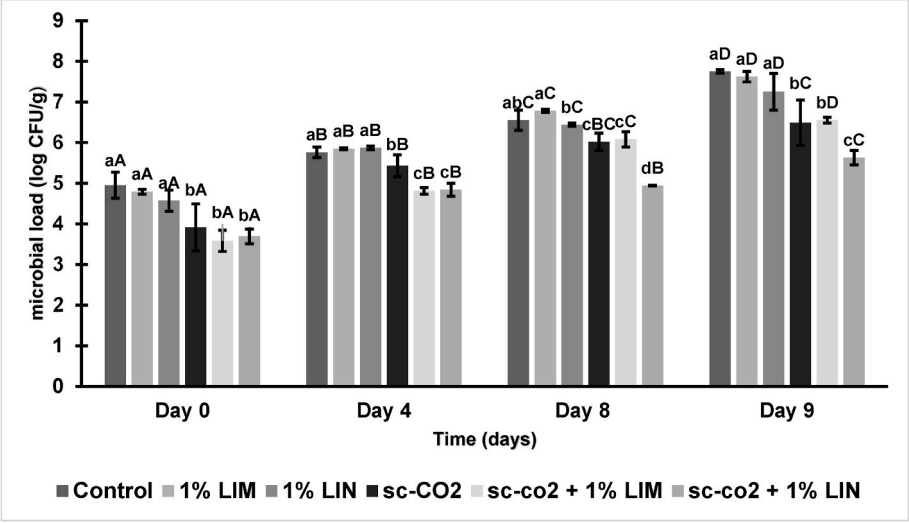

Fig. 1. Total Viable Count load on chicken breast samples during the 9 days storage test.
The counts of the control samples (Control), samples with 1% limonene (1% LIM), samples with 1% linalool (1% LIN), samples treated with supercritical carbon dioxide (sc-CO_2), samples treated with sc-CO2 coupled with 1% limonene (sc-CO_2 + 1% LIM) and samples treated with sc-CO2 coupled 1% linalool (sc-CO_2 + 1% LIN).
Values are mean ± standard deviation population recovered (log cfu/g) (n = 3).
Lower case letters identify differences between the theses on the same day, upper case letters represent the differences between the days of the same thesis ($P < 0.05$).

recorded a load of 4.11 log cfu/g, although their initial load was lower at 4.28 log cfu/g compared to ours. Their study also indicated that microbial growth could be further inhibited by coupling essential oils with a MAP composition of 70% CO_2 and 30% N_2, resulting in a load of 3.21 log cfu/g after 9 days of storage. This suggests that MAP could be also coupled after sc-CO_2 + 1% LIN could enhance the antimicrobial effect during storage. Additionally, other strategies have been explored, such as those by Konuk Takma & Korel (2019), who investigated the antimicrobial properties of active packaging based on cumin essential oil and achieved a load of 7 log cfu/g after 5 days at 4 °C.

3.3.2. *Pseudomonas spp*

During storage in air at refrigeration temperature, *Pseudomonas* spp. is the predominant bacterial population recovered from chicken meat (Salama & Chennaoui, 2024) and can be considered a specific spoilage microorganism (SSO) due to its ability to grow in psychotropic and oligotrophic environments (Saenz-García et al., 2020). As reported by McKee (2012) and Taormina (2021), SSOs develop rapidly during the refrigerated storage, leading to changes in flavor, texture and appearance. The microbial upper limit for the *Pseudomonas* in the chicken breast meat storage at 4 °C is suggested to be 5 log cfu/g (Peter et al., 2023). As shown in Fig. 2, the initial microbial load of the raw chicken was 3.99 ± 0.07 log cfu/g, and treatments with LIN, sc-CO_2, sc-CO_2 + 1% LIN and sc-CO_2 + 1% LIM resulted in a significant reduction of about 0.5 log cfu/g. After 4 days, sc-CO_2 treatments slowed bacterial growth, but by day 8 day, only the combined treatment sc-CO_2 + 1% LIN showed an effective reduction of. After 9 days, the observed microbial loads were: 7.26, 6.73, 6.38, 6.40, 5.90 and 4.75 log cfu/g respectively for the control, 1% LIM, 1% LIN, sc-CO_2, sc-CO_2 + 1% LIM and sc-CO_2 +1% LIN. Our findings indicate that only samples treated with sc-CO_2 + 1% LIN were able to maintain microbial counts below the 5 log cfu/g after 9 days of storage. These results support the enhanced bacteriostatic effect of the LIN coupled with sc-CO_2 also observed in TVC. Choularia et al. (2007) reported a microbial load of 2.32 log cfu/g after 9 days using 1% oregano essential oil, starting from 3.38 log cfu/g; however, direct comparison is challenging due to the initial load difference. Generally, it was observed a lower inactivation on *Pseudomonas* naturally present on chicken breast meat compared to the inactivation obtained on *P. fluorescens* (Table 3). To improve these results and achieve better control of Pseudomonas spp. growth, it might be possible to use, in combination with our treatment, a CO_2-rich modified atmosphere, as proposed by Latou et al. (2014) in their study on extending the shelf life of chicken breast meat by combining a CO_2-rich modified atmosphere with chitosan coating. Hulankova et al. (2018) also reported better results using various MAP compositions with different CO_2 concentrations compared to air storage at 2 °C. As noted by Choularia et al., (2007) *Pseudomonas* spp. is sensitive to presence of CO_2. Another potential solution is the use of the coating, as suggested by Latou et al. (2014). In our case, this strategy could be applied either before (Santi et., (2023) or after the treatment with sc-CO_2. However, using the coating prior to treatment requires further investigation, as the supercritical carbon dioxide treatment may potentially damage the coating itself. Additionally, the use of coatings does not always guarantee better results, as their effectiveness depends on the material used and the type and quantity of active substances incorporated. For instance, Aghababaei et al. (2022) reported that a chitosan and galbanum gum composite coating containing 0.75% cumin essential oil led to Pseudomonas spp. loads exceeding the 5 log cfu/g limit after more than 6 days, whereas a coating with 1.5% cumin essential oil did not exceed the limit even after 9 days.

3.3.3. *Total coliforms*

Coliforms are Gram-negative bacteria which slowly grow at refrigeration temperatures because they are typically mesophilic or even thermophilic bacteria. However, some psychotropic strains have been isolated from poultry meat and their investigations during storage is important as they contribute to food spoilage, including meat ones (Giaccone & Colavita, 2015). Coliforms are an important quality indicator and are among the most significant microorganisms in meat and meat products (Safari et al., 2023).

As shown in Fig. 3, the initial load of coliform was below 4 log cfu/g, and their growth was limited at refrigerated conditions, increasing of about 1 log cfu/g after 9 days. It is noteworthy that all the treated samples significantly differ from the untreated ones. Additionally, samples treated with only 1% of LIN showed a statistical difference from both the control and 1% LIM samples. Peter et al. (2023) suggested that 4 log cfu/g is the limit of acceptability for coliforms. In our study, after 9 days of aerobic refrigerated storage, the control sample and 1% LIM sample were above this limit, specifically 4.47 and 4.28 log cfu/g,

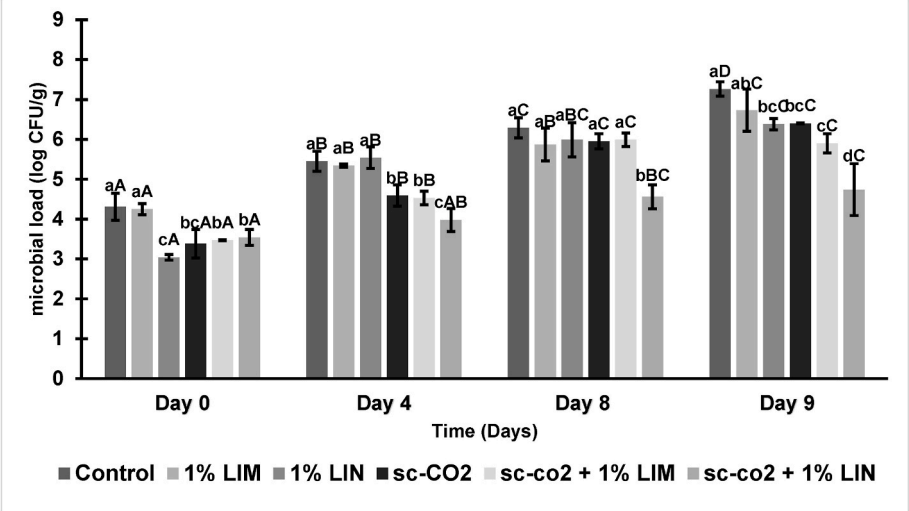

Fig. 2. *Pseudomonas* on chicken breast samples during the 9 days storage test.
The counts of the control samples (Control), samples with 1% limonene (1% LIM), samples with 1% linalool (1% LIN), samples treated with supercritical carbon dioxide (sc-CO_2), samples treated with sc-CO_2 coupled with 1% limonene (sc-CO_2 + 1% LIM) and samples treated with sc-CO_2 coupled 1% linalool (sc-CO_2 + 1% LIN).
Values are mean ± standard deviation population recovered (log cfu/g) (n = 3).
Lower case letters identify differences between the theses on the same day, upper case letters represent the differences between the days of the same thesis ($P < 0.05$).

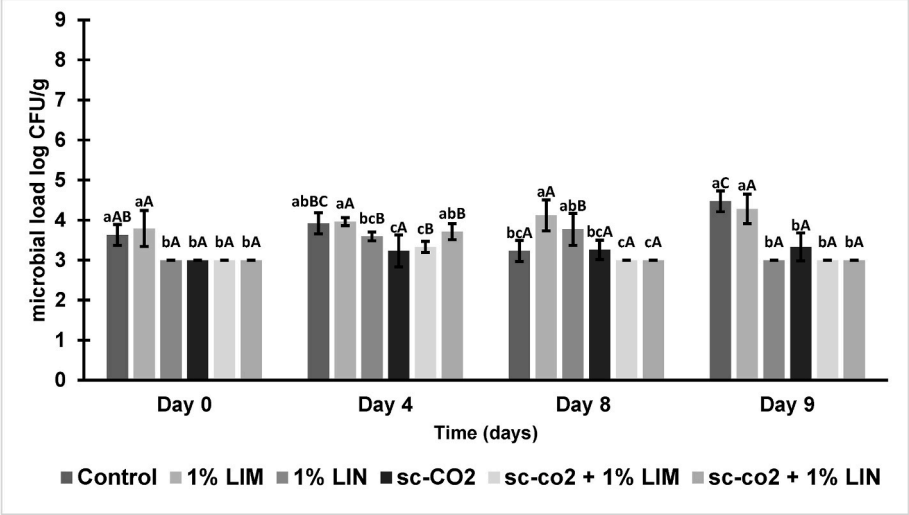

Fig. 3. Total coliforms on chicken breast samples during the 9 days storage test.
The counts of the control samples (Control), samples with 1% limonene (1% LIM), samples with 1% linalool (1% LIN), samples treated with supercritical carbon dioxide (sc-CO_2), samples treated with sc-CO_2 coupled with 1% limonene (sc-CO_2 + 1% LIM) and samples treated with sc-CO_2 coupled 1% linalool (sc-CO_2 + 1% LIN).
Values are mean ± standard deviation population recovered (log cfu/g) (n = 3).
Lower case letters identify differences between the theses on the same day, upper case letters represent the differences between the days of the same thesis ($P < 0.05$).

respectively, while the other thesis was below this threshold. These findings highlight that LIN is a potent antimicrobial agent against coliform growth under refrigerated conditions, a result supported by Mączka et al. (2022), who demonstrated LIN's strong antimicrobial activity against Gram-negative bacteria. Alirezalu et al. (2022) also investigated the use of natural antimicrobial substances on chicken breast meat, specifically examining the combined effect of a calcium-alginate coating and various concentrations of Artemisia fragrance essential oil. However, this approach did not prolong shelf life, as all samples exceeded 4 log cfu/g after 4 days at 4 °C. On the other hand, Xu et al. (2024) achieved better results, maintaining a load of 4.41 log cfu/g (coliform MPN analysis) after 12 days using 0.5% bacterial cellulose/thyme essential oil.

These results suggest that using essential oil alone or in combination with a coating may not be sufficient to reduce coliform levels effectively. The same study noted that other research employing plant extract coatings has reported similar outcomes for coliform inactivation across different food matrices. In conclusion, our data, along with other studies, suggest that coliforms are difficult to eradicate, and their susceptibility depends on the antimicrobial agent used. In this study, 1% LIN demonstrated a strong effect in inactivating coliforms. Terpenes might exhibit improved efficacy when combined with coatings; however, this hypothesis still needs to be demonstrated, and evidence for their effectiveness in chicken meat is limited.

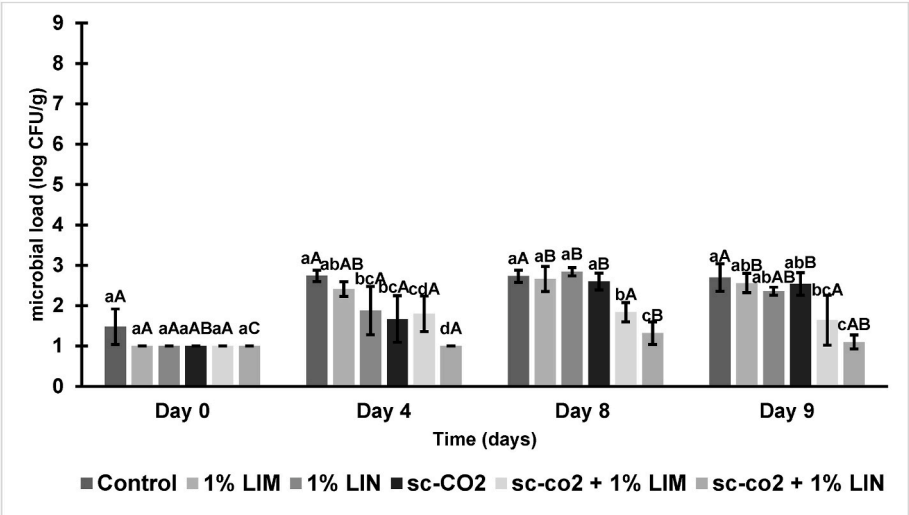

Fig. 4. Lactic Acid Bacteria chicken breast samples during the 9 days storage test.
The counts of the control samples (Control), samples with 1% limonene (1% LIM), samples with 1% linalool (1% LIN), samples treated with supercritical carbon dioxide (sc-CO_2), samples treated with sc-CO_2 coupled with 1% limonene (sc-CO_2 + 1% LIM) and samples treated with sc-CO_2 coupled 1% linalool (sc-CO_2 + 1% LIN).
Values are mean ± standard deviation population recovered (log cfu/g) (n = 3).
Lower case letters identify differences between the theses on the same day, upper case letters represent the differences between the days of the same thesis ($P < 0.05$).

3.3.4. Lactic acid bacteria (LAB)

LAB are involved in the deterioration of chicken breast meat especially when the food is packaged under vacuum or in a modified atmosphere (Chouliara et al., 2007). Being mesophilic or thermotropic bacteria, their growth slows in refrigerated foods and they are sensitive to the competitive effect of other microbial populations (Giaccone & Colavita, 2015).

In untreated samples, LAB were present in low loads (1.48 log cfu/g) and they resulted below the detection limit (<1 log cfu/g) in all the other treated samples (see Fig. 4). Their growth during storage was slow and never exceeded 3 log cfu/g, even in the untreated samples. However, it is important to note that samples treated with sc-CO_2 + 1% LIN and sc-CO2 + 1% LIM showed the slowest growth after 9 days of storage, with counts of 1.64 and 1.10 cfu/g, respectively. Once again, LIN demonstrated a strong bacteriostatic effect in combination with sc-CO_2. Peter et al. (2023) suggested an upper load for LAB of 6 log cfu/g during storage, which was not exceeded under any of the conditions in our study. In a recent study, by Ekonomou et al. (2023) investigated the effect of coating linalool and eugenol on chicken breast meat. The results for the control group in their study were similar to ours control samples. However, the inactivation achieved with the coating was lower, likely due to the lower concentration of terpenes (0.5 and 0.7 mg/mL) available in their coating. LAB are responsible for deterioration of food, and our findings demonstrate that the treatment with sc-CO_2 and 1% LIN or LIM effectively slows their growth, helping to maintain food acceptance and quality.

3.3.5. Yeasts and molds (Y&M)

Y&M are aerobic microorganisms that can also grow in poultry meat. They are resistant to a wide range of both intrinsic and extrinsic food-related factors, including storage temperatures, and their growth on food often leads to significant spoilage (Dagnas & Membré, 2013; Odeyemi et al., 2020). In the untreated samples, Y&M counts were about 3 log cfu/g and increased to nearly 5.5 log cfu/g after 9 days of storage at 4 °C. LIM did not affect the inactivation or the inhibition of the growth during storage, while LIN exhibited an opposite behavior, as shown in Fig. 5. The highest inactivation was achieved with the combined treatment, specifically sc-CO_2 combined with 1% of terpenes demonstrates the ability to inactivate Y&M and prevent the rapid growth throughout the entire storage period. Furthermore, treatments with sc-CO_2 alone and sc-CO_2 + 1% LIN were sufficient to reduce the presence of Y&M. These results are promising, as our technologies can effectively control the growth of these aerobic microorganisms even under air conditions. Similar to other microorganisms, applying a MAP could further enhance these results.

4. Conclusions

This work investigated the synergistic relationship between sc-CO_2 treatment and natural antimicrobial substances in order to improve the safety and the preservation of fresh chicken meat. It was observed that the terpenes (LIN and LIM) have a higher antimicrobial effect than the relative essential oils (LEO, CEO and BEO) at the optimal concentration of 1%. The inactivation was higher for the Gram-negative (*Escherichia coli* and *Pseudomonas fluorescens*) rather than Gram-positive (*Listeria innocua* and *Enterococcus faecium)* bacteria. The synergism was also observed on natural present microorganisms on chicken fresh meat during the storage test. Specifically, when LIN or LIM at 1% is coupled with sc-CO_2, after 9 days of storage the total mesophilic load was below the acceptable limit of 7 log cfu/g (6.55 log cfu/g, 5.63 log cfu/g respectively) after which the food is considered no more suitable for human consumption. Other microorganisms belonging to the natural microflora of chicken meat showed sensitivity against the combined technology, especially sc-CO_2 + 1% LIN achieved the lowest level of growth at 9 days. This preliminary investigation demonstrated the efficacy of combining naturally antimicrobial substances with innovative technology to increase the microbial safety and prolong the shelf life. The combination of the natural antimicrobial substances here studied could also be effective on the inactivation, but it should be further investigated. Additional technologies, such as MAP, might also be coupled after the treatment to increase the storage time. Further studies are needed to confirm the process acceptability in terms of qualitative aspects, including sensorial test and consumers' acceptance. Also, a cost-benefit analysis should be performed to support further optimization studies, since the cost of antimicrobial substances might play a significant role in the total cost of production. Moreover, another aspect to consider is the potential extensibility of our process to other food matrices, including the influence of their composition.

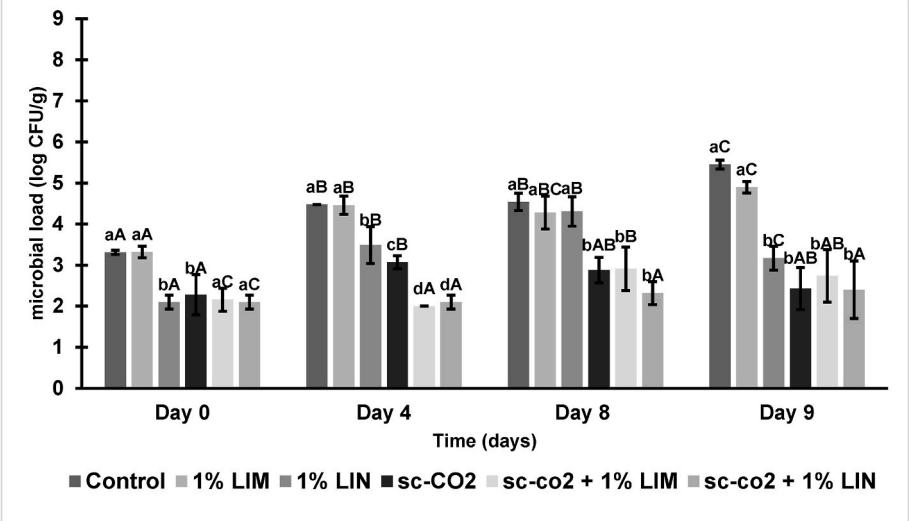

Fig. 5. Yeasts and Molds chicken breast samples during the 9 days storage test.
The counts of the control samples (Control), samples with 1% limonene (1% LIM), samples with 1% linalool (1% LIN), samples treated with supercritical carbon dioxide (sc-CO_2), samples treated with sc-CO_2 coupled with 1% limonene (sc-CO_2 + 1% LIM) and samples treated with sc-CO_2 coupled 1% linalool (sc-CO_2 + 1% LIN).
Values are mean ± standard deviation population recovered (log cfu/g) (n = 3).
Lower case letters identify differences between the theses on the same day, upper case letters represent the differences between the days of the same thesis ($P < 0.05$).

CRediT authorship contribution statement

Santi Fabio: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lincetti Elisa:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Alberghini Giulia:** Writing – original draft, Investigation. **Giaccone Valerio:** Writing – original draft, Supervision, Funding acquisition. **Zambon Alessandro:** Writing – original draft, Methodology, Funding acquisition, Conceptualization, Supervision. **Spilimbergo Sara:** Writing – original draft, Methodology, Funding acquisition, Conceptualization, Supervision.

Funding

Valerio Giaccone, Alessandro Zambon and Sara Spilimbergo used their own Department's funds to support this study. Fabio Santi's PhD fellowship was financed by MUR under the PNRR, Mission 4, Component 1, Investments 3.4 and 4.1, DM 351/2022.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Aghababaei, L., Hasani, M., Shotorbani, P. M., Basti, A. A., & Hamedi, H. (2022). Antioxidant and antimicrobial characteristics of chitosan and galbanum gum composite coating incorporated with cumin essential oil on the shelf life of chicken fillets. *Journal of Food Measurement and Characterization, 16*(3), 1820–1833. https://doi.org/10.1007/s11694-022-01295-4

Alirezalu, K., Moazami-Goodarzi, A. H., Roufegarinejad, L., Yaghoubi, M., & Lorenzo, J. M. (2022). Combined effects of calcium-alginate coating and Artemisia fragrance essential oil on chicken breast meat quality. *Food Science and Nutrition, 10*(8), 2505–2515. https://doi.org/10.1002/fsn3.2731

Allai, F. M., Azad, Z. R. A. A., Mir, N. A., & Gul, K. (2023). Recent advances in non-thermal processing technologies for enhancing shelf life and improving food safety. *Applied Food Research, 3*(1), Article 100258. https://doi.org/10.1016/j.afres.2022.100258

Bagheri, R., Izadi Amoli, R., Tabari Shahndash, N., & Shahosseini, S. R. (2016). Comparing the effect of encapsulated and unencapsulated fennel extracts on the shelf life of minced common kilka (*Clupeonella cultriventris caspia*) and *Pseudomonas aeruginosa* inoculated in the mince. *Food Science and Nutrition, 4*(2), 216–222. https://doi.org/10.1002/fsn3.275

Ben Mhenni, N., Alberghini, G., Giaccone, V., Truant, A., & Catellani, P. (2023). Prevalence and antibiotic resistance phenotypes of Pseudomonas spp. in fresh fish fillets. *Foods, 12*(5). https://doi.org/10.3390/foods12050950. Article 5.

Bibow, A., & Oleszek, W. (2024). Essential oils as potential natural antioxidants, antimicrobial, and antifungal agents in active food packaging. *Antibiotics, 13*(12), 1168. https://doi.org/10.3390/antibiotics13121168

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Cappelletti, M., Ferrentino, G., & Spilimbergo, S. (2015). High pressure carbon dioxide on pork raw meat: Inactivation of mesophilic bacteria and effects on colour properties. *Journal of Food Engineering, 156*, 55–58. https://doi.org/10.1016/j.jfoodeng.2015.02.009

Carlez, A., Jean-Philippe, R., Nicole, R., & Jean-Claude, C. (1993). High pressure inactivation of Citrobacter freundii, Pseudomonas fluorescens and Listeria innocua in inoculated minced beef muscle. *LWT - Food Science and Technology, 26*(4), 357–363. https://doi.org/10.1006/fstl.1993.1071. ISSN 0023-6438.

Chen, H., Guan, Y., Wang, A., & Zhong, Q. (2022). Inactivation of Escherichia coli K12 on raw almonds using supercritical carbon dioxide and thyme oil. *Food Microbiology, 103*, Article 103955.

Chouliara, E., Karatapanis, A., Savvaidis, I. N., & Kontominas, M. G. (2007). Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. *Food Microbiology, 24*(6), 607–617. https://doi.org/10.1016/j.fm.2006.12.005

Dacal-Gutiérrez, A., Tirado, D. F., & Calvo, L. (2022). Inactivation of Clostridium spores in honey with supercritical CO2 and in combination with essential oils. *Processes, 10*(11). https://doi.org/10.3390/pr10112232. Article 11.

Dagnas, S., & Membré, J.-M. (2013). Predicting and preventing mold spoilage of food products. *Journal of Food Protection, 76*(3), 538–551. https://doi.org/10.4315/0362-028X.JFP-12-349

EFSA (European Food Safety Authority). (2022). Story map on foodborne outbreaks. Retrieved from https://storymaps.arcgis.com/stories/ca42d02e580441b79fdfd46a427abaab. (Accessed 15 November 2024).

Ekonomou, S. I., Leech, D. J., Lightfoot, S., Huson, D., & Stratakos, A. C. (2023). Development of novel antimicrobial coatings incorporating linalool and eugenol to improve the microbiological quality and safety of raw chicken. *Lebensmittel-Wissenschaft & Technologie, 182*, Article 114839. https://doi.org/10.1016/j.lwt.2023.114839

Espina, L., Gelaw, T. K., De Lamo-Castellví, S., Pagán, R., & García-Gonzalo, D. (2013). Mechanism of bacterial inactivation by (+)-Limonene and its potential use in food preservation combined processes. *PLoS One, 8*(2), Article e56769. https://doi.org/10.1371/journal.pone.0056769

European Food Safety Authority, & European Centre for Disease Prevention and Control. (2022). The European union one health 2021 zoonoses report. *EFSA Journal, 20*(12), Article e07666. https://doi.org/10.2903/j.efsa.2022.07666

Ferrentino, G., Balzan, S., & Spilimbergo, S. (2013). Supercritical carbon dioxide processing of dry cured ham spiked with Listeria monocytogenes: Inactivation kinetics, color, and sensory evaluations. *Food and Bioprocess Technology, 6*(5), 1164–1174. https://doi.org/10.1007/s11947-012-0819-4

Fratianni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R., & Nazzaro, F. (2010). Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science, 75*(8), M528–M535. https://doi.org/10.1111/j.1750-3841.2010.01791.x

Giaccone, V., & Colavita, G. (2015). Principi di microecologia degli alimenti Le Point Veterinaire. https://www.libreriauniverso.it/.

González-Alonso, V., Cappelletti, M., Bertolini, F. M., Lomolino, G., Zambon, A., & Spilimbergo, S. (2020). Research note: Microbial inactivation of raw chicken meat by supercritical carbon dioxide treatment alone and in combination with fresh culinary herbs. *Poultry Science, 99*(1), 536–545. https://doi.org/10.3382/ps/pez563

Gürbüz, M., Ercan, A., & Omurtag-Korkmaz, B.İ. (2024). The antimicrobial effect of eugenol against *Campylobacter jejuni* on experimental raw chicken breast meat model. *Journal of Food Safety*. https://doi.org/10.1111/jfs.13104

Han, Y., Sun, Z., & Chen, W. (2020). Antimicrobial susceptibility and antibacterial mechanism of limonene against Listeria monocytogenes. *Molecules, 25*(1). https://doi.org/10.3390/molecules25010033. Article 1.

He, R., Chen, W., Chen, H., Zhong, Q., Zhang, H., Zhang, M., & Chen, W. (2022). Antibacterial mechanism of linalool against L. monocytogenes, a metabolomic study. *Food Control, 132*, Article 108533. https://doi.org/10.1016/j.foodcont.2021.108533

Herman, A., Tambor, K., & Herman, A. (2016). Linalool affects the antimicrobial efficacy of essential oils. *Current Microbiology, 72*(2), 165–172. https://doi.org/10.1007/s00284-015-0933-4

Hernandes, C., Taleb-Contini, S. H., Bartolomeu, A. C. D., Bertoni, B. W., França, S. C., & Pereira, A. M. S. (2014). Chemical composition and antifungal activity of the essential oils of Schinus weinmannifolius collected in the spring and winter. *Natural Product Communications*, 1383–1386. https://doi.org/10.1177/1934578X1400900940

Hulankova, R., Borilova, G., Abdullah, F. A. A., & Buchtova, H. (2018). Microbiological quality of organic chicken meat during refrigerated storage in air and modified atmospheres. *British Poultry Science, 59*(5), 506–513. https://doi.org/10.1080/00071668.2018.1496399

Hussein, K. N., Csehi, B., József, S., Ferenc, H., Kiskó, G., Dalmadi, I., & Friedrich, L. (2021). Effect of α-terpineol on chicken meat quality during refrigerated conditions. *Foods, 10*(8). https://doi.org/10.3390/foods10081855. Article 8.

ICMSF, International Commission on Microbiological Specifications for Foods. (1986). In (2nd ed.), *Vol. 2. Sampling for microbiological analysis: Principles and scientific applications*. Toronto: University of Toronto Press.

Javadian, S. R., Shahosseini, S. R., & Ariaii, P. (2017). The effects of liposomal encapsulated thyme extract on the quality of fish mince and Escherichia coli O157:H7 inhibition during refrigerated storage. *Journal of Aquatic Food Product Technology, 26*(1), 115–123.

Ji, J., Shankar, S., Royon, F., Salmieri, S., & Lacroix, M. (2021). Essential oils as natural antimicrobials applied in meat and meat products—a review. *Critical Reviews in Food Science and Nutrition*, 1–17. https://doi.org/10.1080/10408398.2021.1957766

Konuk Takma, D., & Korel, F. (2019). Active packaging films as a carrier of black cumin essential oil: Development and effect on quality and shelf-life of chicken breast meat. *Food Packaging and Shelf Life, 19*, 210–217. https://doi.org/10.1016/j.fpsl.2018.11.002

Latou, E., Mexis, S. F., Badeka, A. V., Kontakos, S., & Kontominas, M. G. (2014). Combined effect of chitosan and modified atmosphere packaging for shelf life extension of chicken breast fillets. *LWT - Food Science and Technology, 55*(1), 263–268. https://doi.org/10.1016/j.lwt.2013.09.010

Mączka, W., Duda-Madej, A., Grabarczyk, M., & Wińska, K. (2022). Natural compounds in the battle against microorganisms—linalool. *Molecules, 27*(20). https://doi.org/10.3390/molecules27206928. Article 20.

Mangalagiri, N. P., Panditi, S. K., & Jeevigunta, N. L. L. (2021). Antimicrobial activity of essential plant oils and their major components. *Heliyon, 7*(4), Article e06835. https://doi.org/10.1016/j.heliyon.2021.e06835

McKee, L. (2012). Microbiological and sensory properties of fresh and frozen poultry. In L. M. L. Nollet (Ed.), *Handbook of meat, poultry and seafood quality*. https://doi.org/10.1002/9781118352434.ch25

Nabrdalik, M., & Grata, K. (2016). Antibacterial activity of *Ocimum basilicum L.* essential oil against Gram-negativebacteria. https://repo.uni.opole.pl/info/article/UO624d99c914ba474291899643a6de9530/.

Odeyemi, O. A., Alegbeleye, O. O., Strateva, M., & Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety, 19*, 311–331. https://doi.org/10.1111/1541-4337.12526

Pateiro, M., Munekata, P. E. S., Sant'Ana, A. S., Domínguez, R., Rodríguez-Lázaro, D., & Lorenzo, J. M. (2021). Application of essential oils as antimicrobial agents against spoilage and pathogenic microorganisms in meat products. *International Journal of Food Microbiology, 337*, Article 108966. https://doi.org/10.1016/j.ijfoodmicro.2020.108966

Peter, A., Mihaly Cozmuta, L., Nicula, C., et al. (2023). Storage of chicken breast meat in paper coated with different types of hydrophobic agents. *Packaging Technology and Science, 36*(2), 97–110. https://doi.org/10.1002/pts.2694

Rouger, A., Tresse, O., & Zagorec, M. (2017). Bacterial contaminants of poultry meat: Sources, species, and dynamics. *Microorganisms, 25*(3), 50. https://doi.org/10.3390/microorganisms5030050, 5.

Saenz-García, C. E., Castañeda-Serrano, P., Mercado Silva, E. M., Alvarado, C. Z., & Nava, G. M. (2020). Insights into the identification of the specific spoilage organisms in chicken meat. *Foods, 9*(2), 225. https://doi.org/10.3390/foods9020225

Safari, R., Yaghoubi, M., Marcinkowska-Lesiak, M., Paya, H., Sun, X., Rastgoo, A., … Alirezalu, K. (2023). The effects of double gelatin containing chitosan nanoparticles–calcium alginate coatings on the stability of chicken breast meat. *Food Science and Nutrition, 11*(12), 7673–7685. https://doi.org/10.1002/fsn3.3592

Salama, Y., & Chennaoui, M. (2024). Understanding microbial contamination in meat and poultry production. *Journal of Research in Agriculture and Food Sciences, 1*, 87–107. https://doi.org/10.5455/JRAFS.20240705062923

Santi, F., Zulli, R., Lincetti, E., Zambon, A., & Spilimbergo, S. (2023). Investigating the effect of rosemary essential oil, supercritical CO2 processing and their synergism on the quality and microbial inactivation of chicken breast meat. *Foods, 12*(9). https://doi.org/10.3390/foods12091786. Article 9.

Schneider, G., Steinbach, A., Putics, Á., Solti-Hodován, Á., & Palkovics, T. (2023). Potential of essential oils in the control of Listeria monocytogenes. *Microorganisms, 11*(6), 1364. https://doi.org/10.3390/microorganisms11061364

Scollard, J., McManamon, O., & Schmalenberger, A. (2016). Inhibition of Listeria monocytogenes growth on fresh-cut produce with thyme essential oil and essential oil compound verbenone. *Postharvest Biology and Technology, 120*, 61–68. https://doi.org/10.1016/j.postharvbio.2016.05.005

Sheerzad, S., Khorrami, R., Khanjari, A., Gandomi, H., Akhondzadeh Basti, A., & Khansavar, F. (2024). Improving chicken meat shelf-life: Coating with whey protein isolate, nanochitosan, bacterial nanocellulose, and cinnamon essential oil. *Lebensmittel-Wissenschaft & Technologie, 197*, Article 115912. https://doi.org/10.1016/j.lwt.2024.115912. ISSN 0023-6438.

Sweet, R., Kroon, P. A., & Webber, M. A. (2022). Activity of antibacterial phytochemicals and their potential use as natural food preservatives. *Critical Reviews in Food Science and Nutrition, 64*(8), 2076–2087. https://doi.org/10.1080/10408398.2022.2121255

Taormina, P. J. (2021). Microbial growth and spoilage. In P. J. Taormina, & M. D. Hardin (Eds.), *Food safety and quality-based shelf life of perishable foods. Food microbiology and food safety*. Cham: Springer. https://doi.org/10.1007/978-3-030-54375-4_3.

Wei, C. I., Balaban, M. O., Fernando, S. Y., & Peplow, A. J. (1991). Bacterial effect of high pressure CO2 treatment on foods spiked with Listeria or Salmonella. *Journal of Food Protection, 54*(3), 189–193. https://doi.org/10.4315/0362-028X-54.3.189

Xu, Y., Xin, J., Lyu, Y., & Zhang, C. (2024). Effects of bacterial cellulose/thyme essential oil emulsion coating on the shelf life of chilled chicken meat. *Journal of the Science of Food and Agriculture, 104*(9), 5577–5587. https://doi.org/10.1002/jsfa.13392