


Research Note: Microbial inactivation of raw chicken meat by supercritical carbon dioxide treatment alone and in combination with fresh culinary herbs

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ABSTRACT The objective of the present study was to assess the potential synergistic effect between supercritical carbon dioxide (SC-CO₂) and fresh culinary herbs (*Coriandrum sativum* and *Rosmarinus officinalis*) on the microbial inactivation of raw chicken meat. The microbiological inactivation was performed on *Escherichia coli* and natural flora (total mesophilic bacteria, yeasts, and molds). High pressure treatments were carried out at 40°C, 80 or 140 bar from 15 to 45 min. Microbial inactivation had a strong dependence on treatment time, achieving 1.4 log CFU/g reduction of *E. coli* after 15 min, and up to 5 log after 45 min, while a pressure increase from 80 up to 140 bar was not significant on the microbial inactivation. Mesophilic microorganisms were strongly reduced (>2.6 log CFU/g) after 45 min, and yeasts and molds were below the

detection limits of the technique (<100 CFU/g) in most cases. The combination of fresh herbs together with SC-CO₂ treatment did not significantly increase the inactivation of either *E. coli* or natural flora, which was similar to the SC-CO₂ alone. The synergistic effect was obtained on the inactivation of *E. coli* using a proper concentration of coriander essential oil (EO) (0.5% v/w), while rosemary EO did not show a significant effect. Color analysis after the treatment showed an increment of lightness (L*), and a decrease of redness (a*) on the surface of the sample, making the product visually similar to cooked meat. Texture analysis demonstrated the modification of the texture parameters as a function of the process pressure making the meat more similar to the cooked one.

Key words: supercritical carbon dioxide, microbial inactivation, chicken meat, culinary herb, essential oil

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INTRODUCTION

Over the last decades, the consumption of poultry meat has increased worldwide and dominates the market with an average annual growth of 2% (OECD-FAO, 2015), owing to its low-fat content and high nutritional value, as well as its low cost of production and few religious impediments (Chouliara et al., 2007). Fresh poultry meat is a highly perishable food due to its physical–chemical characteristics. Because of its higher pH, it is more perishable than pork or beef meats (Jay and Loessner, 2005) and its shelf-life is limited by the growth of different spoilage bacteria during processing, transportation, and storage. Shelf-life can be extended via carcass disinfection, maintenance of the cold chain and appropriate packaging (Amélie et al., 2017). Nevertheless, the shelf-life of raw poultry products remains

short for the demands of the market, and new preservation technologies are desirable.

Microbiological stability is an issue in chicken meat. Indeed, during the slaughtering process, the microbiota present in the gastrointestinal tract, lungs, skin, and feathers can colonize the muscle tissue through a number of routes (Amélie et al., 2017). These microorganisms can multiply at relatively low temperatures and the result of their metabolic activity is evidenced as product spoilage (Singh, 1993). Among them, some pathogens may be present (Del Olmo et al., 2012). *Escherichia coli* O157: H7 is an enterohemorrhagic serotype, which survives well in foods during refrigerated storage, causes hemorrhagic colitis, and has the potential to cause hemolytic uremic syndrome in vulnerable individuals (Del Olmo et al., 2012). *Salmonella* spp. and *Campylobacter* sp. are many times the cause of food infections related to chicken meat, even though their virulence is generally lower than that of *E. coli* O157: H7 (EFSA, 2016).

Low-temperature pasteurization technologies have been investigated to improve the safety while maintaining the food's natural properties. These alternative

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technologies attempt to be mild, energy saving, environmentally friendly to guarantee natural appearance while eliminating pathogens and spoilage microorganisms or by preventing their growth (Zhou et al., 2010). High pressure processing (HPP) has been used for the low-temperature pasteurization of different meat products (Hygreeva and Pandey, 2016); however, it requires very high pressure conditions (>300 MPa), and high investment and operational costs (Picart-Palmade et al., 2019). Pulsed electric fields at high electric field strengths (>20 kV/cm) have been shown to be lethal to many spoilage and pathogenic bacteria in meat, but high-intensity treatments required to inactivate the microbial load in meat have an adverse impact on its sensorial and nutritional quality (Bhat et al., 2018). Recently non-thermal high-voltage dielectric barrier discharge showed inhibition growth of psychrophilic and a reduction of pathogens; however, the treatment may increase pale color in raw chicken breast (Zhuang et al., 2019). Irradiation is an alternative low-temperature pasteurization technology for poultry meat. However, it can cause sensorial changes leading to off-flavors in meat and the label “irradiated” is sometimes met with distrust by consumers (Ahn et al., 2017; Kawasaki et al., 2019). Even though it was regulated in 1999 (Directive 1999/3/EC), its spread is still low and only 26 facilities have been authorized in the EU so far (European Parliament, 2019).

Supercritical carbon dioxide (SC-CO₂) processes have been developed as innovative low-temperature pasteurization for liquid (Perrut, 2012) and solid products (Ferrentino and Spilimbergo, 2011). The inactivation mechanism of SC-CO₂ was studied in depth (Dillow et al., 1999; Spilimbergo and Bertucco, 2003; Damar and Balaban, 2006; Garcia-Gonzalez et al., 2007), and it occurs by several steps involving the solubilization of CO₂ in the free water, diffusion through cell membranes, intracellular solubilization, a rapid drop of the intracellular pH (Giulitti et al., 2011), and consequently the disruption of a number of enzymatic processes that are essential for the cellular metabolism. The permeabilization of the cell membrane also causes the disruption of the cell membrane integrity (Spilimbergo et al., 2009). For this to happen, a combination of the right temperature, pressure, and time is necessary. Process implementation is facilitated due to its low critical point (31°C, 73.9 bar), which allows handling at relatively low-pressure conditions in comparison to HPP, and results in better control of the process pressure and lower investment costs (Garcia-Gonzalez et al., 2007; Ferrentino and Spilimbergo, 2011). In the case of meat products, it has been shown to achieve microbial inactivation in a variety of meat products (Balaban and Duong, 2014). Reductions of 1 to 3 log were achieved in the total mesophilic count after treatments in raw pork meat (Cappelletti et al., 2015), while Ferrentino et al. (2013) reported 3 log reductions in *Listeria monocytogenes* in dry cured ham. Besides, up to 1.7 log and 2.2 log reductions in the total mesophilic count and

Salmonella spp. were observed in ground pork by Bae et al. (2010). Nevertheless, research on applications in chicken meat is limited. Wei et al. (1991) were the first to investigate the inactivation of *Salmonella* spp. and *L. monocytogenes* in spiked chicken meat obtaining 1 to 2 log reductions at 137 bar, 35°C and 2 h, and recently Morbiato et al. (2019) achieved 2.5 log reduction after 15 min and complete pasteurization after 90 min in mesophilic microorganisms, in the frame of SC-CO₂ drying at 100 bar and 40°C.

To improve the microbial inactivation, SC-CO₂ has been combined with other technologies or with additives. Applications with SC-CO₂ and high power ultrasound can be found in chicken (Morbiato et al., 2019) and in cured ham (Spilimbergo et al., 2014). Additives such as lactic or acetic acids were used in combination with SC-CO₂ in fresh pork (Choi et al., 2009), generally obtaining better inactivation results than when using SC-CO₂ alone. Recently, Huang et al. (2017) reported the first work in which a culinary herb (*Rosmarinus officinalis*) was used in combination with SC-CO₂ to improve the shelf-life of raw pork meat. The synergistic effect on microbial reductions, although significant, did not exceed 0.5 log comparing to the SC-CO₂ treatment alone. Fresh herbs contain a large group of substances, including essential oils (EOs), often used instead of synthetic antioxidants to extend the shelf-life of food products (Chouliara et al., 2007; Michalczyk et al., 2012), showing promising results also in the storage stability of vacuum packed low pressure mechanically separated meat (Cegielka et al., 2019), and in the control of *Campylobacter jejuni* on chicken skin (Shrestha et al., 2019). Despite their potential, the use of natural antimicrobial products to improve the inactivation efficacy of SC-CO₂ treatment has not been extensively investigated, and additional studies are needed in order to demonstrate their feasibility in different food products.

Thus, the objective of this study was to assess the synergistic effect of SC-CO₂ in combination with fresh culinary herbs (*R. officinalis* and *Coriandrum sativum*) on the microbial inactivation of chicken meat. Rosemary and coriander are often used as culinary herbs, and they are known for their antimicrobial properties (Delaquis et al., 2002; Perricone et al., 2015). Rosemary contains a large amount of phenolic compounds and terpenoids, such as carnosol, camphor, or borneol (Babovic et al., 2010), that prevent the oxidation of lipids and inhibit bacteria, through a number of ways (Shan et al., 2007). Likewise, EOs of *C. sativum* leaves have been reported to inhibit a broad spectrum of bacteria, demonstrating its efficacy as an antimicrobial agent (Yildiz, 2016), due to the presence of long-chain (C6–C10) alcohols and aldehydes (Delaquis et al., 2002). The inactivation was investigated on spiked *E. coli*, a relevant surrogate microorganism for the presence of fecal contamination and enteric pathogens, and naturally present mesophilic bacteria and yeasts and molds. Instrumental analysis, in terms of color, pH, and

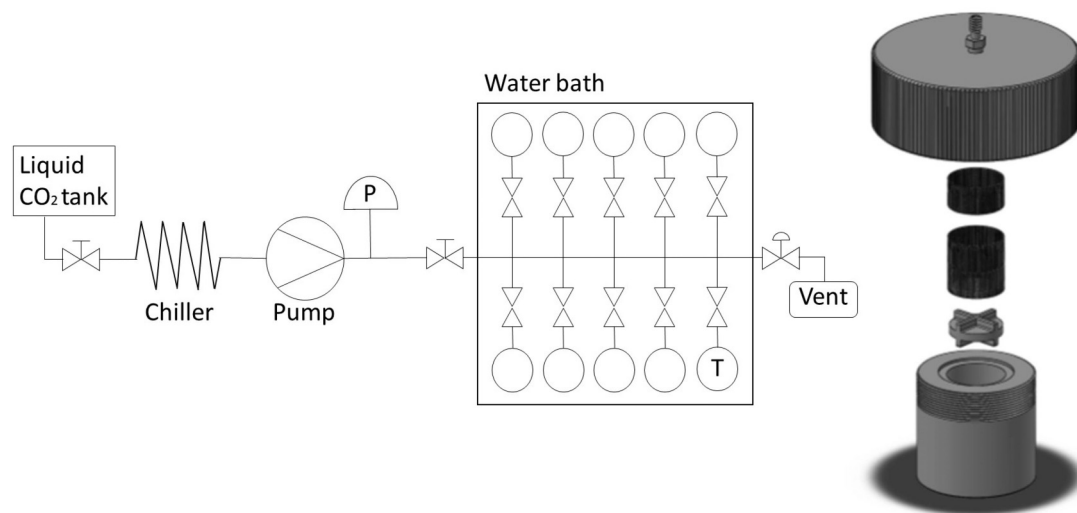


Figure 1. Schematic representation of the supercritical carbon dioxide (SC-CO₂) multibatch apparatus (left); with P and T standing for pressure control and temperature control, respectively. A reactor and its elements (right). From top to bottom: reactor lid, basket for herbs, basket for the inoculated sample, magnetic agitator, reactor body.

texture change before and after the process, was also included to expand and confirm the existing literature on the SC-CO₂ pasteurization of raw chicken meat.

MATERIALS AND METHODS

Culture and Cell Suspension

Escherichia coli (Migula) Castellani and Chalmers (ATCC 25922) strain were inoculated on raw chicken breast meat. The microbial culture was grown in 10 mL Luria–Bertani (LB) medium broth (Lennox, L3022, Sigma-Aldrich, UK) at 37°C overnight, and then transferred to a 100 mL flask of LB and grown at 37°C overnight. Cell growth was done in a shaking incubator (set at 220 rpm) and carefully monitored through measurements of the optical density to achieve the stationary phase. The microbial suspensions were centrifuged at 6,000 rpm for 8 min, the supernatant was removed, and the pellet re-suspended in a measured amount of sterile phosphate-buffered saline (PBS; 0.01 M, pH 7.4; Oxoid, UK), reaching a final concentration of 10⁸ CFU/mL.

Sample Preparation and Microbial Inoculation

In sterility conditions, raw chicken breast meat, purchased from a local market, was cut in small cubes with a weight of 1 ± 0.05 g and subsequently frozen. One hour before the treatment, the samples were taken out of the freezer and left to thaw inside the flow cabinet for 30 min. Then, they were spiked with 20 μ L of *E. coli* suspension, obtaining a concentration of 10⁸ CFU/g. The samples were left 15 min under a laminar flow at room temperature to let the microbial suspension dry, then placed in a sterile stainless-steel basket

(approximately 1 cm high and 1 cm diameter, Figure 1 B), and subsequently treated with SC-CO₂ alone or in combination with herbs (SC-CO₂ + herbs) by means of a multibatch apparatus (Figure 1A); more information can be found in the next section. For the investigation of the natural flora, thawed samples were not inoculated. Fresh herbs, rosemary (*R. officinalis*) and coriander (*C. sativum*) branches, were purchased from a local market in Padua. After being gently washed and dried, 1 g of leaves was chopped by hand and placed in a stainless-steel basket, which in turn was placed over the basket containing the chicken meat samples (Figure 1B). The quantity of herbs was chosen based on preliminary trials (data not shown). Further analyses were carried out to investigate the effects of EOs alone or in combination with SC-CO₂. After *E. coli* inoculation, different concentrations (1, 0.5, and 0.1% v/w) of *R. officinalis* L. (Erbeamea, Perugia, IT) and *C. sativum* (Pranarôm, IT) pure EOs were tested. Concentration was chosen based on the literature (Chouliara et al., 2007). Samples were surface-inoculated and left 15 min under a laminar flow to allow adsorption.

Raw Chicken Meat Treatment With SC-CO₂

SC-CO₂ Multibatch Apparatus SC-CO₂ treatments were carried out in a multi-batch apparatus (Ferrentino et al., 2013). The vessels consisted of ten 15-mL cylinders, provided with a magnetic system for stirring (Vetrotecnica, micro stirrer, Velp 300 rpm, IT). The cylinders were connected in parallel, so that each experimental run provided a set of experimental data taken at identical process conditions but different treatment times. Each reactor was connected to an on-off valve that could be used to pressurize and depressurize it independently from the others. The reactors

were submerged in a single temperature-controlled water bath. Liquid CO₂ (Messer, carbon dioxide 4.0, purity 99.99%, Germany) was fed into the reactors by a volumetric pump (LEWA, mod. LCD1/M910s, Germany) that increased the pressure to the desired processing levels with a rate of about 6 MPa/min. The apparatus was provided with a transducer (Endress + Hauser GmbH, Maulburg, Germany) to control the pressure values, while one cover lid of the 10 reactors was equipped with a fixed thermocouple (Pt 100 Ω) to control the product temperature. At the end of the process, 2 micrometric valves and 1 on-off valve were used to depressurize and release CO₂ from the apparatus that occurred over approximately 1 min. After the treatment, the reactors were disconnected from the pressurization line and opened in a laminar flow hood. The processed samples were collected in sterile containers and cooled down immediately at 4°C until microbial analysis (Spilimbergo et al., 2010).

Process Conditions For *E. coli* inactivation kinetics, different treatment times (15, 30, and 45 min), temperature (40°C), and pressures (80 and 140 bar) were considered. Previous studies on meat showed that pressures around 80 to 160 bar, temperatures between 35 and 50°C, and times below 60 min were optimal values to induce a pasteurization effect (Balaban and Duong, 2014). The range of treatment times tested in this study was between 15 and 45 min, both to ensure a sufficient degree of inactivation and to satisfy the industrial requirements for competitive processes. Temperature was kept at 40°C to limit thermal degradation effects on quality while ensuring the obtention of supercritical CO₂ (Ferrentino et al., 2013). Two different pressure conditions (80 and 140 bar) were considered to assess the effect of pressure on the microbial inactivation. For the study on microbial flora, samples were treated 45 min at 80 or 140 bar based on the results obtained with *E. coli*.

Microbial Analysis

Standard plate count technique was used to determine the initial microbial concentration and the efficiency of the treatment in reducing the number of microorganisms on the surface of the sample. After each treatment, chicken meat samples were collected in sterile Falcon tubes, mixed with 9 mL of Phosphate Buffer Saline (PBS; 0.01 M, pH 7.4; Oxoid, UK), and homogenized at 35 Hz for 1 min (Stomacher 400; International P.B.I., Milan, Italy). The solution was serially diluted (1:10) in PBS; 100 μL of the solution was plated in duplicate onto the selective media Chromatic Coli/Coliform Agar (Liofilchem, Italia) for *E. coli*, and on Rose Bengal (RB) (Microbiol, Cagliari, IT) for yeasts and molds, while 1 mL was pour-plated into Plate Count Agar (PCA, Sacco, Como, IT) for the determination of the total mesophilic count. The incubation temperature and time were 37°C and 24 h for *E. coli*, and 30 and 22°C for 3 to 5 D for PCA and RB plates, respectively. The inactivation degree

was determined by evaluating the $\log(N/N_0)$, where N_0 (CFU/g) is the number of colony forming units per mL initially present in the untreated sample, and N (CFU/g) is the number of survivors after the treatment. At least 3 independent experiments were carried out for each single treatment condition, and the results were expressed as mean and standard deviation. Each experiment was performed at least in triplicate.

Color and pH Measurement

The effect of the treatments on color both internally and externally was studied at 80 or 140 bar and 45 min based on the preliminary microbiological results. Treated samples of 1 g were photographed (1/125s, f 8.0, ISO 200; Canon 550D) along with a white reference. Correction of “brightness and contrast” and further conversion into the SCIE-L*a*b* color space were performed with ImageJ (NIH). The pH values were measured directly in the chicken meat samples with an electronic pH meter (Basic 20; Crison Instruments Sa, Carpi, Italy) equipped with an electrode (cat.5232; Crison Instruments Sa). At least 10 determinations were executed per treatment.

Texture Analysis

Texture analysis was carried out on raw SC-CO₂ treated and cooked meat samples. They were cut from whole chicken breast obtaining pieces of similar shape and dimensions (about 2 × 2 × 4 cm). The cooked meat samples were obtained by putting them in plastic bags and kept in a water bath until they reached 80°C in the inner part (about 1 h). Sc-CO₂ samples were processed in bigger vessels (about 300 mL volume) at 80 and 140 bar, 40°C, 45 min.

The texture analysis was carried out using Texture Profile Analysis (TPA) and cutting effort. The TPA was conducted in a TA-XTplus Texture analyzer (Stable Micro System, London, UK), using a 250 N load cell. A 2-cycle compression test was performed using an aluminum probe (40 × 50 mm), which was used to compress samples to 50% of their original thickness at a compression rate of 1 mm/s, and a preload of 10 g. Hardness, compression, springiness, cohesiveness, gumminess, chewiness, adhesiveness, and resilience were obtained from the force-time curves. Second, a cutting effort test was executed in a Lloyd Instruments LS5 (Ametek), using a load cell of 500 N. A cutting blade of 1 mm thickness cut the samples at a 2 mm/s rate, arriving at a maximum depth of 25 mm. A total of 16 to 20 measurements were performed for each treatment.

Statistical Analysis

Statistical analysis was performed in RStudio. Mean values were used to compare differences between treatments. The existence of significant differences ($\alpha = 95\%$) between different treatments was studied with an ANOVA and pair comparisons within a group with

Table 1. Log CFU/g reductions of “*E. coli*” as a function of time (15, 30, and 45 min) and pressure (80 and 140 bar) and 40°C.

Pressure	Time	SC-CO ₂	Coriander	Rosemary
80 bar	15 min	-1.36 (0.24) ^{A,a}	-1.47 (0.69) ^{A,a}	-1.33 (0.48) ^{A,a}
	30 min	-3.93 (0.61) ^{B,a}	-3.68 (1.36) ^{B,a}	-3.97 (1.32) ^{B,a}
	45 min	-4.68 (0.86) ^{C,a}	-4.47 (0.93) ^{C,a}	-3.64 (1.26) ^{C,a}
140 bar	15 min	-1.53 (0.36) ^{A,a}	-1.84 (0.32) ^{A,a}	-1.73 (0.32) ^{A,a}
	30 min	-3.19 (0.79) ^{D,a}	-2.82 (0.65) ^{D,a}	-2.71 (0.57) ^{D,a}
	45 min	-4.54 (1.48) ^{C,a}	-4.21 (1.17) ^{C,a}	-5.27 (1.92) ^{C,a}

E. coli was inoculated on raw poultry meat and treated with supercritical carbon dioxide (SC-CO₂) in the presence of fresh coriander or rosemary, or treated alone (control).

Values are the mean and SD—in brackets—of at least 3 determinations.

Means with different small letter superscripts in the same row are significantly different ($P < 0.05$).

Means with different capital letter superscripts in the same column are significantly different ($P < 0.05$).

its post hoc analysis (Tukey HSD) where possible, and the Kruskal–Wallis rank-sum test and Wilcoxon rank-sum test were used as their non-parametric alternatives where the assumptions for an ANOVA were not fulfilled.

RESULTS AND DISCUSSION

Microbial Inactivation

The inactivation kinetics of *E. coli* with SC-CO₂ alone or in combination with rosemary or coriander at 40°C and 80 or 140 bar is reported in Table 1. The high-pressure treatments induced a significant ($P < 0.01$) inactivation of *E. coli*. Treatment time was a significant factor, since its increment resulted in a higher inactivation, at either 80 or 140 bar. This evidence is confirmed by previous studies on pork where inactivation of *Salmonella* Typhimurium increased from 1.0 log after 20 min treatment to 1.8 log after 40 min, keeping pressure and temperature constant at 140 bar and 40°C (Bae et al., 2010). On the other hand, an increment of pressure from 80 to 140 bar did not increase the inactivation in our experiments. This is in contrast with published work on ground pork where after 40 min treatment at 40°C, inactivation of *L. monocytogenes* increased from 1 log at 100 bar up to 1.8 log at 140 bar (Bae et al., 2010). Nevertheless, this evidence could be explained by a dependence on the food matrix. Protein content and morphology, and fat content and disposition, can have a decisive impact on the antimicrobial effect of SC-CO₂ (Ferrentino and Spilimbergo, 2011). Previous studies on *E. coli* show variable inactivation results in beef or pork: 1 log reduction was achieved at 310 bar/42.5°C/180 min in ground beef (Sirisee et al., 1998), 1.5 log reduction at 120 bar/35°C/30 min in fresh pork (Choi et al., 2009), while the average inactivation of *E. coli* at 140 bar/40°C/45 min in our experiments was 4.27 log CFU/g. This illustrates the variable results obtained when treating *E. coli* in different matrixes. Besides, our results also showed a higher inactivation when compared to the experiments in chicken by Wei et al. (1991), who reported microbial reductions up to

Table 2. Log CFU/g reductions of “*E. coli*” as a function of time (15, 30, and 45 min) at 140 bar and 40°C.

Pressure	Time	SC-CO ₂	Coriander	Rosemary
140 bar	15 min	-1.68 (0.22) ^{A,a}	-1.66 (0.87) ^{A,a}	-1.72 (0.83) ^{A,a}
	30 min	-2.12 (0.71) ^{B,a}	-2.74 (1.05) ^{B,a}	-2.26 (1.04) ^{B,a}
	45 min	-4.74 (1.05) ^{C,a}	-4.13 (2.21) ^{B,a}	-3.87 (0.65) ^{C,a}

“*E. coli*” was inoculated on raw poultry meat and treated with supercritical carbon dioxide (SC-CO₂) in the presence of fresh coriander or rosemary; or treated alone (control), and then stored for 7 D at 4°C in a closed container.

Values are the mean and SD—in brackets—of at least 3 determinations.

Means with different small letter superscripts in the same row are significantly different ($P < 0.05$).

Means with different capital letter superscripts in the same column are significantly different ($P < 0.05$).

1 to 2 log for *Salmonella* and <1 log for *L. monocytogenes*, treating for 120 min at 137 bar and 35°C. Nevertheless, their inoculation procedure was different. They dipped the chicken samples for 1 min in a solution containing the bacteria, as opposed to pipette spiking. Remaining for some time in solution might have caused the bacteria to permeate deeper into the chicken muscle, making it less accessible for CO₂.

When SC-CO₂ was coupled with herbs, no additional inactivation was observed if compared to SC-CO₂ alone. Although not significant due to large standard deviations, SC-CO₂ + rosemary at 140 bar for 45 min caused a higher reduction of *E. coli* compared to the control and the coriander-treated samples. Huang et al. (2017) reported a small additional effect of rosemary in the microbial inactivation on raw pork meat. In their study, a longer process time (2 h) was used, which might have helped extracting active components. Indeed published work with EOs on meat explores the antimicrobial effect of herbs. Gouveia et al. (2016) reported 2 log additional reductions achieved by 6.25% (vol/vol) rosemary EOs of *L. monocytogenes* inoculated on beef after sous-vide cooking, which were sustained during a 28-D storage experiment. In another study on beef, an antimicrobial film containing oregano EO was able to first reduce the load of *E. coli* O157: H7 and then also inhibit its growth along a 7-D experiment at 4°C (Oussalah et al., 2004). To investigate the possible inactivation effect of the extracted EOs from the herbs onto the surface of the sample over time, we performed a shelf-life study at 4°C up to 1 wk (Table 2). However, our tests did not show any further reduction of *E. coli* for neither the treatment with herbs nor the SC-CO₂ alone during storage.

We further continued the investigation with the inactivation of natural flora in terms of mesophilic microorganisms, and yeast and molds. Because the highest inactivation of *E. coli* was achieved at longer treatment times (45 min), shorter experiments were not considered for the investigation of natural flora since they were not sufficient to reach an inactivation close to 5 to 6 log that is required for pasteurization. Results of the inactivation with SC-CO₂ alone and in combination with fresh herbs are shown in Table 3. The

Table 3. Log CFU/g reductions of chicken natural flora as a function of pressure (80 and 140 bar) for 45 min and 40°C.

Pressure		SC-CO ₂	Coriander	Rosemary
80 bar	Mesophiles	-2.96 (0.38)	-2.60 (0.47)	-2.62 (0.48)
	Yeasts and molds	-3.24 (1.11)	-3.00 (1.03)	-3.24 (0.64)
140 bar	Mesophiles	-2.99 (0.49)	-3.00 (0.78)	-2.64 (0.32)
	Yeasts and molds	-4.01 (0.58)	-3.41 (0.09)	-2.82 (0.87)

Raw poultry meat and treated with supercritical carbon dioxide (SC-CO₂) in the presence of fresh coriander or rosemary, or treated alone (control). Samples were plated on either Plate Count Agar (30°C) and Rose Bengal Agar (22°C) to evaluate mesophiles, and yeasts and molds, respectively.

Values are the mean and SD—in brackets—of at least 3 determinations.

initial load was 5.63 (0.52) log CFU/g for mesophiles and 5.29 (0.46) log CFU/g for yeasts and molds. Inactivation after 45 min of treatment ranged between 2.6 and 3.0 log CFU/g for the mesophiles, and 2.82 and 4 log CFU/g for yeasts and molds. Significant differences ($P < 0.01$) were found in all cases when comparing the untreated control with the treated groups. The inactivation of yeasts and molds was higher than the total mesophilic count. This has been reported previously for SC-CO₂ treatments in coriander (Zambon et al., 2018), in liquid whole egg (Garcia-Gonzalez et al., 2009), and in chicken (Morbiato et al., 2019). Similarly, to what was observed with *E. coli*, no significant differences ($P > 0.05$) were found when comparing samples treated at 80 or 140 bar. The inactivation level of the natural microbiota was comparable or higher than previous works from the literature with different types of meat. Microbial reductions of 1 to 3 log in mesophilic microorganisms were achieved after conditions of 60 to 160 bar, 20 to 60 min, and 40°C in pork raw meat (Cappelletti et al., 2015), and 0.5 to 1.7 log reduction in total mesophiles were reported after 100 to 140 bar, 20 to 40 min, and 40 to 45°C in ground pork (Bae et al., 2010). Morbiato et al. (2019) showed an inactivation of mesophilic bacteria comparable to this work, achieving 3.5 log inactivation after 45 min, and a complete inactivation after 90 min in chicken breast samples. However, in their study, an extraction of water was induced with the drying, and therefore, different inactivation kinetics might have taken place compared to our research. When fresh rosemary and coriander were combined with SC-CO₂, no additional inactivation effect was observed ($P > 0.05$) for either mesophilic microorganisms or yeasts and molds.

Our findings suggest that the amount of EOs extracted from the herbs during the treatment could not be enough to exert a further antimicrobial effect during treatment. Besides, supercritical fluid extraction of herbal EOs and antioxidants comprises processes, including fractionation steps, up to 2 to 4 h to reach an acceptable yield (Ahmed et al., 2012; Fornari et al., 2012; Vicente et al., 2012). In less time, 90 min, it has been shown that complete microbial inactivation in chicken can be achieved by SC-CO₂ alone (Morbiato

Table 4. Log CFU/g inactivation of “*E. coli*” inoculated on raw poultry meat after treatment with herbal essential oils (EOs) alone or in combination with supercritical carbon dioxide (SC-CO₂).

	EOs	Rosemary	Coriander
Control	1.0%	-1.08 (0.33)	-0.98 (0.18)
	0.5%	-1.23 (0.15)	-0.65 (0.09)
	0.1%	-0.11 (0.04)	-0.44 (0.06)
SC-CO ₂	-	-3.96 (1.58)	-3.96 (1.58)
	1.0%	-4.10 (1.63)	-4.56 (1.88)
	0.5%	-4.29 (0.35)	-6.65 (0.70)
	0.1%	-4.67 (0.32)	-3.36 (0.52)

Three concentration of EOs were tested: 1, 0.5, and 0.1% v/w. “-” refers to the control when no EOs were added. Treatment was 140 bar/40°C/45 min.

Values are the mean and SD—in brackets—of at least 2 determinations.

et al., 2019); therefore, extending treatment time further is not necessary.

To demonstrate the effect of concentration of EOs on the inactivation, we performed some proof-of-concept experiments using different concentrations of pure EOs. Table 4 reports the antimicrobial effect on *E. coli* of SC-CO₂ in combination with EOs of rosemary or coriander inoculated on the surface of raw poultry samples at different concentrations. EOs alone have a limited inactivation capacity for *E. coli*, and the maximum inactivation achieved was 1.23 and 0.98 log CFU/g for rosemary and coriander, respectively. The highest inactivation in combination with SC-CO₂ was achieved at the EO concentration of 0.5% (v/w). At this concentration, coriander EO showed a synergistic effect compared to the treatment alone, while at lower (0.1% v/w) and higher (1% v/w) concentrations an inactivation improvement was not achieved. At lower concentration the amount of EO was probably not sufficient to induce a synergistic effect as seen for the fresh herbs, while at higher concentration there might be a barrier effect caused by an excess of EO on the surface that limited the availability of SC-CO₂ at the sample’s surface. Interestingly, the synergistic effect was not obtained in case of rosemary EO for all the concentrations tested suggesting that also the type and therefore EO chemical composition are important for the synergic inactivation. These preliminary data are interesting, and they open a wide possibility of investigation for the optimization of the use of EOs for the reduction of process time and improvement of microbial inactivation for the SC-CO₂ treatment.

Texture Analysis

The effect of SC-CO₂ in the structure and color of meats and its conformational proteins has been reported earlier in the literature (Zhou et al., 2015; Xie et al., 2018). Table 5 presents the effect of SC-CO₂ treatment on the texture profile of chicken breast meat. Two different pressure conditions were explored (80 and 140 bar), at 40°C for a 45 min duration treatment.

Table 5. Texture descriptors of Texture Profile Analysis (TPA), and cutting effort performed on chicken breast.

	Control	Heat-treated	SC-CO ₂ 80 bar	SC-CO ₂ 140 bar
Hardness (N)	44.7 (27.8) ^a	109.7 (33.1) ^b	57.2 (28.6) ^a	82.8 (25.9) ^c
Cohesiveness	0.55 (0.07) ^{a,c}	0.60 (0.06) ^a	0.50 (0.13) ^{b,c}	0.56 (0.05) ^{a,c}
Springiness	1.33 (0.49) ^{a,c}	1.12 (0.41) ^a	1.85 (0.50) ^b	1.66 (0.37) ^{b,c}
Gumminess (N)	26.3 (19.7) ^a	66.2 (23.8) ^b	29.6 (15.8) ^a	46.1 (14.6) ^d
Chewiness (N)	38.8 (36.4) ^a	69.8 (22.3) ^b	51.7 (28.0) ^{a,b}	76.7 (32.5) ^b
Adhesiveness	-1.66 (0.81) ^a	-0.02 (0.02) ^b	-0.31 (0.22) ^c	-0.44 (0.34) ^c
Resilience	0.64 (0.13) ^a	0.66 (0.09) ^a	0.46 (0.12) ^b	0.46 (0.11) ^b
Cutting effort (N)	41.6 (21.6) ^a	28.5 (8.7) ^a	43.4 (22.1) ^a	54.5 (40.2) ^a

Values are the mean and SD—in brackets—of 16 to 20 determinations. Different superscripts within a row represent significant differences ($P < 0.05$).

Comparisons can be drawn with an untreated control and a heat-treated group. The table shows the results of 2 different tests: a TPA and cutting effort test. The latter test did not show significant differences between the test groups, although heat-treated samples were easier to cut than control or SC-CO₂ and had a lower variability. Moreover, it could be argued that treatment at higher pressures increased the resistance to cut, although it also increased variability. Regarding the TPA descriptors, SC-CO₂ at 140 bar and heat treatment significantly increase the hardness of chicken samples in comparison to the untreated control, and SC-CO₂ at 80 bar increases it, although not significantly. This is in agreement with Ros-Polski et al. (2015), who reported that with increasing pressure the hardness parameter tends to be higher because of the increase of muscle compactness after high-pressure treatment (Sun and Holley, 2010). It is noteworthy that heat treatment increases overall hardness while decreasing the resistance to cut. In fact, as reported by Palka and Daun (1999), the increase in meat hardness after heat treatment may be due to the greater compactness assumed by the myofibrils structure when, with thermal denaturation, they coagulate with diminishing water-retention capacity. During heat treatment, there is a loss of water linked to the tissues and myosin denaturation. This causes the contraction of the protein and the hardening of the fibers with the expulsion of water. Furthermore, with thermal treatment, the myofibrillar disintegration and the decrease in fiber diameter occur, and this could explain the decrease in the resistance to cut (shear strength) observed in this study conducted on cooked poultry meat.

SC-CO₂-treated samples were only significantly different between each other for hardness and gumminess. Differences were, in consequence, between untreated, heat-treated, and SC-CO₂-treated groups. In general, the heat treatment caused an increment in the descriptors that correlate to the meat becoming tougher and more difficult to masticate (gumminess, chewiness, resilience), while decreasing its ability to return to its original shape after compression (springiness). In general, springiness of raw meat Palka and Daun (1999) could be related to the degree of fiber swelling which

Table 6. Effect of supercritical carbon dioxide (SC-CO₂) on pH of raw chicken as a function of pressure after 45 min treatment at 40°C.

	pH
Control	5.85 (0.10) ^a
SC-CO ₂ 80 bar	5.75 (0.05) ^c
SC-CO ₂ 140 bar	5.76 (0.07) ^{a,c}

Values are the mean and SD—in brackets—of 10 determinations.

Means with different small letter superscripts in the same column are significantly different ($P < 0.05$).

in turn should be reflected in the fiber diameter. After thermal treatment, the water loss of muscle fiber and the thinning of fiber diameter could explain the slight decrease in springiness (Table 6). SC-CO₂-treated samples were in a middle ground between control and heat-treated samples, with 80 bar-treated samples slightly closer to the control. Adhesiveness, which is the degree with which a sample adheres to the measuring probe after the first compression, was found to be significantly larger (in negative value) for the untreated control, intermediate for the SC-CO₂-treated samples, and minimum for the heat-treated group, in which the muscle protein has been completely polymerized and the degree of stickiness is expected to be lower (Bouton and Harris, 1972).

Color and pH Measurement

The effect of the treatments on the pH is reported in Table 6. SC-CO₂ treatment resulted in a small acidification. The effect of SC-CO₂ on the color of raw chicken meat is shown in Table 7. Significant differences ($P > 0.05$) were observed between the treated and non-treated samples. In general, after treatment, an increase in lightness (L*), and a decrease in redness (a*) and yellowness (b*) were seen in the measures taken at the surface of the chicken samples. Morbiato et al. (2019) investigated the effect of SC-CO₂ drying on the color of raw chicken meat. They also reported an increase in lightness and a decrease in redness of the samples, which resulted in a sample appearance close to a “cooked” one. That much has been previously reported in the literature (Wei et al., 1991; Sirisee et al., 1998;

Table 7. Effect of supercritical carbon dioxide (SC-CO₂) treatment on instrumental color parameters (CIE-L*, a*, b*) of raw chicken as a function of pressure after 45 min treatment.

	Parameters	Control	80 bar	140 bar
Outer (x = 1)	L*	51.70 (1.60) ^{A,a}	84.59 (2.86) ^{A,b}	80.68 (3.38) ^{A,b}
	a*	9.83 (1.73) ^{A,a}	2.21 (0.71) ^{A,b}	1.45 (1.05) ^{A,b}
	b*	44.86 (1.65) ^{A,a}	42.89 (1.16) ^{A,b}	41.92 (1.46) ^{A,a,b}
Inner (x = 0)	L*	51.70 (1.60) ^{A,a}	60.25 (3.22) ^{B,b}	58.53 (0.19) ^{B,a,b}
	a*	9.83 (1.73) ^{A,a}	12.76 (1.08) ^{B,a}	12.87 (0.62) ^{B,a}
	b*	44.86 (1.65) ^{A,a}	54.14 (4.61) ^{B,b}	49.32 (0.77) ^{B,a,b}

Values are the mean and SD—in brackets—of at least 3 determinations.

Means with different small letter superscripts in the same row are significantly different ($P < 0.05$).

Means with different capital letter superscripts in the same column are significantly different ($P < 0.05$).

Comparisons reflect only a parameter with its equal in another group.

Cappelletti et al., 2015). The study by Fletcher et al. (2000), also reported an increase in lightness, decrease in redness, and increase of pH when cooking poultry meat.

Besides, the effect of SC-CO₂ treatment at the surface and at the center of the sample was investigated in. All 3 parameters of the color profile were significantly different ($P < 0.05$) when comparing the center with the surface in treated samples. Lightness (L*) at the surface was much higher than at the center for treated samples, and the lightness at the center was similar to the untreated control, although still significantly higher. As reported by Carlez et al. (1995), high pressure on meat lead to an increase in the L* parameter as a result of the denaturing of myoglobin with the release of the heme group and the coagulation of myofibrillar proteins (Goutefongea et al., 1995). Redness (a*) at the center increased, rather than decreased because of the treatment, being significantly higher than the surface of the treated samples and the control. The decrease in the a* value, found only on the surface of the sample treated with SC-CO₂, could be due to the effect of high pressure on enzymes that reduce (metmyoglobin) or oxidize (oxymyoglobin) the myoglobin of meat sample (Jung et al., 2003). Furthermore, the yellowness (b*) significantly increased at the center of the treated samples compared to the surface of treated samples and the control. No significant differences in the color profile were found between treated samples at 80 or 140 bar. The data observations suggest that 45 min treatment time is not enough to allow diffusion through the entire sample to cause a significant change in the protein matrix, which would be observed as color change. Additional studies should further explore the extent to which SC-CO₂ is able to penetrate within high protein matrixes like chicken and other meat samples to understand how this can affect future commercialization of these products.

In conclusion, the present work investigated SC-CO₂ application as an innovative technology for the pasteurization of raw chicken meat. The process induced up to 3.25 log reductions in mesophilic microorganisms, 4 log in yeasts and molds, and up to 5 log reductions in *E. coli*. The combination of fresh herbs and SC-CO₂

did not show any synergistic effect. However, the use of 0.5% v/w pure EO's instead of fresh herbs showed increased inactivation for coriander, but not for rosemary. Texture and color changed to a state closer to cooked samples. Results of this research confirm SC-CO₂ technology as a viable decontamination technology for raw chicken meat. Future work should focus on the use of EO extracts rather than fresh herbs and perform sensory tests to validate the consumer acceptance.

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