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Different Pressures, Low Temperature, and Short-Duration Supercritical Carbon Dioxide Treatments: Microbiological, Physicochemical, Microstructural, and Sensorial Attributes of Chill-Stored Chicken Meat

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Featured Application: We approached supercritical carbon dioxide (SC-CO₂) treatment with different levels of pressure (7.4, 11.4 and 15.4 MPa) on raw chicken meat stored for seven days at 4 °C. We emphasize the lower temperature (31 °C) and shorter time (10 min) used as compared to previous studies by other researchers. Through analysis of microbiological, physicochemical, microstructural, and sensorial data, the importance of a lower temperature and shorter time with suitable pressure was revealed. This determined SC-CO₂ treatment has potential in the chicken meat industry as a tool to improve microbial safety while retaining meat quality.

Abstract: This work aimed to determine the microbiological, physicochemical, microstructural, and sensorial attributes of chicken meat treated with different pressures of supercritical carbon dioxide (SC-CO₂) at a low temperature for a short duration. The raw chicken meat was subjected to three pressures: 7.4, 11.4 and 15.4 MPa at 31 °C for 10 min and then stored at 4 °C for seven days. The 11.4 and 15.4 MPa treatments on the zeroth day reduced the microbial load compared to the control and 7.4 MPa treatment. Similarly, the higher pressure resulted in a decrease in the total count of yeast and mold. The SC-CO₂ had a lesser effect on the lipid peroxidation, pH, cooking loss, and water holding capacity of the treated chicken meats. Color analysis showed an increase in lightness (*L*^{*}) and a reduction in redness (*a*^{*}) on the sample surface. Both texture and color results were within acceptable ranges. SC-CO₂ treatment with 11.4 or 15.4 MPa at a low temperature for a short time improve microbiological safety while retaining the quality of chicken meat. These findings can be expanded and applied as an alternative for non-thermal processing of chicken meat.

Keywords: supercritical carbon dioxide; SC-CO₂; non-thermal technology; microbial reduction; raw chicken meat

1. Introduction

Chicken meat is one of the most important sources of protein in the human diet apart from beef and pork. According to a report by the United States Department of Agriculture (USDA), the total production

of chicken meat in the United States and several selected countries increased from 91,148,000 metric tons in 2016 to 100,026,000 metric tons in 2020 [1]. The storage of raw chicken meat is related to microbial deterioration and lipid peroxidation, which have been implicated as the main factors in reducing the quality and shelf-life of muscle foods [2–4]. Lipid peroxidation changes the meat quality parameters such as color, flavor, odor, texture, and nutritional value [5]. The rich nutrients in meat that have suitable water activity and pH for the survival of bacteria can lead to microbial spoilage [6].

Thermal methods have been used for deactivation of microorganisms inside meat and meat products; however, they have negative effects on the flavor and aroma of the treated meat [7]. Supercritical carbon dioxide (SC-CO₂) is a non-thermal technology applied in the food, pharmaceutical, and other related industries. SC-CO₂ has been recognized as an alternative method for the inhibition of microorganisms and effective food pasteurization [8]. The mechanism of bactericidal activity of SC-CO₂, which may be related to pressure, temperature, and exposure time, is still not well understood. Higher pressure, for example, raises the solubility of CO₂ and promotes the acidification of the cellular membrane. SC-CO₂ is assumed to enhance the fluidity of the cell membrane, improving its permeability and promoting the extraction of membrane components such as phospholipids [9]. The critical point of SC-CO₂ starts at 7.4 MPa at 31.1 °C. Several studies have reported the application of SC-CO₂ on pork meat in different conditions conducted between 7.4 and 15.2 MPa [10–12].

Chicken meat and chicken meat products could be negatively affected by high temperatures such as during cooking or other treatments involving heat. For example, 90% soluble protein, 43% myofibrillar protein, and the weight of chicken patties decreased when the temperature was between 23 and 80 °C [13]. González-Alonso et al. [14] performed microbial inactivation in chicken meat using SC-CO₂ at 140 bars (14 MPa), but at 40 °C from 15 to 45 min. However, the chicken meat texture hardened and the color changed as compared to the control. Similar results were obtained by Jauhar et al. [15], where the total plate count and total yeast and mold count reduced after SC-CO₂ treatment at 14 MPa at 45 °C for 40 min and then stored for seven days; however, the color and texture had negative effects. Various levels of pressure could result in different chicken meat quality. A lower temperature and shorter time might be suitable for the application of SC-CO₂ to improve the quality of chicken meat. Therefore, this work aimed to determine the microbial, physicochemical, microstructural, and sensorial properties of chicken meat treated at three levels of SC-CO₂ pressure (7.4, 11.4, and 15.4 MPa) at 31 °C for 10 min and then stored at 4 °C for seven days.

2. Materials and Methods

2.1. Materials

The plate count agar (PCA; Oxoid™), potato dextrose agar (PDA; Oxoid™), peptone water (Oxoid™), petri dishes, and stomacher bags were from Thermo Fisher Scientific (Shah Alam, Malaysia). Whatman 4 filter paper, ethylenediaminetetraacetic acid (EDTA), malondialdehyde (MDA), ethanol, methanol, and trichloroacetic acid (TBA) were from Sigma-Aldrich (M) Sdn Bhd (Malaysia).

2.2. Sample Preparation

Raw broiler chicken breast meat (*pectoralis major* muscle) was collected from Azli Chicken Meat Supplier, Seri Kembangan, Selangor, three hours post-slaughter and aseptically transported in a cold box to the lab. Four treatments were carried out—the control and three different levels of SC-CO₂ (Supercritical Fluid Extraction Lab Scale Plant, Deven Supercriticals Pvt Ltd, Mumbai, Maharashtra, India) (7.4, 11.4 and 15.4 MPa)—at 31.1 °C for 10 min. The chicken meat was cut into 1.5 × 8.0 × 2.0 cm pieces. It was then placed in an SC-CO₂ machine in the above-mentioned conditions. The vessel was cleaned using a 70% ethanol solution after every treatment. All treatments were conducted in aerobic conditions with the samples packed in low-density polyethylene bags. The treated chicken meats were kept at 4 °C aseptically for seven days. The samples were analyzed on days 0, 3 and 7 of storage.

2.3. Microbiological Quality Evaluation

The total count of bacteria, and total count of yeast and mold were measured using plate count agar (PCA) and potato dextrose agar (PDA), respectively. A 10 mg aliquot was homogenized with 90 mL peptone water and then diluted with 0.1% peptone water. The sample was inoculated into the PCA and PDA. The dilutions were carried out four times (10^{-2} – 10^{-5}) for each sample in triplicate. The readings of plates were carried out after the incubation at 37 °C for 48 h (PCA) and 72–120 h (PDA) [16].

2.4. Lipid Peroxidation Measurement

A 5 g aliquot was mixed with 25 mL 7.5% (*w/v*) trichloroacetic acid containing 0.1% ethylenediaminetetraacetic acid (EDTA). Then, it was homogenized at 15,000 rpm using a kitchen type grinder (Panasonic MX-GM1011, Malaysia). The mixture was then centrifuged for 20 min at $3600\times g$ at room temperature. The supernatant filtered by Whatman 4 (5 mL) was then mixed with 5 mL TBA 0.02 mol/L reagent, heated for 30 min using a boiling bath and cooled at room temperature. A blank sample was prepared by mixing 5 mL TBA with 5 mL distilled water. The absorbance of the supernatant was recorded at 532 nm against the blank sample. The thiobarbituric acid reactive substances (TBARS) values were measured based on the malondialdehyde standard curve developed and are reported as mg malondialdehyde per kg of the chicken meat sample [17].

2.5. Color Analysis

A Chroma meter (CR-410, Japan) was used for color measurement, and the results are described by lightness (L^*), redness (a^*), and yellowness (b^*) [17].

2.6. PH Analysis

A 10 g aliquot was homogenized with distilled water (100 mL) and, after filtration, the pH was measured using a pH meter (SevenMulti, Mettler-Toledo GmbH 8603, Schwerzenbach, Switzerland) [17].

2.7. Determination of Water Holding Capacity

The samples were prepared in a cylindrical shape (approximately 1 cm in diameter and 1.5 cm in length), wiped, weighed, and enfolded in Whatman 4 filter paper. This was followed by centrifugation at $10,000\times g$ for 10 min at 10 °C. The sample was then weighed again [18], and the water holding capacity was calculated according to the following formula:

$$\text{Water loss\%} = [(\text{weight before centrifuge} - \text{weight after centrifuged})/\text{weight before centrifuge}] \times 100 \quad (1)$$

2.8. Cooking Loss

Samples were blotted dry with paper towel, weighed (10 ± 2 g) and cooked for 40 min at 120 °C using an electric oven (CEO-S22BL, Malaysia). Then, the cooked samples were blotted dry and re-weighed. The cooking loss was calculated based on the method by Komoltri and Pakdeechanuan [19], as shown below:

$$\text{Cooking loss (\%)} = [(\text{pre-cooked weight} - \text{post cooked weight})/\text{pre-cooked weight}] \times 100 \quad (2)$$

2.9. Texture Profile Analysis

The samples were cut into 24 mm diameter and 20 mm height pieces. They were double compressed by a 40% double-cycle test through a cylindrical probe (50 mm flat bottom) at 25 °C using a 25 kg load cell. The Stable Micro Analyzer TA-XT2i, U.K. was used for analyzing texture with test speed of a 1 mm/s pre-test speed and 2 mm/s post-test speed [20]. The hardness, adhesiveness,

springiness, cohesiveness, gumminess, chewiness and resilience of the samples were then calculated from the recorded force–time plot.

2.10. Microstructural Analysis

The samples were cut into $1 \times 1 \times 0.5$ cm pieces and treated with SC-CO₂. The method was modified to dehydrating meat samples at 45 °C for 24 h. This was followed by attaching them to aluminum stubs and coating them with gold. A scanning electron microscope SEM (JEOL JSM-IT100 (JEOL Germany GmbH, Freising, Germany) was used for microstructure determination with an accelerated voltage of 1 kV. The samples were imaged at 300× magnification [19].

2.11. Sensory Evaluation

Thirty untrained panelists evaluated the samples on the third day of storage based on the results of the microbiological evaluation and for safety purposes. The chicken meats were cooked without any additional spices in the electric oven CEO-S22BL (Cornell Sales & Service Sdn. Bhd., Petaling Jaya, Selangor, Malaysia) for 40 min at 120 °C, labeled with a random three-digit number and served warm to the panelists. A 9-point hedonic scale was used with 1—dislike extremely, 2—dislike very much, 3—dislike moderately, 4—dislike slightly, 5—neither like nor dislike, 6—like slightly, 7—like moderately, 8—like very much, and 9—like extremely. The attributes of interest were color, aroma, flavor, tenderness, juiciness, springiness, and overall acceptability [19].

2.12. Statistical Analysis

All treated meat samples were measured in triplicate (except for the sensory evaluation) and analyzed for comparison using two-way ANOVA. The sensory data were analyzed using one-way ANOVA. Significant differences are expressed as $p < 0.05$ with the results presented as mean \pm standard deviation. Minitab Statistical Software version 17 (MiniTab Inc., State College, PA, USA) was used for the statistical analysis.

3. Results and Discussion

3.1. Microbiological Quality

The results of the microbiological evaluation of the raw chicken meat treated with SC-CO₂ (7.4, 11.4 and 15.4 MPa) at 31 °C for 10 min during the seven-day storage period are shown in Table 1. Differences ($p < 0.05$) are observed on the zeroth day of storage, where increasing the SC-CO₂ pressure caused the log CFU/g of the treated samples to decrease. In this case, 15.4 MPa resulted in lower bacterial, and yeast and mold count. The log₁₀ CFU/g of total yeast and mold also decreased ($p < 0.05$) compared to the control sample on the seventh day of storage. However, no differences ($p > 0.05$) were detected between the total plate count on the third and seventh day of storage. The muscle from a healthy animal that is turned into food could also be contaminated in the slaughterhouse environment, or as a result of dirty knives, intestinal content, or polluted water [21]. The high microbial count on the zeroth day might be the result of such contaminated conditions.

Choi et al [22] studied the microbial reduction of fresh pork and observed a reduction in microbes under high-pressure SC-CO₂ treatments. Solid foods, including meat and meat products, have a lower microbial reduction compared to liquid foods because of the partial diffusion of CO₂ into solid food [23]. Two stages were detected in the survival curves of microorganisms after the application of SC-CO₂. In the early stage, the microbial reduction was sluggish and then declined rapidly during the later stage [24]. The results of this research showed that the inactivation rate increases with increased pressure. The reasons for the increase in log₁₀ CFU/g on the third and seventh day of storage might be that the lower diffusion of CO₂ into the chicken meat's deep structure and the inactivation of vital enzyme and cellular metabolism resulted in the germination of some remaining spores.

Table 1. Effects of three levels (7.4, 11.4 and 15.4 MPa) of SC-CO₂ at 31 °C for 10 min on the total plate count, total yeast and mold count, and lipid peroxidation of raw chicken meat stored at 4 ± 1 °C for seven days.

Parameters	Treatments	Day 0	Day 3	Day 7
Total Plate Count (log CFU/g)	Control	6.72 ± 0.25 ^{Aa}	7.35 ± 1.17 ^{Aa}	7.32 ± 0.13 ^{Aa}
	7.4 MPa	6.68 ± 0.17 ^{Aa}	6.4 ± 0.47 ^{Aa}	6.66 ± 0.49 ^{Aa}
	11.4 MPa	4.02 ± 0.55 ^{Bb}	5.92 ± 0.47 ^{Aa}	7.00 ± 0.48 ^{Aa}
	15.4 MPa	2.00 ± 0.00 ^{Cc}	5.8 ± 0.40 ^{Ab}	6.98 ± 0.28 ^{Aa}
Total Yeast and Mold (log CFU/g)	Control	5.98 ± 0.53 ^{Ab}	6.06 ± 0.72 ^{Aab}	7.40 ± 0.40 ^{Aa}
	7.4 MPa	5.72 ± 0.63 ^{Aa}	5.56 ± 0.51 ^{Aa}	6.20 ± 0.31 ^{Ba}
	11.4 MPa	3.58 ± 0.09 ^{Bb}	5.69 ± 0.81 ^{Aa}	5.82 ± 0.08 ^{Ba}
	15.4 MPa	2.00 ± 0.00 ^{Cc}	5.19 ± 0.18 ^{Ab}	5.77 ± 0.09 ^{Ba}
Lipid Peroxidation (mg/kg)	Control	0.29 ± 0.05 ^{Aa}	0.20 ± 0.01 ^{Ab}	0.23 ± 0.01 ^{Aab}
	7.4 MPa	0.27 ± 0.03 ^{Aa}	0.23 ± 0.01 ^{Aa}	0.24 ± 0.03 ^{Aa}
	11.4 MPa	0.25 ± 0.01 ^{Aa}	0.25 ± 0.04 ^{Aa}	0.28 ± 0.03 ^{Aa}
	15.4 MPa	0.24 ± 0.01 ^{Aa}	0.25 ± 0.04 ^{Aa}	0.23 ± 0.01 ^{Aa}

Means with different small letters of the same treatment (effect of time) are significantly different ($p < 0.05$). Means with different capital letters of the varied treatment (effect of treatment) are significantly different ($p < 0.05$); CFU: Colony Forming Unit.

3.2. Lipid Peroxidation

The lipid peroxidation results of this research are presented in Table 1. The results revealed that the application of SC-CO₂ did not change ($p > 0.05$) the peroxidation values of the meat samples throughout all seven days of storage. The TBARS values were all below 0.29 mg/kg. The lack of changes in lipid peroxidation could be due to the removal of the visible fat from the chicken breast meat samples, thus limiting the oxidation of the lipid process. A similar situation was seen in a study by Xiong et al. [4] on chicken samples that were cooked up to 60 °C, kept at 4 °C for zero, two, and four days and showed no significant changes in TBARS values, which all were below 0.2 mg/kg. However, Huang et al. [24] reported that the application of SC-CO₂ at 13.8 MPa under 35 °C for 2 h on ground pork meat increased its TBARS value after five days of storage at 4 °C. The lipids are commonly dispersed as triacyl glycerides, phospholipids, and sterols in both the intra- and extra-cellular spaces of meat. Lipids are, however, chemically unstable and, consequently, easily oxidizable, particularly when being stored and handled post-mortem. The rancid smell, off-flavor growth, droplet losses, discoloration, nutrient value loss, and shelf-life decline are causes of lipid oxidation that negatively affect the meat. Several factors, including iron content, distribution of unsaturated fatty acids, pH, and antioxidant levels, influence the rates and degree of lipid oxidation [25].

3.3. Color Properties

Table 2 shows the effects on raw chicken meat color when treated with SC-CO₂ and stored at 4 ± 1 °C for seven days. The results revealed that the lightness (L^* value) increased ($p < 0.05$) compared to the control on the zeroth, third, and seventh day of storage, while comparison between days of storage for each SC-CO₂ treatment showed no changes ($p > 0.05$). Nevertheless, the values can be considered better with 55.84–56.78 for control samples and 58.51–67.09 for treated samples throughout the storage times as compared to other studies using 14 MPa for 45 min at 40 °C, which resulted in higher L^* values such as 80.68 [14] and 77.17 [15]. The redness (a^*) values of treated chicken meats were lower ($p < 0.05$) compared to the control, but no changes ($p > 0.05$) were observed between days of storage. The values can be considered as acceptable with 10.72–12.64 for control samples and 8.21–10.98 for treated samples as compared to the study by González-Alonso et al. [14] with 2.21 and 1.45 for 8 and 14 MPa, respectively. The yellowness (b^*) showed minimal changes and the results agree with the results of González-Alonso et al. [14].

Several works have shown that the treatment of pressure slowly changes the surface color of meats. For example, studies conducted on ground pork meat and the porcine *longissimus dorsi* muscle also confirm the results of this research, claiming that SC-CO₂ significantly changed the meat color [17,24,26]. The discoloration shown in the production at high pressure is a result of globin denaturation and ferrous myoglobin oxidation to ferrous metmyoglobin [11]. In conclusion, the lower temperature and shorter time used in this study helped to maintain the color of the treated chicken meats even at different levels of pressure.

Table 2. Effects of three levels (7.4, 11.4 and 15.4 MPa) of SC-CO₂ at 31 °C for 10 min on *L**, *a** and *b** values of raw chicken meat stored at 4 ± 1 °C for seven days.

Parameters	Treatments	Day 0	Day 3	Day 7
<i>L*</i>	Control	56.78 ± 0.79 ^{Ca}	55.84 ± 0.63 ^{Ba}	56.50 ± 3.50 ^{Ba}
	7.4 MPa	62.02 ± 0.72 ^{Ba}	61.97 ± 1.55 ^{Aa}	58.51 ± 1.32 ^{ABb}
	11.4 MPa	67.09 ± 2.1 ^{Aa}	64.89 ± 1.77 ^{Aa}	63.62 ± 2.03 ^{Aa}
	15.4 MPa	63.41 ± 0.91 ^{Ba}	62.55 ± 0.72 ^{Aa}	61.53 ± 0.74 ^{ABa}
<i>a*</i>	Control	12.23 ± 0.67 ^{Aa}	12.64 ± 0.29 ^{Aa}	10.72 ± 1.31 ^{Aa}
	7.4 MPa	10.00 ± 0.28 ^{Ba}	10.98 ± 0.92 ^{ABa}	9.81 ± 0.34 ^{ABa}
	11.4 MPa	8.28 ± 0.15 ^{Ca}	8.70 ± 1.22 ^{Ca}	8.21 ± 1.10 ^{Ba}
	15.4 MPa	9.68 ± 0.50 ^{Ba}	10.02 ± 0.72 ^{BCa}	9.29 ± 0.78 ^{ABa}
<i>b*</i>	Control	12.52 ± 0.39 ^{Aa}	12.38 ± 0.52 ^{ABa}	9.57 ± 2.05 ^{Aa}
	7.4 MPa	11.59 ± 0.72 ^{Aab}	13.30 ± 0.32 ^{Aa}	11.18 ± 1.12 ^{Ab}
	11.4 MPa	13.18 ± 0.63 ^{Aa}	10.80 ± 0.97 ^{Bb}	11.95 ± 1.10 ^{Aab}
	15.4 MPa	12.87 ± 1.73 ^{Aa}	11.61 ± 1.13 ^{ABa}	12.33 ± 1.61 ^{Aa}

Means with different small letters of the same treatment (effect of time) are significantly different ($p < 0.05$). Means with different capital letters of the varied treatment (effect of treatment) are significantly different ($p < 0.05$).

3.4. The pH, Water Holding Capacity (WHC), and Cooking Loss

The effects of SC-CO₂ on the pH values of raw chicken meat stored at 4 ± 1 °C for seven days are presented in Table 3. The result revealed that low- and medium-pressure (7.4 and 11.4 MPa, respectively) SC-CO₂ affected ($p < 0.05$) the pH of the meat samples on the zeroth day of storage compared to the control and high-pressure (15.4 MPa) SC-CO₂. However, no changes ($p > 0.05$) were recorded between the control and the application of SC-CO₂ on the third and seventh day of storage. A similar study confirmed the current results, that is, no changes in pH were observed after the application of SC-CO₂ on the meat of the porcine *longissimus dorsi* muscle [7,10]. The increase in the pH values from the zeroth day to the third and seventh days may be due to the exposure of the acidic amino acid groups as proteins unfolded during the pressure application. Treatment of high pressure and temperature can affect molecular interactions and protein conformation, resulting in protein denaturation and meat aggregation [11].

The effects of water holding capacity (WHC) of the raw chicken meat in chilled storage after the application of SC-CO₂ are shown in Table 3. The results showed that the treated samples were not affected similarly as the control sample. The denaturation of the muscle protein occurs as a result of the combination of temperature and low pH, which then becomes responsible for a reduction in WHC. For example, the pale breast fillets displayed slightly lower absolute pH and WHC than the control meat, demonstrating a close relationship between both the color of meat and the ultimate pH and WHC [27]. Based on the lower temperature used during the treatments and less affected pH values of the chicken meat, the result of the WHC subsequently was not affected by the SC-CO₂ treatment.

Table 3 shows the cooking loss values after the application of SC-CO₂ on the raw chicken meat stored at 4 ± 1 °C for seven days. No difference ($p > 0.05$) was recorded in all the samples. This result is confirmed by a similar research study conducted on porcine *longissimus dorsi* muscles [10]. Meat lost both volume and weight by fluid removal during the cooking process. This increase in fluid content contributes to changes in meat's textural characteristics which are in addition to the changes

in protein and fat that are heat-induced. Furthermore, fluctuations in the yield of cooked meat are an economic problem for meat processors. Temperature and time are key factors in the heat and mass transfer processes, protein denaturation, and, in some cases, protein solubilization occurring during cooking [28].

Table 3. Effects of three levels (7.4, 11.4 and 15.4 MPa) of SC-CO₂ at 31 °C for 10 min on the pH, water holding capacity, and cooking loss of chicken meat stored at 4 ± 1 °C for seven days.

Parameters	Treatments	Day 0	Day 3	Day 7
pH	Control	6.12 ± 0.06 ^{Aa}	5.96 ± 0.24 ^{Aa}	6.19 ± 0.10 ^{Aa}
	7.4 MPa	5.88 ± 0.06 ^{Bb}	6.26 ± 0.05 ^{Aa}	6.17 ± 0.01 ^{Aa}
	11.4 MPa	5.87 ± 0.01 ^{Bb}	6.13 ± 0.02 ^{Aa}	6.15 ± 0.03 ^{Aa}
	15.4 MPa	6.07 ± 0.02 ^{Aa}	6.14 ± 0.02 ^{Aa}	6.42 ± 0.43 ^{Aa}
Water Holding Capacity (%)	Control	21.55 ± 3.52 ^{Aa}	18.5 ± 2.18 ^{Aa}	26.76 ± 5.84 ^{Aa}
	7.4 MPa	22.19 ± 0.38 ^{Aa}	21.27 ± 0.59 ^{Aa}	23.34 ± 1.50 ^{Aa}
	11.4 MPa	27.29 ± 5.35 ^{Aa}	25.77 ± 5.80 ^{Aa}	29.24 ± 4.84 ^{Aa}
	15.4 MPa	20.98 ± 2.72 ^{Aa}	19.03 ± 4.44 ^{Aa}	24.39 ± 0.28 ^{Aa}
Cooking Loss (%)	Control	52.2 ± 2.18 ^{Aa}	54.23 ± 4.84 ^{Aa}	50.79 ± 0.78 ^{Aa}
	7.4 MPa	50.98 ± 2.40 ^{Aa}	55.11 ± 3.21 ^{Aa}	48.49 ± 3.69 ^{Aa}
	11.4 MPa	52.25 ± 1.43 ^{Aa}	52.61 ± 0.82 ^{Aa}	51.88 ± 2.49 ^{Aa}
	15.4 MPa	48.8 ± 5.10 ^{Aa}	47.20 ± 5.57 ^{Aa}	50.18 ± 4.76 ^{Aa}

Means with different small letters of the same treatment (effect of time) are significantly different ($p < 0.05$). Means with different capital letters of the varied treatment (effect of treatment) are significantly different ($p < 0.05$).

3.5. Texture Properties

Table 4 shows the texture values of the raw chicken meat after the SC-CO₂ treatment. The results show that some important attributes were affected ($p < 0.05$) during the storage period. For example, hardness, cohesiveness, gumminess, chewiness, and resilience are among the factors that were influenced in some days of the storage period. The most affected attribute was hardness, which changed ($p < 0.05$) for all storage times after SC-CO₂ application. Medium-pressure (11.4 MPa) SC-CO₂ hardened the meat samples to 6360 ± 846 g, followed by high-pressure (15.4 MPa) SC-CO₂, which hardened it to 4646 ± 650 g in contrast to low-pressure (7.4 MPa) SC-CO₂, which produced a meat hardness of 1473 ± 281 g. The hardness degree of the control sample (2030 ± 629 g) was not significantly different compared to the low-pressure SC-CO₂. The medium-pressure (11.4 MPa) SC-CO₂ hardened the meat on the third day of evaluation, but the hardness of the meat was increased even more with high SC-CO₂ pressure (15.4 MPa) on the seventh day of storage. Regardless of the hardened texture, these results can be considered acceptable compared to the study by González-Alonso et al. [14] with 57.2 N (5832 g) and 82.8 N (8443 g) for treatment at 8 and 14 MPa for 45 min at 40 °C, respectively. Another finding that proves that using a higher temperature and longer treatment time of SC-CO₂ could negatively affect the texture was reported by Jauhar et al. [15], where the hardness values of the treated chicken meat were recorded at 7248, 8822, and 8808 g after storage at zero, three and seven days, respectively.

There was high cohesiveness of the control meat samples at the zeroth and seventh day of storage followed by the low-pressure SC-CO₂ on the zeroth day and high-pressure SC-CO₂ on the seventh day of storage, but cohesiveness did not change ($p > 0.05$) on the third day of storage. The 15.4 MPa SC-CO₂ pressure showed high gumminess on the zeroth and seventh day of treatment, whereas the control sample revealed the lowest degree of gumminess during storage time. The meat treated with medium- and high-pressure SC-CO₂ showed a notably ($p < 0.05$) high degree of chewiness at the zeroth day, but this did not significantly change on the seventh day of treatment. The low-pressure SC-CO₂ indicated a high degree of resilience in comparing the control sample with the other pressure-treated samples on the zeroth and seventh day of storage. The remaining affected factors were adhesiveness and springiness, both of which were not influenced ($p > 0.05$) over all the days of texture evaluation; one of the reasons for this finding could be that the meat hardness after SC-CO₂ treatment had

experienced water loss, i.e., about $20\% \pm 5\%$ of water was lost after the application of SC-CO₂ on the meat samples. A similar study confirmed that marinated pork also hardened after the application of SC-CO₂ at 7.4, 12.2 and 15.4 MPa at 31.1 °C for 10 min [11].

Table 4. The effects of three levels (7.4, 11.4 and 15.4 MPa) of SC-CO₂ at 31 °C for 10 min on the texture of raw chicken meat stored at 4 ± 1 °C for seven days.

Treatment	Day	Hardness (g)	Adhesiveness (g s)	Springiness (mm)	Cohesiveness	Gumminess (g)	Chewiness (g mm)	Resilience
Control	0	2030 ± 629 ^{Ca}	-136 ± 83 ^{Aa}	0.70 ± 0.08 ^{Aa}	0.79 ± 0.05 ^{Aa}	1578 ± 398 ^{Ba}	1190 ± 410 ^{BCa}	0.55 ± 0.06 ^{Aa}
	3	1186 ± 92 ^{Ba}	-187 ± 1.8 ^{Aa}	0.73 ± 0.08 ^{Aa}	0.80 ± 0.05 ^{Aa}	957 ± 93 ^{Aa}	710 ± 127 ^{Aa}	0.55 ± 0.09 ^{Aa}
	7	1350 ± 381 ^{Ba}	-127 ± 54 ^{Aa}	0.76 ± 0.08 ^{Aa}	0.76 ± 0.06 ^{Aa}	1025 ± 263 ^{Ba}	780 ± 221 ^{Aa}	0.53 ± 0.09 ^{Aa}
7.4 MPa	0	1473 ± 281 ^{Ca}	-93 ± 8 ^{Aa}	0.8 ± 0.04 ^{Aa}	0.80 ± 0.04 ^{Aba}	1125 ± 172 ^{Ba}	870 ± 116 ^{Ca}	0.50 ± 0.05 ^{Aba}
	3	2788 ± 1754 ^{Aba}	-150 ± 32 ^{Aab}	0.72 ± 0.05 ^{Aa}	0.74 ± 0.05 ^{Aa}	2061 ± 1296 ^{Aa}	1460 ± 813 ^{Aa}	0.48 ± 0.02 ^{Aa}
	7	3030 ± 1388 ^{Ba}	-161 ± 32 ^{Ab}	0.78 ± 0.08 ^{Aa}	0.78 ± 0.01 ^{Aa}	2400 ± 1103 ^{ABa}	1902 ± 1011 ^{Aa}	0.53 ± 0.03 ^{Aa}
11.4 MPa	0	6360 ± 846 ^{Aa}	-19 ± 5 ^{Aa}	0.63 ± 0.04 ^{Ab}	0.60 ± 0.04 ^{Ca}	3663 ± 741 ^{Aa}	2356 ± 603 ^{Aa}	0.33 ± 0.04 ^{Ca}
	3	4989 ± 658 ^{Aa}	-163 ± 49 ^{Ab}	0.63 ± 0.01 ^{Ab}	0.55 ± 0.19 ^{Aa}	2841 ± 1289 ^{Aa}	1825 ± 849 ^{Aa}	0.35 ± 0.15 ^{Aa}
	7	5250 ± 1374 ^{ABa}	-156 ± 53 ^{Ab}	0.78 ± 0.08 ^{Aa}	0.61 ± 0.04 ^{Ba}	3228 ± 811 ^{ABa}	2511 ± 634 ^{Aa}	0.39 ± 0.02 ^{Ca}
15.4 MPa	0	4646 ± 650 ^{Ba}	-98 ± 69 ^{Aa}	0.70 ± 0.03 ^{Aa}	0.65 ± 0.05 ^{BCa}	2996 ± 194 ^{Aa}	2118 ± 211 ^{Aba}	0.37 ± 0.06 ^{BCa}
	3	4761 ± 1345 ^{Aa}	-196 ± 52 ^{Aa}	0.65 ± 0.01 ^{Aa}	0.61 ± 0.04 ^{Aa}	2886 ± 668 ^{Aa}	1810 ± 433 ^{Aa}	0.36 ± 0.05 ^{Aa}
	7	6357 ± 2446 ^{Aa}	-214 ± 83 ^{Aa}	0.63 ± 0.10 ^{Aa}	0.65 ± 0.03 ^{Ba}	4093 ± 1426 ^{Aa}	2679 ± 1183 ^{Aa}	0.40 ± 0.01 ^{BCa}

Means of triplicate ± standard deviation (SD). Means with different capital letters of the varied treatment are significantly different ($p < 0.05$). Means with different small letters of the same treatment are significantly different ($p < 0.05$).

3.6. Microstructure of Chicken Meat

The microstructure of the raw chicken meat treated with three levels of SC-CO₂ (7.4, 11.4 and 15.4 MPa) at the zeroth day of storage is presented in Figure 1. The untreated chicken meat showed a smooth surface compared to the treated samples. At 7.4 MPa, the chicken meat surface started to show a dense surface. At 11.4 MPa, the chicken was more affected with a denser surface; ultimately at 15.4 MPa, the chicken meat had the most compact and dense surface compared to other treatments. These changes are indicators of the increasing pressures applied to the chicken meat affecting the protein of the muscle [10].

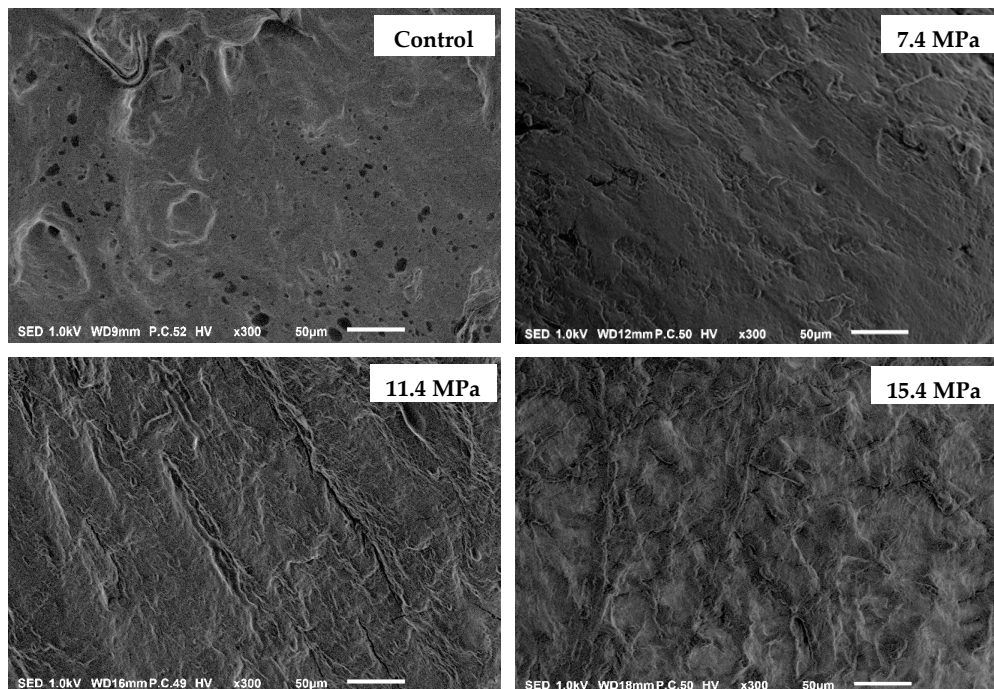


Figure 1. The microstructure (300× magnification) of raw chicken meat (zeroth day storage) treated with SC-CO₂ at three levels (7.4, 11.4 and 15.4 MPa) at 31 °C for 10 min.

3.7. Sensory Properties

Figure 2 shows the effects of SC-CO₂ (7.4, 11.4 and 15.4 MPa at 31 °C for 10 min) on the sensory analysis of raw chicken meat stored at 4 ± 1 °C for three days and cooked for 40 min at 120 °C in the oven. The data showed that all samples did not differ ($p > 0.05$) in terms of color, aroma, flavor, tenderness, juiciness, and springiness. The results proved that different levels of pressure with a lower temperature and shorter SC-CO₂ treatments did not affect the acceptance of the chicken meat. A similar study on the effects of 15.2 MPa SC-CO₂ treatment on some bacteria in marinades and marinated pork also found no significant differences between the treatments [7].

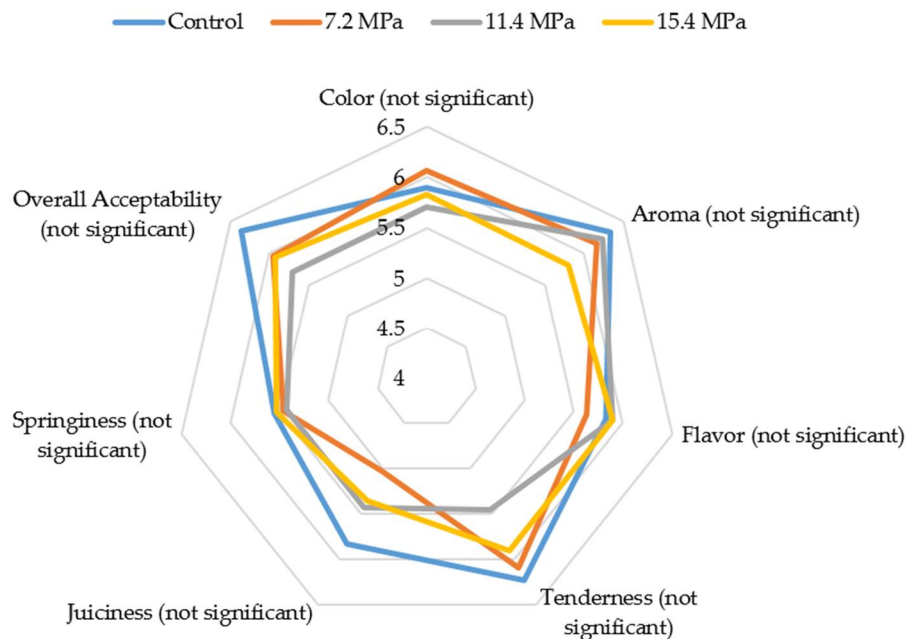


Figure 2. The effects of three levels (7.4, 11.4 and 15.4 MPa) of SC-CO₂ at 31 °C for 10 min on sensory analysis of cooked chicken meat stored at 4 ± 1 °C for three days.

4. Conclusions

Alternative treatments of SC-CO₂ at a lower temperature (31 °C) and shorter time (10 min) with various pressures (7.4, 11.4 and 15.4 MPa) were successfully conducted on raw chicken meat. The microbial load was reduced, and the quality of the chicken meat was simultaneously preserved. The microbial count of chicken meat chill-stored up to seven days increased for the treated chicken meat, but not much of a difference was seen against the untreated meat at the zeroth day of storage. The color and texture of the treated chicken meats were in the acceptable ranges when compared to the untreated chicken meat due to the lower temperature and shorter time used for the SC-CO₂ treatments in this study. The results of the texture and color analyses were also better compared to other works conducted previously using a higher temperature and longer SC-CO₂ treatment. The microstructure became denser as the pressure levels increased, but this was not obvious as the panelists rated the treated chicken meat similar to the untreated chicken meat during the sensory evaluation. In conclusion, the SC-CO₂ treatment can be applied in the chicken meat industry as non-thermal processing with a lower temperature and shorter time at various levels of pressure.

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