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CHICKEN PASTEURIZATION WITH SUPERCRITICAL CO_2

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-CO_2) 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, and yeasts and molds). High pressure treatments were carried out at 40 ºC, 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-CO_2 treatment did not significantly increase the inactivation of either *E. coli* or natural flora, which was similar to the SC-CO_2 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*

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 & 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 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 & 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 (PEF) at high electric field strengths (> 20 kV/cm) have been shown to be lethal to many spoilage and pathogenic bacteria in meat, but the 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 (HVDBD) 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-CO_2) processes have been developed as innovative low temperature pasteurization for liquid (Perrut, 2012), and solid products (Ferrentino & Spilimbergo, 2011). The inactivation mechanism of SC-CO_2 was studied in depth (Dillow et al., 1999; Spilimbergo & Bertucco, 2003; Damar & Balaban, 2006; Garcia-Gonzalez et al., 2007) and it occurs by several steps involving the solubilisation of CO_2 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 are necessary. Process implementation is facilitated due to its low critical point (31 ºC, 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 & Spilimbergo, 2011). In the case of meat products, it has

been shown to achieve microbial inactivation in a variety of meat products (Balaban & Duong, 2014). Reductions of 1-3 log were achieved in the total mesophilic count after treatments in raw pork meat (Cappelletti et al., 2015), while Ferrentino et al. (2012) reported 3 log reduction 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 1-2 log reductions at 137 bar, 35 ºC 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-CO_2 drying at 100 bar and 40 ºC.

To improve the microbial inactivation, SC-CO_2 has been combined with other technologies or with additives. Applications with SC-CO_2 and High Power Ultrasound (HPU) 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-CO_2 in fresh pork (Choi et al., 2009), generally obtaining better inactivation results than when using SC-CO_2 alone. Recently Huang et al. (2017), reported the first work in which a culinary herb (*Rosmarinus officinalis*) was used in combination with SC-CO_2 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-CO_2 treatment alone. Fresh herbs contain a large group of substances, including EO's, 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 (MSM) (Cegiełka 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-CO_2 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-CO_2 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, EO's of *Coriandrum 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 texture change before and after the process, were also included to expand and confirm the existing literature on the SC-CO_2 pasteurization of raw chicken meat.

MATERIALS AND METHODS

Culture and Cell Suspension

Escherichia coli (Migula) Castellani and Chalmers (ATCC 25922) strain was inoculated on raw chicken breast meat. The microbial culture was grown in 10 ml Luria Bertani (LB) medium broth (Lennox, L3022, Sigma-Aldrich) at 37 °C overnight, 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 6000 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 10^8 CFU/ml.

146 ***Sample Preparation and Microbial Inoculation***

147 In sterility conditions, raw chicken breast meat, purchased from a local market, was cut in small
148 cubes with a weight of 1 ± 0.05 g and subsequently frozen. One hour before the treatment, the
149 samples were taken out of the freezer and left to thaw inside the flow cabinet for 30 min. Then, they
150 were spiked with 20 µl of *E. coli* suspension, obtaining a concentration of 10^8 CFU/g. The samples
151 were left 15 minutes under a laminar flow at room temperature to let the microbial suspension dry,
152 then placed in a sterile stainless-steel basket (approximately, 1 cm high and 1 cm diameter, Figure
153 1B) and subsequently treated with SC-CO_2 alone or in combination with herbs (SC-CO_2 + herbs) by
154 means of a multibatch apparatus (Figure 1A); more information can be found in the next section.
155 For the investigation of the natural flora, thawed samples were not inoculated. Fresh herbs,
156 rosemary (*Rosmarinus officinalis*) and coriander (*Coriandrum sativum*) branches, were purchased
157 from a local market in Padua. After being gently washed and dried, 1 g of leaves were chopped by
158 hand and placed in a stainless-steel basket, which in turn was placed over the basket containing the
159 chicken meat samples (Figure 1B). The quantity of herbs was chosen based on preliminary trials
160 (data not shown). Further analyses were carried out to investigate the effects of EO's alone or in
161 combination with SC-CO_2. After *E. coli* inoculation, different concentrations (1, 0.5 and 0.1% v/w)
162 of *Rosamarinus officinalis L.* (Erbamea, IT) and *Coriandrum sativum* (Pranarôm, IT) pure EO's
163 were tested. Concentration was chosen based on literature (Chouliara et al., 2007). Samples were
164 surface-inoculated and left 15 minutes under a laminar flow to allow adsorption.

165 ***Raw Chicken Meat Treatment with SC-CO_2***

166 ***SC-CO_2 Multibatch Apparatus.*** SC-CO_2 treatments were carried out in a multi-batch
167 apparatus (Ferrentino et al., 2012). The vessels consisted of ten 15 ml-cylinders, provided with a
168 magnetic system for stirring (Vetrotecnica, micro-stirrer, Velp, 300 rpm). The cylinders were
169 connected in parallel, so that each experimental run provided a set of experimental data taken at
170 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 CO_2 (Messer, carbon dioxide 4.0, purity 99.99%) was fed into the reactors by a volumetric pump (LEWA, mod. LCD1/M910s), 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, two micrometric valves and one on–off valve were used to depressurize and release CO_2 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 80-160 bar, temperatures between 35-50 ºC, and times below 60 min were optimal values to induce a pasteurization effect (Balaban & 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 ºC to limit thermal degradation effects on quality while at the same time ensuring the obtention of supercritical CO_2 (Ferrentino et al., 2012). Two different pressure conditions (80 bar 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 PBS, 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, IT) for Yeasts and Molds, while 1 ml was pour-plated into Plate Count Agar (PCA, Sacco, 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 ºC and 22 ºC for 3-5 days 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 three 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 the colour 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 was performed with ImageJ (NIH, US). The pH values were measured directly in the chicken meat samples with a electronic pH-meter (Basic 20; Crison Instruments Sa, Carpi, Italy) equipped with an electrode (cat.5232; Crison Instruments Sa). At least ten determination were executed per treatment.

Texture Analysis

Texture analysis were carried out on raw, SC-CO_2 treated and cooked meat samples. They were cut from whole chicken breast obtaining pieces of similar shape and dimensions (about 2x2x4 cm).

220 The cooked meat samples were obtained by putting them in plastic bags and kept in a water bath
221 until they reached 80 °C in the inner part (about 1h). Sc-CO_2 samples were processed in bigger
222 vessels (about 300 mL volume) at 80 and 140 bar, 40°C, 45 min.

223 The texture analysis were carried out using Texture Profile Analysis (TPA), and effort-to-cut.
224 The TPA was conducted in a TA-XTplus Texture analyzer (Stable Micro System, London, UK),
225 using a 250 N load cell. A two-cycle compression test was performed using an aluminum probe
226 (40x50 mm), which was used to compress samples to 50% of their original thickness at a
227 compression rate of 1 mm/s, and a preload of 10 g. Hardness is obtained from the compression,
228 springiness, cohesiveness, gumminess, chewiness, adhesiveness, and resilience were obtained from
229 the force-time curves. Secondly, a cutting effort test was executed in a Lloyd Instruments LS5
230 (Ametek, US), using a load cell of 500 N. A cutting blade of 1 mm thickness, cut the samples at a 2
231 mm/s rate, arriving at a maximum depth of 25 mm. 16-20 measurements were performed for each
232 treatment.

233 ***Statistical Analysis***

234 Statistical analysis was performed in RStudio. Mean values were used to compare differences
235 between treatments. The existence of significant differences (α = 95%), between different
236 treatments were studied with an ANOVA and the pair comparisons within a group with its post-hoc
237 analysis (Tukey HSD) where possible, and Kruskal-Wallis Rank-sum test and Wilcoxon Rank-sum
238 test as their non-parametric alternative where the assumptions for an ANOVA were not fulfilled.

239 **RESULTS AND DISCUSSION**

240 ***Microbial Inactivation***

241 The inactivation kinetics of *E. coli* with SC-CO_2 alone or in combination with rosemary or
242 coriander at 40 ºC and 80 or 140 bar is reported in Table 1. The high-pressure treatments induced a
243 significant ($P < 0.01$) inactivation of *E. coli*. Treatment time was a significant factor, since its

244 increment resulted in a higher inactivation, at either 80 or 140 bar. This evidence is confirmed by
245 previous studies on pork where inactivation of *Salmonella* Typhimurium increased from 1.0 log
246 after 20 min treatment to 1.8 log after 40 min, keeping pressure and temperature constant at 140 bar
247 and 40 ºC (Bae et al., 2010). On the other hand, an increment of pressure from 80 to 140 bar did not
248 increase the inactivation in our experiments. This is in contrast with published work on ground pork
249 where after 40 min treatment at 40 ºC, inactivation of *L. monocytogenes* increased from 1 log at 100
250 bar up to 1.8 log at 140 bar (Bae et al., 2010). Nevertheless, this evidence could be explained by a
251 dependence on the food matrix. Protein content and morphology, and fat content and disposition,
252 can have a decisive impact on the antimicrobial effect of SC-CO_2 (Ferrentino & Spilimbergo,
253 2011). Previous studies on *E. coli* show variable inactivation results in beef or pork: 1 log reduction
254 was achieved at 310 bar/42.5 ºC/180 min in ground beef (Sirisee et al., 1998), 1.5 log reduction at
255 120 bar/35 ºC/30 min in fresh pork (Choi et al., 2009), while the average inactivation of *E. coli* at
256 140 bar/40 ºC/45 min in our experiments was 4.27 log CFU/g. This illustrates the variable results
257 obtained when treating *E. coli* in different matrixes. Besides, our results also showed a higher
258 inactivation when compared to the experiments in chicken by Wei et al. (1991), who reported
259 microbial reductions up to 1-2 log for *Salmonella* and < 1 log for *L. monocytogenes*, treating for
260 120 min at 137 bar and 35 ºC. Nevertheless, their inoculation procedure was different. They dipped
261 the chicken samples for 1 min in a solution containing the bacteria, as opposed to pipette-spiking.
262 Remaining for some time in solution might have caused the bacteria to permeate deeper into the
263 chicken muscle, making it less accessible for the CO_2.

264 When SC-CO_2 was coupled with herbs, no additional inactivation was observed if compared to
265 SC-CO_2 alone. Although not significant due to large standard deviations, SC-CO_2 + rosemary at
266 140 bar – 45 min caused a higher reduction of *E. coli* compared to the control and the coriander-
267 treated samples. Huang et al. (2017), reported a small additional effect of rosemary in the microbial
268 inactivation on raw pork meat. In their study, a longer process time (2 h) was used, which might
269 have helped extracting active components. Indeed published work with EO's on meat explores the

antimicrobial effect of herbs. Gouveia et al. (2016) reported 2 log additional reductions achieved by 6.25% (vol/vol) rosemary EO's of *L. monocytogenes* inoculated on beef after sous-vide cooking, which were sustained during a 28-day 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-day experiment at 4 ºC (Oussalah et al., 2004). To investigate the possible inactivation effect of the extracted EO's from the herbs onto the surface of the sample over time, we performed a shelf life study at 4°C up to 1 week (Table 2). However, our tests did not show any further reduction of *E. coli* for neither the treatment with herbs nor the SC-CO_2 alone during storage.

We further continued the investigation with the inactivation of natural flora in terms of mesophilic microorganisms, and yeast and moulds. 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 5-6 log that is required for pasteurization. Results of the inactivation with SC-CO_2 alone and in combination with fresh herbs are shown in Table 3. The 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 2.6-3.0 log CFU/g for the mesophiles, and 2.82-4 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-CO_2 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 literature with different type of meat. Microbial reductions of 1-3 log in mesophilic microorganisms were achieved after conditions of 60-160 bar, 20-60 min, and 40 ºC in pork raw meat (Cappelletti et al., 2015), and 0.5-1.7 log reduction in total

296 mesophiles were reported after 100-140 bar, 20-40 min, and 40-45 ºC in ground pork (Bae et al.,
297 2010). Morbiato et al. (2019) showed an inactivation of mesophilic bacteria comparable to this
298 work, achieving 3.5 log inactivation after 45 min, and a complete inactivation after 90 min in
299 chicken breast samples. However, in their study, an extraction of water was induced with the
300 drying, and therefore, different inactivation kinetics might have taken place compared to our
301 research. When fresh rosemary or coriander were combined with SC-CO_2 no additional inactivation
302 effect was observed ($P > 0.05$) for either mesophilic microorganisms or yeasts and molds.
303 Our findings suggest that the amount of essential oils extracted from the herbs during the
304 treatment could not be enough to exert a further antimicrobial effect during treatment. Besides,
305 herbs EO's and antioxidants supercritical fluid extraction comprises processes, including
306 fractionation steps, up to 2-4 h to reach an acceptable yield (Ahmed et al., 2012; Fornari et al.,
307 2012; Vicente et al., 2012). In less time, 90 min, it has been shown that complete microbial
308 inactivation in chicken can be achieved by SC-CO_2 alone (Morbiato et al., 2019) therefore
309 extending treatment time further is not necessary.
310 To demonstrate the effect of concentration of EO's on the inactivation, we performed some
311 proof-of-concept experiments using different concentration of pure EO's. Table 4 reports the
312 antimicrobial effect on *E. coli* of SC-CO_2 in combination with EO's of rosemary or coriander
313 inoculated on the surface of raw poultry samples at different concentrations. EO's alone have a
314 limited inactivation capacity for *E. coli*, and the maximum inactivation achieved was 1.23 and 0.98
315 log CFU/g for rosemary and coriander respectively. The highest inactivation in combination with
316 SC-CO_2 was achieved at the EO concentration of 0.5% (v/w). At this concentration, coriander EO
317 showed a synergistic effect compared to the treatment alone, while at lower (0.1 % v/w) and higher
318 (1% v/w) concentration an inactivation improvement was not achieved. At lower concentration the
319 amount of essential oil was probably not sufficient to induce a synergistic effect as seen for the
320 fresh herbs, while at higher concentration there might be a barrier effect caused by an excess of EO
321 on the surface that limited the availability of SC-CO_2 at the sample's surface. Interestingly the

322 synergistic effect was not obtained in case of rosemary EO for all the concentration tested
323 suggesting that also the type and therefore EO chemical composition is important for the synergic
324 inactivation. These preliminary data are interesting, and they open a wide possibility of
325 investigation for the optimization of the use of EO's for the reduction of process time and
326 improvement of microbial inactivation for the SC-CO_2 treatment.

327 ***Texture Analysis***

328 The effect of SC-CO_2 in the structure and color of meats and its conformational proteins has
329 been reported before in the literature (Zhou et al., 2015; Yan et al., 2018). Table 5 presents the
330 effect of SC-CO_2 treatment on the texture profile of chicken breast meat. Two different pressure
331 conditions were explored (80 and 140 bar), at 40 ºC for a 45 min duration treatment. Comparisons
332 can be drawn with an untreated control, and a heat-treated group. The table shows the results of two
333 different tests: a TPA and cutting effort test. The latter test did not show significant differences
334 between the test groups, although heat-treated samples were easier to cut than control or SC-CO_2
335 and had a lower variability. Moreover, it could be argued that treatment at higher pressures
336 increased the resistance to cut, although it also increased variability. Regarding the TPA descriptors
337 SC-CO_2 at 140 bar and heat-treatment significantly increase the hardness of chicken samples in
338 comparison to the untreated control, and SC-CO_2 80 bar increases it, although not significantly.
339 This is in agreement with Ros-Polski et al., (2015) who report that with increasing pressure the
340 hardness parameter tends to be higher because of the increase of muscle compactness after high
341 pressure treatment (Sun et al, 2010). It is noteworthy that heat-treatment increases overall hardness
342 while decreasing the resistance to cut. In fact, as reported by Palka and Daun (1999), the increase in
343 meat hardness after heat treatment may be due to the greater compactness assumed by the
344 myofibrils structure when, with thermal denaturation, they coagulate with a diminishing of their
345 water retention. During heat treatment there is a loss of water linked to the tissues, and myosin
346 denaturation. This causes the contraction of the protein and the hardening of the fibers with the

347 expulsion of water. Furthermore, with thermal treatment, the myofibrillar disintegration and the
348 decrease in fiber diameter occur and this could explain the decreasing of the resistance to cut (shear
349 strength) observed in this study on cooked poultry meat.

350 SC-CO_2-treated samples were only significantly different between each other for Hardness, and
351 Gumminess. Differences were, in consequence, between Untreated, Heat-treated and SC-CO_2-
352 treated groups. In general, the heat treatment caused an increment in the descriptors that correlate to
353 the meat becoming tougher and more difficult to masticate (Gumminess, Chewiness, Resilience),
354 while decreasing its ability to return to its original shape after compression (Springiness). In
355 general, springiness of raw meat (Palka et al., 1999) could be related to the degree of fiber swelling
356 which in turn should be reflected in the fiber diameter. After thermal treatment, the water loss of
357 muscle fiber and the thinning of fiber diameter could explain the slight decrease in springiness
358 (Table 6). SC-CO_2-treated samples were in a middle ground between control and heat-treated
359 samples, with 80 bar-treated samples slightly closer to the control. The adhesiveness, which is the
360 degree with which a sample adheres to the measuring probe after the first compression, was found
361 to be significantly larger (in negative value) for the untreated control, intermedium for the SC-CO_2-
362 treated samples, and minimum for the heat-treated group, in which the muscle protein has been
363 completely polymerized and the degree of stickiness is expected to be lower (Bouton & Harris,
364 1972).

365 ***Color and pH Measurement***

366 The effect of the treatments on the pH is reported in Table 6. SC-CO_2 treatment resulted in a
367 small acidification. The effect of SC-CO_2 on the color of raw chicken meat is shown in Table 7.
368 Significant differences ($P > 0.05$) were observed between the treated and non-treated samples. In
369 general, after treatment, an increase in lightness (L*), and a decrease in redness (a*) and yellowness
370 (b*) was seen in the measures taken at the surface of the chicken samples. Morbiato et al. (2019),
371 investigated the effect of SC-CO_2 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; 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-CO_2 treatment at the surface and at the center of the sample was investigated in. All three 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-CO_2, 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-CO_2 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-CO_2 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-CO_2 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-CO_2 technology as a viable decontamination technology for raw chicken meat. Future work should focus on the use of EO's extracts rather than fresh herbs and perform sensory tests to validate the consumer acceptance.

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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 ºC. 'E. coli was inoculated on raw poultry meat and treated with Supercritical Carbon Dioxide (SC-CO_2) in the presence of fresh coriander or rosemary; or treated alone (control)

Pressure	Time	SC-CO_2	Coriander	Rosemary
80 bar	15 min	-1.36 (0.24) ^{Aa}	-1.47 (0.69) ^{Aa}	-1.33 (0.48) ^{Aa}
	30 min	-3.93 (0.61) ^{Ba}	-3.68 (1.36) ^{Ba}	-3.97 (1.32) ^{Ba}
	45 min	-4.68 (0.86) ^{Ca}	-4.47 (0.93) ^{Ca}	-3.64 (1.26) ^{Ca}
140 bar	15 min	-1.53 (0.36) ^{Aa}	-1.84 (0.32) ^{Aa}	-1.73 (0.32) ^{Aa}
	30 min	-3.19 (0.79) ^{Da}	-2.82 (0.65) ^{Da}	-2.71 (0.57) ^{Da}
	45 min	-4.54 (1.48) ^{Ca}	-4.21 (1.17) ^{Ca}	-5.27 (1.92) ^{Ca}

[1] Values are the mean and SD - in brackets - of at least three determinations.
[2] Means with different small letter superscripts in the same row are significantly different ($P < 0.05$)
[3] Means with different capital letter superscripts in the same column are significantly different ($P < 0.05$)

Table 2. Log CFU/g reductions of 'E. coli' as a function of time (15, 30 and 45 min) at 140 bar and 40 ºC. 'E. coli' was inoculated on raw poultry meat and treated with Supercritical Carbon Dioxide (SC-CO_2) in the presence of fresh coriander or rosemary; or treated alone (control), and then stored for 7 days at 4 ºC in a closed container.

Pressure	Time	SC-CO_2	Coriander	Rosemary
140 bar	15 min	-1.68 (0.22) ^{Aa}	-1.66 (0.87) ^{Aa}	-1.72 (0.83) ^{Aa}
	30 min	-2.12 (0.71) ^{Ba}	-2.74 (1.05) ^{Ba}	-2.26 (1.04) ^{Ba}
	45 min	-4.74 (1.05) ^{Ca}	-4.13 (2.21) ^{Ba}	-3.87 (0.65) ^{Ca}

[1] Values are the mean and SD - in brackets - of at least three determinations.
[2] Means with different small letter superscripts in the same row are significantly different ($P < 0.05$)
[3] Means with different capital letter superscripts in the same column are significantly different ($P < 0.05$)

615 *Table 3. Log CFU/g reductions of chicken natural flora as a function of pressure (80, and 140 bar)*
616 *for 45 min and 40 ºC. Raw poultry meat and treated with Supercritical Carbon Dioxide (SC-CO_2) in*
617 *the presence of fresh coriander or rosemary; or treated alone (control). Samples were plated on*
618 *either Plate Count Agar (30 ºC) and Rose Bengal Agar (22 ºC) to evaluate mesophiles, and yeasts*
619 *and molds respectively.*

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)

620 [1] Values are the mean and SD - in brackets - of at least three determinations.
621
622
623 *Table 4. Log CFU/g inactivation of 'E. coli' inoculated on raw poultry meat after treatment with*
624 *herbal Essential oils (EO's) alone or in combination with Supercritical Carbon Dioxide (SC-*
625 *CO_2).Three concentration of EO's were tested: 1, 0.5 and 0.1% v/w." –" refers to the control when*
626 *no EO's were added. Treatment was 140 bar/40 ºC/ 45 min.*

	EO's	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)

627 [1] Values are the mean and SD - in brackets - of at least two determinations.
628
629
630

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) [ac]	0.60 (0.06) [a]	0.50 (0.13) [bc]	0.56 (0.05) [ac]
Springiness	1.33 (0.49) [ac]	1.12 (0.41) [a]	1.85 (0.50) [b]	1.66 (0.37) [bc]
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) [ab]	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]

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

Table 6. Effect of Supercritical Carbon Dioxide (SC-CO_2) on pH of raw chicken as a function of pressure after 45 min treatment at 40 ºC.

	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) [ac]

[1] Values are the mean and SD - in brackets - of ten determinations.
[2] Means with different small letter superscripts in the same column are significantly different ($P < 0.05$).

650 *Table 7. Effect of Supercritical Carbon Dioxide (SC-CO_2) treatment on instrumental color*
651 *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) ^{Aa}	84.59 (2.86) ^{Ab}	80.68 (3.38) ^{Ab}
	a*	9.83 (1.73) ^{Aa}	2.21 (0.71) ^{Ab}	1.45 (1.05) ^{Ab}
	b*	44.86 (1.65) ^{Aa}	42.89 (1.16) ^{Ab}	41.92 (1.46) ^{Aab}
Inner (x=0)	L*	51.70 (1.60) ^{Aa}	60.25 (3.22) ^{Bb}	58.53 (0.19) ^{Bab}
	a*	9.83 (1.73) ^{Aa}	12.76 (1.08) ^{Ba}	12.87 (0.62) ^{Ba}
	b*	44.86 (1.65) ^{Aa}	54.14 (4.61) ^{Bb}	49.32 (0.77) ^{Bab}

652 [1] Values are the mean and SD - in brackets - of at least three determinations.
653 [2] Means with different small letter superscripts in the same row are significantly different ($P < 0.05$)
654 [3] Means with different capital letter superscripts in the same column are significantly different ($P <$
655 0.05). Comparisons reflect only a parameter with its equal in another group.
656

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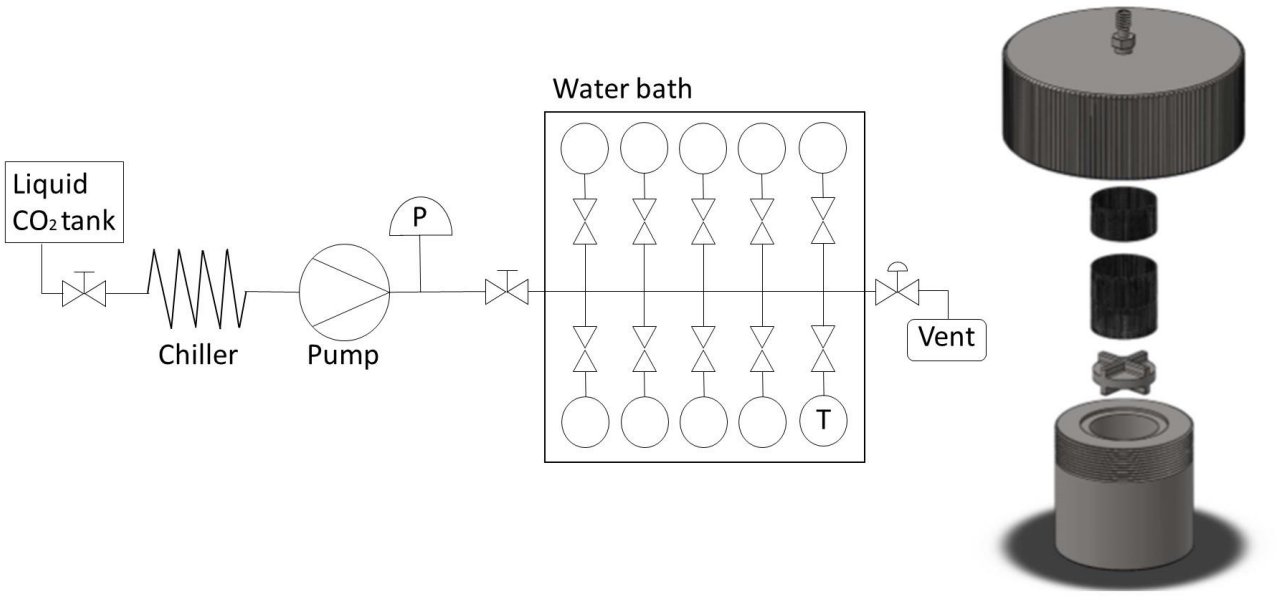

Figure 1. Schematic representation of the Supercritical Carbon Dioxide (SC-CO_2) multibatch apparatus (left); with P and T standing for Pressure Control and Temperature control respectively. A reactor and its elements (right). From top to bottom: reactor lid, basket for herbs, basket for the inoculated sample, magnetic agitator, reactor body.