



## Application of supercritical carbon dioxide (SC-CO<sub>2</sub>) on the microbial and physicochemical quality of fresh chicken meat stored at chilling temperature

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### Abstract

Supercritical carbon dioxide (SC-CO<sub>2</sub>) is a non-thermal technique implemented by food, pharmaceutical, and similar industries with the aim of inhibiting the microorganisms and apply effective sterilisation. Presently, limited number of studies has reported the application of SC-CO<sub>2</sub> on fresh chicken meat. The present work therefore aimed to reveal the microbial and physicochemical quality of the SC-CO<sub>2</sub>-treated fresh chicken meat. The fresh chicken meat was subjected to the SC-CO<sub>2</sub> at 14 MPa and 45°C for 40 min and was stored at 4°C for 0, 3, and 7 days. The obtained results indicated that the treatment with SC-CO<sub>2</sub> significantly decreased the total plate count and, yeast and mould count from log<sub>10</sub> 5.90 to 2.00 CFU/g and from log<sub>10</sub> 5.02 to 2.00 CFU/g at day 7 of storage, respectively. The values of pH, cooking loss, and water holding capacity were not affected by the treatment. The results revealed that the SC-CO<sub>2</sub>-treated samples displayed harder texture, higher lightness and yellowness, and lower redness. In addition, lipid peroxidation of SC-CO<sub>2</sub> and control samples resulted in values of 1.9 and 0.5 MDA/mg of meat at day 7 of storage time and did not significantly change in the rest of the evaluation days. In summary, the application of SC-CO<sub>2</sub> was capable of enhancing the microbial quality and certain physicochemical attributes. However, alteration of certain parameters of SC-CO<sub>2</sub> might enhance the overall meat quality.

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### Keywords

Supercritical carbon dioxide (SC-CO<sub>2</sub>), fresh chicken meat, non-thermal technology, lipid peroxidation, microbial count reduction

### Introduction

Meat as a delicate food with high nutritional ingredients is highly susceptible to quality deterioration. This muscle tissue, which is obtained from slaughtered animals, is composed of lipids, proteins, water, minerals, and some carbohydrates (Devatkal *et al.*, 2014). In general, meat degradation is approached from chemical and microbial alterations, specifically the oxidation of lipids inside the meat. The chemical composition of meat, oxygen, light, and storage temperature are the main causes of a complex process known as lipid oxidation. In addition, the processing procedures could also affect the quality of meat and result in changes in flavour, colour, texture, and nutritional value (Shah *et al.*, 2014).

Non-thermal approaches of food quality enhancements have been implemented by food, pharmaceutical, and similar industries in order to inhibit the growth of microorganisms and apply effective sterilisation. For instance, supercritical

carbon dioxide (SC-CO<sub>2</sub>) has been extensively utilised as an alternative low-temperature microbial inactivation technique in food and bioactive materials, and has been recognised as an effective method in food sterilisation (Erkmen, 2000) which is capable of inactivating microorganisms at relatively moderate pressure range (7.3 - 50.0 MPa). The leading factors of SC-CO<sub>2</sub> during microbial inactivation are temperature, exposure time, and pressure. Generally, by increasing the pressure or temperature, the inactivation process accelerates as a result of higher CO<sub>2</sub> solvating power, which facilitates both cellular contact and acidification throughout the treatment. The CO<sub>2</sub> diffusivity is a result of higher temperature, which increases the fluidity and diffusion (Wimmer and Zarevúcka, 2010). The higher microbial reduction was observed by means of longer treatment time and higher temperature or pressure, in comparison to a lower temperature, pressure, and shorter time (Jung *et al.*, 2009). The application of SC-CO<sub>2</sub> on fresh chicken meat and its effects on the meat quality and microbial reduction has been rarely studied. Therefore, the

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present work aimed to study the effect of SC-CO<sub>2</sub> on the quality and microbial reduction of fresh chicken meat stored at chilling temperature. Since the optimum effects of SC-CO<sub>2</sub> were reported at high temperature, pressure, and time (Choi *et al.*, 2009b), the highest conditions (14 MPa and 45°C for 40 min) were applied to the chicken meat samples in the present work.

## Materials and methods

### Materials

Stomacher bags, potato dextrose agar (PDA), total plate count agar (PCA), peptone, Petri dishes, malondialdehyde, trichloroacetic acid, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). The fresh chicken breast meat was purchased from Azli chicken meat supplier from a local market and was transported to the Laboratory of Food Technology, FSTM in a cold box 3 h post-slaughter at 4°C.

### Sample preparation and treatment

The fresh chicken breast meat was divided into six portions in aseptic condition. The samples were prepared in two treatments, namely the control sample and the SC-CO<sub>2</sub>-treated sample. Each portion of meat was cut into 1.5 cm thickness, 2 cm width, and 8 cm height. The meat pieces were then introduced to SC-CO<sub>2</sub> machine at 14 MPa and 45°C for 40 min. The design of the machine is represented in Figure 1. After the treatment of samples, the vessel was cleaned by 70% ethanol solution and the treated samples were placed in low-density polyethylene bags and were packed in aerobic condition. The samples were then stored at 4°C under aseptic conditions and were immediately assigned for meat quality and microbial measurements. The evaluation of treatments was carried out at days 0, 3 and 7.

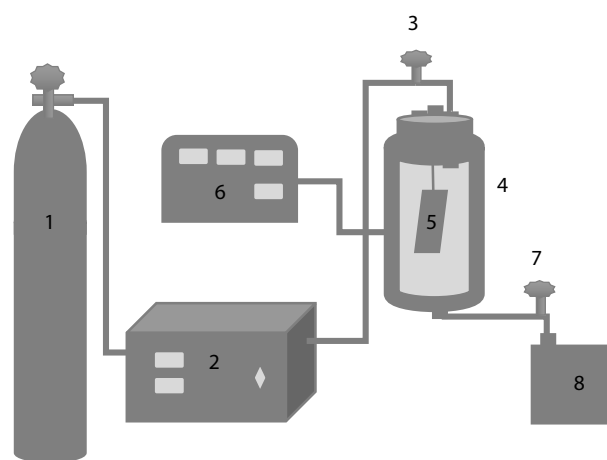


Figure 1. Diagrammatic representation of the SC-CO<sub>2</sub> system: (1) gas cylinder; (2) pump; (3) valve; (4) pressure vessel; (5) meat sample; (6) temperature controller; (7) valve; (8) disposable CO<sub>2</sub> and meat water.

### Microbial quality evaluation

The microbial quality of the samples was assessed through a method reported by Vaithiyanaathan *et al.* (2011). All the samples were assessed by plate count agar (PCA) for total plate count and potato dextrose agar (PDA) for yeast and mould count. The samples were homogenised in stomacher with sterile peptone water in 10 mg/90 mL, meat/sterile peptone water. The mixture was further diluted with 0.1% peptone water before inoculation onto PCA and PDA. Four dilutions (10<sup>-2</sup> - 10<sup>-5</sup>) were prepared for each sample in triplicates. Following incubation at 37°C for 2 days (PCA) and for 5 days (PDA), colonies appearing on the plates were enumerated.

### Colour analysis

The colour parameters [lightness (L\*), yellowness (b\*) and redness (a\*)] of the meat samples were measured by a method described by Aslinah *et al.* (2018) and Zhang *et al.* (2016) with slight modifications, using a Chromameter (CR-410). The measurement was carried out vertically on the surface of the meat in triplicate in order to obtain an average value.

### Texture profile analysis

The texture profile analysis (TPA) of the meat samples was carried out according to Zheng *et al.* (2015), and the texture analyser (Stable Micro Analyser TA-XT2i) was used to determine the TPA. An aluminium cylindrical probe P/50 SMP with 50 mm flat bottom diameter was used at 25°C surrounding temperature. Sample of meat was prepared in a cylindrical shape at 200 mm height and 24 mm diameter. The samples were analysed by axially double compressed cycle test at 40% of its original height. A 5 g trigger force, 1 mm/s pre-test speed, 1 mm/s test speed, and 2 mm/s post-test speed were used, while the time across two compression cycles was 5 s and the Exponent Stable Microsystem software was employed for data generation. All the samples were evaluated for adhesiveness, chewiness, resilience, cohesiveness, springiness, hardness, and gumminess.

### pH analysis

The pH value of all samples was determined as reported by Zhang *et al.* (2016). A 10 g of meat sample was retained in stomacher bag and was homogenised in 100 mL of distilled water through stomacher machine. After the filtration of the mixture, the value of pH was measured using a pH meter (SevniMulti, Mettler-Toledo GmbH 8603 Scherzenbach).

### Lipid peroxidation

The lipid peroxidation was measured following the method employed by Zhang *et al.* (2016). A 5 g slice of meat sample was homogenised with 25 mL of 7.5% (w/v) trichloroacetic acid which included 0.1% ethylenediaminetetraacetic acid (EDTA) at 15,000 rpm through simple kitchen-type grinder (Panasonic MX-GM1011). This was followed by centrifugation at 3,600 g for 20 min at room temperature (25°C) and was filtered through a Whatman 4 filter. The supernatant was mixed with 5 mL of TBA 0.02 mol/L reagent and the mixture was heated in a boiling bath for 30 min. Consequently, the sample was cooled at room temperature while the UV-visible spectrophotometer was used at 532 nm to measure the absorbance against the blank sample prepared with 5 mL TBA and 5 mL distilled water. The result of TBARS was reported as mg malondialdehyde per kg of meat sample.

### Determination of water holding capacity

The water holding capacity (WHC) of meat samples was determined as reported by Zheng *et al.* (2018). The meat slice was cut into 1 cm length cylinders, followed by weighing the slice and enfolded in a filter paper. The centrifugation process was performed at 10,000 g for 10 min at 10°C. Then, the filter paper was removed, and the sample was re-weighed. The value of WHC was indicated by the percentage of water loss from the total weight.

### Cooking loss

All the samples were evaluated for cooking loss as described by Komoltri and Pakdeechanuan (2012). The samples were slightly blotted with a tissue paper and were pre-weighed. After cooking for 40 min at 120°C internal temperature in an electric oven (CEO-S22BL), the samples were blotted and post-weighed, and calculated using Eq. 1:

$$\text{Cooking loss (\%)} = [(w1 - w2) / w1] \times 100 \quad (\text{Eq. 1})$$

where, w1 = pre-weight, and w2 = post-weight of meat samples.

### Statistical analysis

All the meat samples were measured in three-time replication while the significant differences were determined by Minitab 17 (Minitab Inc., State College, PA, USA) software. The results were presented as means  $\pm$  SD while the comparison between the variables was made by two-way ANOVA and the significant difference was expressed as ( $p < 0.05$ ).

## Results and discussion

### Microbial evaluation

The total plate count (TPC) and yeast and mould count (YMC) of fresh chicken meat were evaluated at days 0, 3, and 7 following the application of SC-CO<sub>2</sub> at 14 MPa and 45°C for 40 min. Table 1 displays the effect of SC-CO<sub>2</sub> on TPC of the meat samples, where it was evident that SC-CO<sub>2</sub> had substantial effect on the inhibition of bacterial growth at day 7 of storage and reduced the bacterial growth to 2 log<sub>10</sub> CFU/g. The treatment with SC-CO<sub>2</sub> had a slight effect at day 0 of storage, but it was not significantly different as compared to the control sample. In addition, TPC demonstrated high log<sub>10</sub> CFU/g at day 0 which indicated the contaminated slaughtering condition of the supplier. Both control and SC-CO<sub>2</sub> treatments had insignificant growth at day 3 of storage, and this was further reduced at day 7. Similar researches have been conducted on fresh and marinated pork, which demonstrated the reduction of bacteria such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *E. coli* O157:H7 (Choi *et al.*, 2009a; 2009b).

Table 1 also displays the effects of SC-CO<sub>2</sub> on YMC of the meat, which indicated that SC-CO<sub>2</sub> had a significant influence on the inhibition of fungal growth at day 7 of storage with up to 2 log<sub>10</sub> CFU/g reduction. SC-CO<sub>2</sub> had a minor effect at day 0 of storage, but this was not significantly different as compared to the control sample. The high value of YMC at day 0 indicated the contaminated slaughtering condition of the supplier. The YMC in the SC-CO<sub>2</sub> treatment increased at day 3 and was significantly different in comparison to the control sample. This could be due to the fact that complete inactivation of microbial spores is challenging to attain in the applied 14 MPa pressure and 45°C temperature. Black *et al.* (2007) reported that comprehensive inactivation of bacteria, yeasts and moulds is a “holy grail”, which is difficult to achieve. However, high pressure such as 300 to 600 MPa and > 50°C temperature may have great reduction power against microbial spores.

Microbial growth is directly related to the appropriate condition such as nutrients, temperature, pH, and water activity. When the optimal condition is altered, the growth of microorganisms will also be affected (Chavasit *et al.*, 2018). The precise mechanism by which SC-CO<sub>2</sub> inhibits microbial growth is yet to be known; however, the following theories are hypothesised: the CO<sub>2</sub> might dissolve in the cell membrane of microorganisms which leads to cell membrane modification followed by a reduction in

Table 1. Effects of SC-CO<sub>2</sub> on total plate count and total yeast and mould count of fresh chicken meat stored at 4 ± 1°C for seven days.

Parameter	Treatment	Day 0	Day 3	Day 7
Total plate count (Log CFU/g)	Control	6.28 ± 2.27 <sup>Aa</sup>	6.53 ± 1.67 <sup>Aa</sup>	5.93 ± 0.05 <sup>Aa</sup>
	SC-CO <sub>2</sub>	4.01 ± 1.75 <sup>Aa</sup>	7.41 ± 1.35 <sup>Ab</sup>	2.00 ± 0.00 <sup>Ba</sup>
Total yeast and mould count (Log CFU/g)	Control	6.11 ± 3.58 <sup>Aa</sup>	6.35 ± 0.74 <sup>Aa</sup>	5.03 ± 0.43 <sup>Aa</sup>
	SC-CO <sub>2</sub>	3.04 ± 1.81 <sup>Ab</sup>	6.56 ± 1.27 <sup>Aa</sup>	2.00 ± 0.00 <sup>Bb</sup>

Means within the same row with different small letters (effect of days) are significantly different ( $p < 0.05$ ). Means within the same column with different capital letters (effect of treatments) are significantly different ( $p < 0.05$ ).

intracellular pH. As a result of the reduction in pH value, the essential enzymes and cellular metabolism would be affected while CO<sub>2</sub> and HCO<sub>3</sub> directly altered the molecular metabolism. Consequently, the intracellular electrolyte balance would be disrupted and ultimately vital components would be leaked from the cell (Garcia-Gonzalez *et al.*, 2007). The TPC results obtained in the present work supports the bacterial growth theory. As earlier described, the TPC and YMC were fluctuating, which corresponded to the unfavourable condition for their growth. It can therefore be concluded that the microbial growth was significantly impaired in treated samples at day 7 of storage. The results obtained in the present work demonstrated that the application of SC-CO<sub>2</sub> at 14 MPa and 45°C for 40 min on fresh chicken meat was capable to significantly inhibit the microbial growth and increase the shelf life, especially for long period of storage.

### Colour

The colour changes of fresh chicken meat stored at 4 ± 1°C for seven days are presented in Table 2, which indicates that the measured colour parameters [lightness (L\*), redness (a\*), and yellowness (b\*)] were significantly different

( $p < 0.05$ ) in each day. The (L\*) and (b\*) values of SC-CO<sub>2</sub> treatment were significantly higher in comparison to the control, while the (a\*) values indicated opposite behaviour throughout the storage period. It could be observed that the application of temperature and pressure led to an increase in the lightness and yellowness of the meat but decreased its redness. Similar results and alterations in the meat was reported on porcine *longissimus dorsi* muscle at 7.4 and 15.2 MPa, 31°C for 10 min (Choi *et al.*, 2008). Similar results were also observed in a study conducted on ground pork meat which reported that SC-CO<sub>2</sub> had a significant effect on changing the colour of meat (Huang *et al.*, 2017). In another study, Omana *et al.* (2011) examined the effect of different ingredients on colour and oxidative characteristics of high pressure processed chicken breast meat with some additives. The results indicated similar behaviour to the findings obtained in the present work. Moreover, Orlien (2017) reported that the application of high pressure on beef, lamb, pork, and chicken meats resulted in discolouration due to myoglobin molecule modification and meat microstructure alterations. The main causes of colour changes are myoglobin denaturation, dislocation of heme, and oxidation of bright red oxymyoglobin to brownish metmyoglobin,

Table 2. Effects of SC-CO<sub>2</sub> on colour values of fresh chicken meat stored at 4 ± 1°C for seven days.

Colour value	Treatment	Day 0	Day 3	Day 7
L*	Control	62.03 ± 1.82 <sup>Aa</sup>	58.48 ± 1.07 <sup>Ab</sup>	59.26 ± 0.39 <sup>Bab</sup>
	SC-CO <sub>2</sub>	77.17 ± 3.96 <sup>Ba</sup>	73.13 ± 2.63 <sup>Ba</sup>	77.05 ± 0.67 <sup>Aa</sup>
a*	Control	10.57 ± 0.50 <sup>Aa</sup>	10.06 ± 0.35 <sup>Aa</sup>	10.70 ± 0.46 <sup>Aa</sup>
	SC-CO <sub>2</sub>	6.08 ± 1.58 <sup>Ba</sup>	7.31 ± 0.86 <sup>Ba</sup>	5.24 ± 0.18 <sup>Ba</sup>
b*	Control	11.27 ± 1.39 <sup>Ba</sup>	12.23 ± 0.66 <sup>Ba</sup>	12.66 ± 1.19 <sup>Ba</sup>
	SC-CO <sub>2</sub>	15.13 ± 0.35 <sup>Aa</sup>	15.74 ± 1.27 <sup>Aa</sup>	15.33 ± 0.68 <sup>Aa</sup>

Means within the same row with different small letters (effect of days) are significantly different ( $p < 0.05$ ). Means within the same column with different capital letters (effect of treatments) are significantly different ( $p < 0.05$ ).

while the significant factor in the colour stability of meat is temperature (Bekhit *et al.*, 2019). As the samples were treated at 45°C, the main reason for colour alteration could be the temperature, which could have accelerated the myoglobin denaturation and metabolic process.

### Texture

Texture evaluation of fresh chicken meat after treatment with SC-CO<sub>2</sub> is exhibited in Table 3. It could be observed that important attributes such as hardness, springiness, gumminess, and resilience were influenced in some days of storage period. The totally affected attribute was hardness, which was notably altered after the application of SC-CO<sub>2</sub>. Hardness is defined as a force that the food requires for its constriction or deformation (Sañudo-Barajas *et al.*, 2019). Evidently, the application of SC-CO<sub>2</sub> hardened the meat samples from 4480 ± 5730 g of the control sample to 7248 ± 1002 g in SC-CO<sub>2</sub>-treated samples at day 0 of storage. It is worth mentioning that around 20% of the water was lost in the samples after the application of SC-CO<sub>2</sub> treatment, which could have contributed to the increase in the meat hardness. In a similar study, the hardness of marinated pork after application of SC-CO<sub>2</sub> was reported at 7.4, 12.2, and 15.2 MPa at 31.1°C for 10 min (Young *et al.*, 2013). Moreover, other important factors such as the springiness, gumminess, and resilience were significantly influenced in some days of texture evaluation. The score for the springiness of control samples was 0.59 ± 0.05, which was significantly different from the score of 0.68 ± 0.01 obtained by SC-CO<sub>2</sub>-treated meat samples at day 3 of storage. In addition, gumminess and resilience were notably altered at day 0 of storage from 2756 ± 2050 g and 0.371 ± 0.049 to 4389 ± 7370 and 0.2623 ± 0.038,

respectively.

In summary, it was observed that the SC-CO<sub>2</sub>-treated fresh chicken meat which was stored at 4 ± 1°C for seven days had significant alteration on their texture, where the hardness was significantly different from the control throughout the storage period. In addition, other parameters such as springiness, gumminess, and resilience were also affected on some days of storage. However, some characteristics such as adhesiveness, cohesiveness, and chewiness were not significantly influenced by the treatments.

### pH analysis

The effect of SC-CO<sub>2</sub> treatment on pH values of fresh chicken meat stored at 4 ± 1°C for seven days is shown in Table 4. It could be observed that the application of SC-CO<sub>2</sub> did not have a significant effect on the pH value of both control and treated samples. These results were also reported in a similar study where no alterations in the pH value and the meat quality was observed after application of SC-CO<sub>2</sub> on porcine *longissimus dorsi* muscle (Choi *et al.*, 2008; 2009b). Some studies have suggested that through the application of CO<sub>2</sub> on meat, the pH level decreases due to the dissolution of CO<sub>2</sub> inside the meat muscles and production of bicarbonate and protons (Jakobsen and Bertelsen, 2002). The value of pH starts to decrease from about 7.0 in live animal muscles to 5.3 - 5.8 in the slaughtered animal, which is known as an ultimate pH. The alterations in pH level of muscle are initiated by anaerobic glycolytic pathway and transformation of glycogen to lactic acid. As can be seen, the notions and findings of the mentioned results were confirmed in the present work. Furthermore, the minimum value of pH in the present work was not

Table 3. Effects of SC-CO<sub>2</sub> on texture profile analysis parameters of fresh chicken meat stored at 4 ± 1°C for seven days.

Treatment	Control			SC-CO <sub>2</sub>		
	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
Hardness (g)	4480 ± 5730 <sup>Aa</sup>	6232 ± 3499 <sup>Aa</sup>	5432 ± 1083 <sup>Aa</sup>	7248 ± 1002 <sup>Ba</sup>	8822 ± 1317 <sup>Ba</sup>	8808 ± 2466 <sup>Ba</sup>
Adhesiveness	-86.8 ± 81.7 <sup>Aa</sup>	-162.6 ± 167.3 <sup>Aa</sup>	-60.3 ± 22.8 <sup>Aa</sup>	-82.3 ± 46.4 <sup>Aa</sup>	-112.3 ± 100.3 <sup>Aa</sup>	-87.4 ± 107.1 <sup>Aa</sup>
Springiness	0.69 ± 0.08 <sup>Aa</sup>	0.59 ± 0.05 <sup>Aa</sup>	0.63 ± 0.09 <sup>Aa</sup>	0.67 ± 0.04 <sup>Aa</sup>	0.68 ± 0.01 <sup>Ba</sup>	0.66 ± 0.03 <sup>Aa</sup>
Cohesiveness	0.62 ± 0.08 <sup>Aa</sup>	0.56 ± 0.08 <sup>Aa</sup>	0.52 ± 0.07 <sup>Aa</sup>	0.60 ± 0.03 <sup>Aa</sup>	0.57 ± 0.02 <sup>Aa</sup>	0.61 ± 0.03 <sup>Aa</sup>
Gumminess (g)	2756 ± 2050 <sup>Aa</sup>	3622 ± 2234 <sup>Aa</sup>	2899 ± 7950 <sup>Aa</sup>	4389 ± 7370 <sup>Ba</sup>	5096 ± 6780 <sup>Aa</sup>	5387 ± 1510 <sup>Aa</sup>
Chewiness (g)	1904 ± 3520 <sup>Aa</sup>	2211 ± 1470 <sup>Aa</sup>	1887 ± 7220 <sup>Aa</sup>	2959 ± 6580 <sup>Aa</sup>	3490 ± 4000 <sup>Aa</sup>	3606 ± 1077 <sup>Aa</sup>
Resilience	0.371 ± 0.049 <sup>Aa</sup>	0.316 ± 0.074 <sup>Aa</sup>	0.297 ± 0.043 <sup>Aa</sup>	0.2623 ± 0.038 <sup>Ba</sup>	0.2617 ± 0.016 <sup>Aa</sup>	0.2653 ± 0.018 <sup>Aa</sup>

Means within the same row with different small letters (effect of treatments) are significantly different ( $p < 0.05$ ). Means within the same row with different capital letters (effect of days) are significantly different ( $p < 0.05$ ).

Table 4. Effects of SC-CO<sub>2</sub> on cooking loss, water holding capacity, pH, and peroxidation of fresh chicken meat stored at 4 ± 1°C for seven days.

Parameter	Treatment	Day 0	Day 3	Day 7
Cooking loss (%)	Control	23.33 ± 3.5 <sup>Ab</sup>	49.08 ± 16.85 <sup>Aa</sup>	42.26 ± 1.55 <sup>Aab</sup>
	SC-CO <sub>2</sub>	26.06 ± 3.84 <sup>Aa</sup>	50.8 ± 17.30 <sup>Aa</sup>	37.73 ± 4.02 <sup>Aa</sup>
Water holding capacity (%)	Control	20.86 ± 2.94 <sup>Aa</sup>	19.27 ± 7.83 <sup>Aa</sup>	24.03 ± 1.95 <sup>Aa</sup>
	SC-CO <sub>2</sub>	18.26 ± 4.93 <sup>Aa</sup>	23.29 ± 4.69 <sup>Aa</sup>	26.51 ± 2.82 <sup>Aa</sup>
pH	Control	5.75 ± 0.04 <sup>Aa</sup>	5.83 ± 0.11 <sup>Aa</sup>	5.76 ± 0.06 <sup>Aa</sup>
	SC-CO <sub>2</sub>	5.83 ± 0.05 <sup>Aa</sup>	5.76 ± 0.13 <sup>Aa</sup>	5.76 ± 0.05 <sup>Aa</sup>
Lipid peroxidation	Control	0.03 ± 0.08 <sup>Aa</sup>	0.37 ± 0.34 <sup>Aa</sup>	0.57 ± 0.25 <sup>Ba</sup>
	SC-CO <sub>2</sub>	0.16 ± 0.12 <sup>Aa</sup>	0.47 ± 0.17 <sup>Aa</sup>	1.62 ± 0.19 <sup>Ab</sup>

Means within the same row with different small letters (effect of days) are significantly different ( $p < 0.05$ ). Means within the same column with different capital letters (effect of treatments) are significantly different ( $p < 0.05$ ).

below  $5.76 \pm 0.05$  and was not significantly affected by the application of SC-CO<sub>2</sub>. It is known that pH is an important factor in the quality measurement of meat since it could affect various viable aspects of meat such as shelf life, water holding capacity, flavour, tenderness, and colour.

#### Lipid peroxidation

The results of lipid peroxidation are presented in Table 4. Although no significant changes ( $p > 0.05$ ) at days 0 and 3 of storage was observed, the mean value for treated samples with SC-CO<sub>2</sub> was slightly higher in comparison to the control. In contrast, a significant change ( $p < 0.05$ ) at day 7 of storage for SC-CO<sub>2</sub> treated samples was observed, which resulted in higher MDA level as compared to the control sample. Similar results were reported in another study, where the application of SC-CO<sub>2</sub> on the ground pork meat increased the value of TBARS after several days of the application (Huang *et al.*, 2017). Lipid peroxidation is a complex process which affects unsaturated lipids of meat products and results in degradation of sensorial and nutritional values. Several factors such as myoglobin, meta catalysts, enzymes, oxidative enzymes, storage, processing, and water activity could directly affect the lipid oxidation degree. The lipid peroxidation is a type of oxidative damage which is initiated by peroxy-radicals (oxygen-oxygen bond). Several mechanisms might be included in the production of a high amount of MDA after seven days of storage. It is known that meat possesses a natural defence mechanism against oxidation stress. The defence mechanism is inhibited through decreasing the pH level, antioxidant enzymes inactivation, iron discharge from heme pigments, decreasing some compounds such as nicotinamide adenine dinucleotide,

tocopherols, and system of non-enzymatic antioxidant defence (Villalobos-Delgado *et al.*, 2019). The defence mechanism of the meat samples treated by SC-CO<sub>2</sub> at day 7 of storage might be affected through high pressure or temperature for a long time which resulted in the significantly high level of MDA per kg of meat.

#### Water Holding Capacity (WHC)

The water holding capacity of fresh chicken meat following the application of SC-CO<sub>2</sub> is presented in Table 4. The results indicated that the treated samples were not significantly altered in comparison to the control sample. The amount of water which was discharged during the treatment of SC-CO<sub>2</sub> on the meat and the applied samples was about 20% of weight as a result of application of long term temperature and pressure. In a similar study conducted on porcine *longissimus dorsi* muscle, it was confirmed that the application of SC-CO<sub>2</sub> did not have a significant effect on the value of WHC (Choi *et al.*, 2008). It is known that water in the intermolecular spaces between the salt-soluble proteins (myosin and actin) in muscle tissue possesses high WHC due to the capillary force (Brewer, 2014). The main mechanism of water discharge from the meat has been differently hypothesised, while factors which lead to water purging are reduction in the pH level, proteolysis, protein oxidation, and early slaughtering. Cell structures including the intra and extra myofibrillar spaces of muscle are the places which contain high content of water (Huff-Lonergan and Lonergan, 2005). Since the pH level of the treated meat sample did not reveal any notable difference in comparison to the control sample and that the utilised sample was obtained from fresh meat, it could be concluded that the value of WHC was not affected by the application of SC-CO<sub>2</sub>.

### Cooking loss

Table 4 indicates that the application of SC-CO<sub>2</sub> did not significantly affect the cooking loss of fresh chicken meat. These results are similar to those obtained from porcine *longissimus dorsi* muscles (Choi *et al.*, 2008). Cooking loss is an important attribute of meat, which includes weight and volume losses during the cooking process as well as alterations in its textural qualities and economic concerns (Purslow *et al.*, 2016). Several factors could affect the cooking loss of meat such as muscle pH, cooking method, and the final temperature of cooking (Kerth, 2013). According to the mentioned cooking loss factors, the treated meat samples by the SC-CO<sub>2</sub> did not reveal any changes in comparison to the control sample, which indicates that the SC-CO<sub>2</sub> treatment did not have any apparent effect on cooking loss attribute of chicken meat.

### Conclusion

In the present work, the effect of SC-CO<sub>2</sub> treatment on fresh chicken meat regarding its microbial inhibition and quality upgrade were evaluated. The microbial load, colour, texture, and lipid peroxidation parameters were significantly altered after the application of SC-CO<sub>2</sub>. The microbial loads (total bacteria, moulds, and yeasts) of the treated samples were significantly reduced as compared to the control sample at day 7 of storage. The a\* and b\* values of colour of the treated samples notably decreased while its L\* value increased. Lipid peroxidation did not have any significant alterations on days 0 and 3 of evaluation; however, it possessed notable variations on day 7 of storage. The adhesiveness, cohesiveness, and chewiness of the treated samples did not have any notable variations while the means of hardness of the treated samples was significantly higher than the control sample during all days of storage time. The springiness, gumminess, and resilience only changed on certain days of storage. Water holding capacity, cooking loss, and pH level were among the parameters which did not have any significant alterations during the storage period. As a conclusion, the application of SC-CO<sub>2</sub> at 14 MPa, 45°C for 40 min on fresh chicken meat could enhance major parameters such as inhibition of microbial growth. However, adjustment to the treatment parameters of SC-CO<sub>2</sub> such as the temperature and time are required in order to produce chicken meat with superior quality.

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