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Research Article

Quality and Shelf Life of White Shrimp (*Litopenaeus vannamei*) Processed with High-Pressure Carbon Dioxide (HPCD) at Subcritical and Supercritical States

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Phase changes of carbon dioxide (supercritical or subcritical) depend on its proximity to a pressure of 7.35 MPa and temperature of 31.1°C. Carbon dioxide becomes supercritical and subcritical when it is above and slightly below its critical point, respectively. This study aims to determine the effect of high-pressure CO_2 treatments at a pressure of 900 psi, 950 psi (subcritical), and 1100 psi (supercritical) and at holding times of 5, 10, and 15 min on the quality parameters of white shrimp (*Litopenaeus vannamei*) and to determine the shelf life of white shrimp processed with the best treatment. The results showed that the interaction between pressure and holding time had a significant ($p < 0.05$) effect on cholesterol, protein, moisture content, and $b*$ value, but pressure had a significant effect on carotene content. The best treatment was a supercritical CO_2 treatment at 1100 psi for 10 min, which was determined based on a significant reduction in the number of microorganisms and no significant changes in color, texture, and fat content were observed compared with control. The best treatment was applied to process shrimps, which were then stored at 4°C to evaluate the effectiveness of $scCO_2$ treatment on the shelf life. No significant changes were found in PV and lipid in treated and $scCO_2$-treated shrimps during storage, but the treatment significantly affected pH, TVBN, and microbial counts. Among the samples, there was no hedonic difference in all sensory attributes. Supercritical CO_2 treatment at 1100 psi for 10 min can be an alternative method for preservation of shrimps.

1. Introduction

White shrimp (*Litopenaeus vannamei*) has highly distinctive nutritional value and powerful antioxidants but is of fast deterioration, which results in an unpleasant odor, color, soft texture, and loss of acceptability. Several research works have been carried out to prevent shrimp deterioration during handling, including the application of conventional and nonthermal high-pressure carbon dioxide (HPCD) processing methods. However, there were some drawbacks to applying the first techniques, such as the occurrence of temperature abuse, loss of flavor and acceptability of drying shrimp, destruction of nutritional value, and loss of vitamins and bioactive components during heat treatment [1, 2]. A new method of nonthermal HPCD technology has also been investigated as alternatives. This technique may overcome some weaknesses of conventional methods of shrimp handling techniques because it maintains the nutritional and sensorial quality of the foods while keeping freshness and flavor. High-pressure carbon dioxide is a nonthermal processing or a cold pasteurization method, where CO_2 is above its critical point (supercritical region) and becomes supercritical fluid. CO_2 in the form of supercritical fluid has a density like liquid, so it has a high solubility and diffusion due to the loss of boundary between the liquid and gas phases. The CO_2 fluid density is highly compressible, in

which a slight increase in pressure can lead to a large increase in fluid, making it possible to produce changes in the macromolecule and micromolecules of foods. Moreover, supercritical CO_2 fluid tends to be nonpolar organic compounds, owing to its low polarity and lack of capacity for specific molecule interactions [3, 4]. Several studies on the application of high-pressure CO_2 for shrimp processing have been carried out, mainly with changing of temperature and CO_2 pressure. HPCD at 310 bar (31 MPa), 1875 L of CO_2, and 37°C was to produce low-cholesterol shrimps [5], and that at 20 MPa was for the prevention of shrimp melanosis [6]. HPCD in the range of 20–25 MPa and 37°C was performed for kinetic inactivation of PPO [7], while extraction of shrimp-waste astaxanthin was performed at 200 MPa for 5 min with the use of ethanol cosolvent [8].

From those findings, the CO_2 pressure used to process the shrimp was quite high (14–31 MPa) and 37°C. Additionally, use of a very high CO_2 pressure at 800 MPa resulted in toughening of shrimp texture and loss of flavor [9]. In fact, the critical point of CO_2 is at a pressure of 7.38 MPa and a temperature of 31.1°C [10], meaning that, at and above its critical point, CO_2 exists as a gas and liquid in equilibrium, where an increase in temperature or pressure above this critical point does not result in further phase changes [11]. The CO_2 fluid density is highly compressible; therefore, a slight change in temperature and pressure values can cause changes in the macromolecular and micromolecular structure of the shrimp, which, in turn, affect the quality of the shrimps. Additionally, the use of a very high CO_2 pressure level may also lead to higher costs. Taking into account the above findings, our study used CO_2 pressure treatments at supercritical CO_2 (1100 psi/7.58 MPa) and subcritical CO_2 (900 psi/6.21 MPa and 950 psi/6.55 MPa). This study aimed to determine the effect of subcritical (900 and 950 psi) and supercritical (1100 psi) CO_2 treatment at holding times of 5, 10, and 15 min on changes in shrimps' quality. The evaluated quality parameters included basic nutrients, color, microbiological, total carotene, cholesterol, texture, and muscle microstructure. The best treated shrimp was determined from the quality parameters that were not significantly different or the least changed compared with untreated shrimp. Then, it was stored at 4°C for 10 days to determine whether HPCD processing could prolong its shelf life. Spoilage indications, including the changes of lipid, PV, TVBN, pH, and microbial levels, were monitored during storage.

2. Materials and Methods

2.1. Time and Place of Sampling. Freshly caught and additive-free white shrimp (*Litopenaeus vannamei*) (25–30 shrimps/kg) were purchased from a local farm in Palembang, Indonesia. The shrimps were kept in a sterile box ice (shrimps: ice = 1 : 2) and transported to the laboratory within 45 min. Upon arrival, the shrimps were rinsed with tap water and stored in a low temperature (2°C). Then, the fresh shrimps were run for high-pressure CO_2 processing in the Laboratory of Processing, University of Sriwijaya, Palembang, Indonesia, where the unit of high-pressure CO_2 processing instrument was located. Thereafter, processed shrimps were prepared for analytical tests or quickly packed in plastic container and kept in freezer −20°C until they were needed. Following that, processed and unprocessed shrimps were analyzed for color, texture, and basic nutrients. For other analyses, such as cholesterol, carotene total, and microstructure (SEM), frozen samples were taken to the University of Lampung, Bandar Lampung, Indonesia, within 4 hours. It took 2 months to get all the data analyzed and find the best sample treatment. Thereafter, a storage study was performed in the Laboratory of Microbiology, University of Lampung, after previously processing HPCD samples in the processing laboratory. The whole study was conducted for 4 months from July to October 2020.

2.2. High-Pressure Carbon Dioxide (HPCD) Processing. The high-pressure CO_2 installation used for experimental treatment consists of a CO_2 gas cylinder, cylindrical pressure chamber, pressure gauges, and water bath at a constant temperature [12] (see Figure 1). Fresh shrimps were placed in a pressure chamber, which has been previously disinfected with 70% alcohol. Then, the chamber was tightly closed. When the designated temperature in the water bath was reached and all pipe connections were secured, commercially available CO_2 (Pertamina, Jakarta, Indonesia) was injected through the gas inlet valve from the gas cylinder into the pressure chamber until it reached the desired pressures of 900, 950, and 1100 psi (shown in pressure gauge) within 1 min. After being subjected to high-pressure CO_2 treatment for a defined pressurized time (5, 10, and 15 min), the pressure was lowered to atmospheric pressure within 3 minutes by slowly opening the gas outlet valve. Then, the shrimps were aseptically removed from the pressure chamber using a sterilized tong, placed in the sterilized container, and stored in a freezing temperature (−20°C) before conducting an analysis such as SEM, but the samples were directly analyzed for microorganisms. The experiment was conducted in a full factorial design with the following factors: supercritical CO_2 ($scCO_2$) treatment at 1100 psi for 5, 10, and 15 min and subcritical CO_2 (sub-CO_2) treatment at 950 psi and 900 psi. The holding time for each of the pressure treatments was 5, 10, 15, and 20 min. Each treatment was replicated three times.

2.3. Scanning Electron Microscope (SEM). Analysis of shrimp microstructure was conducted by scanning electron microscope (SEM, JSM-6310LV, JEOL Ltd.) following the procedures of Plascencia Jatomea and Viniegra et al. [13], adjusted to the shrimps' sample. Cross- and longitudinal sections of shrimp muscle (0.5×0.5 cm) were chemically prefixed with 2.5% (v/v) glutaraldehyde in 0.1 mol/L at pH 7.0 PBS (phosphate-buffered saline), overnight at 4°C. Then, postfix the sample with 1.0% (w/v) osmium tetra oxide at room temperature for 1 hour. The specimens were washed in 50 mM of PBS, pH 7.0 three times and then dehydrated for 15 min in a series of ethanol solutions graded at 30%, 50%, 60%, 70%, 80%, 90%, 95%, and 100%, respectively. Following drying, the specimen then was coated in a gold coater by

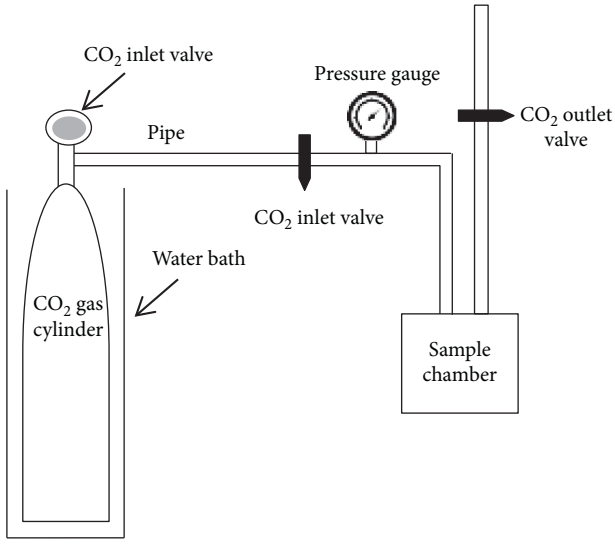


Figure 1: Schematic diagram of HPCD treatment equipment.

using IB3 ion coater tool for 5 minutes with an ions' current of 7-8 milliampere. Finally, the specimen was observed with ACC 20 kV-voltage devices at 100x, 250x, and 500x magnification.

2.4. Microbial Analysis. Microbial analysis in treated shrimp was expressed in terms of the relative survivor fraction, log (N/No), where N is the number of colony-forming units per gram of sample (CFU/g) after treatment and No is the number of colony-forming units per gram (CFU/g) of sample before treatment. Each experiment was performed in duplicate, and the average values were reported. Total aerobic count was performed using the spread plate agar technique. First, the shrimp samples (10.0 ± 0.2 g) were collected aseptically and placed in the sterile stomacher bag homogenized with 90 mL of 0.85% sterile saline solution for 2 min. Next, serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were prepared with 0.85% sterile saline solution. Subsequently, 0.1 mL of each dilution was spread on to plate count agar (Difco, Detroit, MI, USA) for total aerobes.

2.5. Color and Texture Analysis. The colors of untreated shrimps and treated ones with high-pressure CO_2 were measured using a colorimeter (Konica Minolta C-400, Tokyo, Japan) using CIELAB system. In this color system, the L^* (lightness) variable represents the brightness, in which $L^* = 0$ for dark and $L^* = 100$ for white. The a^* (redness) scale ranges from negative values ($-a^*$) for green to positive values ($+a^*$) for red, and b^* (yellowness) scale ranges from negative values ($-b^*$) for blue to positive values ($+b^*$) for yellow. The chroma (C) value reflects the color intensity, where the brighter the color, the lower the C value. The notation of hue ($^\circ H$) indicates the dominant color, where negative $^\circ H$ has a range color of yellow-green; positive $^\circ H$ values have a range color of yellow-red. The colorimeter employed was firstly calibrated with a black standard followed by a white standard to obtain the final setting. The shrimp meat was placed in an optically clear Petridis glass. For each sample, three measurements were performed at the ventral body (second segment) of shrimp muscle, and the average values of the three samples were recorded.

The texture in terms of hardness was measured by using a LFRA Texture Analyzer (Brookfield AMETEK CT3-4500-115CT3). Immediately after processing with HPCD, the sample was placed on a platform and a needle probe (50 mm diameter) attached to a 25 kg load cell. The texture analyzer was set to a test speed of 5.0 mm/s, posttest speed of 10 mm/s, target distance of 30.0 mm, and acquisition rate of 200 pps. A measurement was in gram force (m^2/probe diameter), where a high value of measurement indicates an increase in shrimp hardness. For each sample, three measurements were performed, and the average value was recorded.

2.6. Cholesterol Analysis. The extraction of cholesterol was following the methodology essayed by Saldanha et al. [14] and derivatization with TMS (trimethylsilyl) ethers. The TMS derivatives were diluted in 1 mL hexane, filtered through a 22 μM filter (Millipore, Maryland, MD, USA) and injected into a gas chromatograph (Shimadzu GC 2010, Tokyo, Japan) equipped with a split injector (1 : 50) and a flame ionization detector and capillary column Rtx-5-MS (30 m × 0.25 mm x 0.25 μm Restek, Bellefonte, USA). The operation conditions were as follows: an initial temperature of 230°C (0 min); heating rate of 2°C/min up to 264°C (5 min); and then a heating rate of 1°C/min up to a final temperature of 275°C (2 min). The injector and detector temperatures were 350°C and 290°C, respectively. The carrier gas used was hydrogen at a flow rate of 1 mL/min. Cholesterol identification was performed by comparing sample retention times with standard retention times (Sigma, Milford, MA, USA). The quantification was performed by an external calibration curve with 10 points, at concentrations ranging from 10 to 1000 μg/mL.

2.7. Total Carotenoid Analysis. The concentration of total carotenoids was measured using the spectrophotometric modified method from treated and untreated shrimp [15]. To prepare the standard graph, different volumes 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml stock solutions of astaxanthin (1%) were made, and the solutions were diluted up to 10 ml with n-hexane. The absorbance was measured at 472 nm using n-hexane as the blank. The experiment was performed in triplicate; the standard graph for astaxanthin was constructed.

2.8. Basic Nutrient Analysis. Water content, crude protein, fat, and ash were measured following the standard methods [16]. Weight differences were recorded after drying the samples in a hot air oven at 100 ± 5°C overnight to determine the water content. Protein content was measured by using the micro-Kjeldahl method. Crude fat was measured by Soxhlet extraction with diethyl ether. Ashing was done by incineration in a muffle furnace at 550 ± 50°C until white ash was obtained.

2.9. Storage Study

2.9.1. Determination of pH, TVB-N, and PV during Storage. Determination of pH was performed using a pH meter (Seven Go Duo™, Mettler Toledo Inc., Columbus, OH, USA).The pH of all shrimp samples was determined at ambient temperature. Approximately 20 g of shrimp was homogenized with 80mL distilled water using Warring® Blender (USA). Prior to use, calibration of the Seven Go Duo pH Meter probes was done using standard buffers of pH 4.0.

The determination of TVB was performed following the National Standardization Body [17]. Approximately 5 g of shrimp flesh was homogenized with 15 mL of 4% trichloroacetic acid (TCA) (w/v) and centrifuged at 3000 g for 3 min and then filtered through filter paper. An aliquot of 5 mL was removed and mixed with 5 mL of 2M NaOH. The mixture was poured into a semi-microdistillation tube and steam distillation was performed. The distillate was collected in a beaker containing 15 mL of 0.01 HCl containing 0.1 mL rosolic indicators. Titration was performed using 0.01 M NaOH to a pale end point.

Determination of peroxide value (PV) was done following the procedure IFRA [18]. Approximately 5 g of shrimp flesh was homogenized with 30 ml of solution of glacial acetic acid: chloroform (3 : 2) in 250 mL Erlenmeyer flask for 30 sec. To the homogenate, 1 mL of saturated potassium iodide (KI) solution was added, and it was allowed to react for 1 min by agitating. Then, 30 mL aquades was added. An equivalent (50 mL) amount of distilled water was added to the mixture to release the iodine and solution titrated with 0.01 M sodium thiosulphate solution against blank. PV was calculated and expressed as mEq active O_2/kg lipids.

2.9.2. Sensory Analysis of Shrimps during Storage. Sensory analysis was performed to determine the effect of high-pressure CO_2 (HPCD) processing on the preferences and organoleptic properties of shrimps during storage. Hedonic test was used to determine the preference level of untreated and treated shrimps based on hedonic scale. Duo-Trio test was used to determine whether a perceptible sensory difference exists between samples of two products, untreated and treated shrimp with HPCD. All samples including untreated and treated shrimps with HPCD were assessed. For the Hedonic test, all samples were evaluated by 10 untrained panelists using a five-point hedonic scale, ranging from 5 = like extremely to 1 = dislike extremely [19]. They were asked to evaluate overall likeness, texture, and aroma. For the Duo-Trio test, a set of three samples of shrimps was served to each of the 20 panels, of which one sample was labeled as a reference and the other two samples had different codes. The panels were informed that one of the coded samples was the same as the reference and one was different, and then, they are asked to report which of the coded samples they believe to be different most from the reference (control) and write down the differences in terms of each of the three parameters: color, texture, and odor. The number of correct responses is counted, and the significance is determined by reference to a statistical table [20]. The study was run twice, and the average value was taken. This study does not require ethical approval.

2.10. Statistical Analysis. Statistical analyses were performed using SPSS package (SPSS 23.0 for windows, SPSS Inc., Chicago, IL, USA). The data of the parameters, including basic nutrient, carotenoids, cholesterol, color values, texture, and microbial counts, obtained from the treatments were subjected to an analysis of variance (one-way ANOVA) to determine the significance between samples. Data obtained from the storage study, including parameters such as pH, TVBN, microbial counts, lipid, and PV, were also run for one-way ANOVA. A Duncan's test was used to compare the means of parameter analysis in the experiment. The means with different letters were significantly different at $p \leq 0.05$. Data represent the means ± SD of measurement for three replicates.

3. Results and Discussion

The ANOVA and *t*-test (Table 1) indicated that high-pressure CO_2 (subcritical and supercritical) significantly affected carotene, microbial reduction, moisture, protein, and chrome color ($p \leq 0.05$). However, holding time did not significantly affect the hardness, fat, L^*, a^*, and hue color of treated shrimp ($p \geq 0.05$). The interaction of the pressure and holding time significantly affected the cholesterol, moisture, ash, protein, b^*, and chrome color of treated shrimp ($p \leq 0.05$).

3.1. Effect of High-Pressure Treatment on Color, Texture, and Microstructure. High-pressure CO_2 treatments did not significantly ($p \geq 0.05$) change the color and texture of the shrimps. Table 2 shows the effect of the treatments on the color changes. Raw shrimps appeared slightly opaque and translucent with low color intensity (C) and very low dominant color ($^\circ H$) of yellow-red. After treatment at 900 psi, the shrimps turned slightly pink, low color intensity (C), and low dominant of yellow-red. When pressure treatment increased, the samples were dominant in slight pink color, with higher intensity in pale white, which was more pronounced at 1100 psi. This was related to the decrease in carotenoid (Table 3), meaning that astaxanthin was released. This result can be explained that the more release of astaxanthin in shrimp matrix, the more intense the color change. Youn et al. [21] and Sánchez-Camargo et al. [22] reported that the higher supercritical CO_2 pressure resulted in a significant increase of astaxanthin solubility and extract yield. CO_2 was a good solvent for astaxanthin due to its similar polarity, and therefore, the higher the pressure, the more intense the extraction, releasing free astaxanthin. In our study, the slight discoloration of shrimp may be due to the weakening of physical interactions in the carotenoprotein molecules in the shrimp meat. Diffused CO_2 reduces water density and creates hydrophobic microenvironments. It can break the interaction between protein and astaxanthin by dissolving isoprene units linked by

Table 1: Statistical analysis of the effects of HPCD parameters on the qualities of treated shrimps.

Parameters	p values		
	Pressure (P)	Time (T)	$P \times T$
Carotene	0.000	—	—
Microbial reduction	0.000	0.000	0.200
Cholesterol	0.063	0.024	0.000
Hardness	0.350	0.985	0.989
Basic nutrient			
Moisture	0.019	0.005	0.011
Ash	0.853	0.008	0.064
Fat	0.053	0.854	0.809
Protein	0.047	0.000	0.000
Carbohydrate	0.006	0.003	0.493
Color			
L	0.143	0.315	0.855
a^*	0.178	0.280	0.575
b^*	0.622	0.002	0.004
C	0.016	0.003	0,001
H	0.974	0.065	0.132

Significantly different at $p \leq 0.05$.

conjugated double bonds and produce a slight pink color ($a^* = 5.85$, $?L^* = 61.35$). In shrimp and salmon, astaxanthin is conjugated with protein to form carotenoprotein molecules. Astaxanthin does not bind to protein but only physically interacts, where there is an intermolecular force between astaxanthin and protein molecules [23]. Hydroxyl and ketone groups in the end ring structure, which are bound to the hydrophilic part of the protein membrane, are polar molecules, while the isoprene unit is attached to the hydrophobic part of the protein membrane and is nonpolar. Colors produced by carotenoid pigments ranges from blue to red due to the presence of double bonds structure [24].

This color changes could relate to the change of texture and microstructure found in this study. In the microstructure view (Figure 2), we found that the supercritical CO_2 caused the muscle fiber structure to swell due to protein denaturation. This was associated with a decrease in protein content (Table 4). Monhemi and Dolatabadi [4] reported the complete denaturation occurred between 800 and 1400 MPA. Proteins are completely denatured when their secondary structure consisting of amino acids linked by hydrogen bonds is broken. Denaturation affects the shrinkage of the sarcomere and the breaking of muscle fibers. As CO_2 diffused in the aqueous meat matrix, it reduced water density protein, and muscle fiber structure became dense forming a larger sarcomeric space. Yan et al. [25] found that denaturation of sarcoplasmic protein produced paler color of meat treated with high-pressure CO_2.

The changes of shrimp's texture and microstructure of the shrimps are related to protein content and water-holding capacity. Figure 3 displays the histogram of the hardness of treated and untreated shrimp at various pressures and holding time. When the protein denatured and collagen shrank, shrimp muscles lost their water-holding capacity, leading to changes of hardness. In our study, there was the possibility of partial denaturation of shrimp protein in our experiment. At a pressure of 900, 950, and 1100 psi, high-compressibility CO_2 interacts with the hydrophobic bonds in the protein, which causes unfolding of its tertiary structure. The unfolded protein structure increases their surface hydrophobicity allowing new intra- and interprotein interaction, thereby resulting in a dense protein structure. During the release of CO_2 pressure, the unfolded protein structure may return to its native structure and allow the rehydration of protein, resulting in the less hard texture. This may explain the slight change in the texture of the shrimps.

3.2. Effect of High-Pressure Treatment on Basic Nutrients. A whole shrimp consists of head, shell, meat muscle, and tail. The main part is the meat, accounting for 48% of the shrimp [26]. The chemical analysis of fresh and treated shrimp is presented in Table 4. It showed that the range of values for protein, fat, and ash content in all of the processed shrimps was 16.04–18.89%, 0.23–0.53%, and 0.7–1.54% (w/w, wet-weight basis), respectively. The results were similar to those reported by Venugopal [27], who found the protein and fat levels of *L. vannamei* were ranging from 17.0 to 23.0 and 0.37 to 0.88, respectively, with myosin and actin being the predominant proteins. Tabitha and Anand [26] showed that protein, carbohydrate, lipid, moisture, and ash content of *L. vannamei* from India were 35.69, 3.20, 19, 76.2, and 1.2% (dry basis), respectively.

As shown in Table 4, high-pressure CO_2 (HPCD) treatment significantly affected the protein content of shrimp ($p \leq 0.05$) with the fluctuating value. The protein of untreated shrimp was 18.09%, and the treated shrimp at 1100 psi for 15 min was 18.21%. The lowest content, 16.04%, was shrimp treated at 900 psi for 5 min. In this study, the change in protein content can be due to molecular polarity and interactions of supercritical CO_2 with amino acid side chains. Shrimp proteins have an appreciable amount of the essential amino acids, that is, leucine, isoleucine, lysine, valine, threonine, methionine, phenyl alanine, and cysteine, and good content of glycine, glutamic acid, alanine, and proline [27]. Leucine, isoleucine, valine, methionine, phenyl alanine, alanine, and cysteine are hydrophobic residues of nonpolar molecules, while threonine, lysine, glycine, and glutamic acid are hydrophilic residues of polar molecules. During the high-pressure CO_2 treatment, CO_2 molecules diffused to the protein matrix, made a hydrophobic microenvironment, and lead to adsorption of nonpolar hydrophobic amino acids. This causes reduction in protein content. Polar hydrophilic amino acid residues escape from the hydrophobic microenvironment and form nonnative hydrogen bonds with other hydrophilic residues. This nonnative interactions lead to the protein denaturant in the high-pressure CO_2-treated protein. This may explain the fluctuated value of protein in the study. CO_2 is regarded as hydrophobic fluid at supercritical CO_2 and is a proper solvent for most nonpolar and slightly polar molecules [4, 28]. Meanwhile, subcritical CO_2 (900 psi) tends to attract slight polar molecules [3].

There was no significant difference ($p \geq 0.05$) in fat content in all samples, meaning that there was no correlation

Table 2: Effects of various pressures and holding times on color attributes of white shrimps (*Litopenaeus vannamei*).

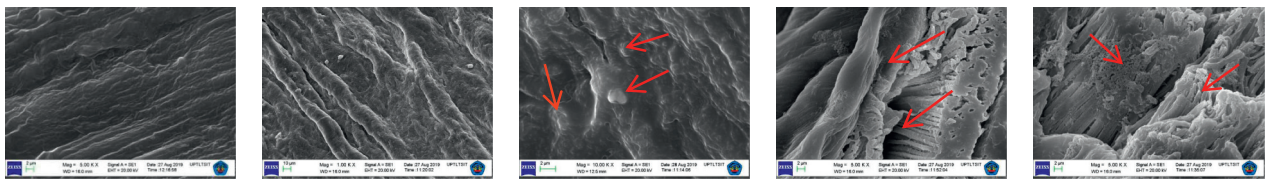
Pressure (psi)/holding time (min)	Color				
	ΔL^*	a^*	b^*	Chrome	Hue
Untreated	51.55 ± 0.77a	4.15 ± 0.07a	4.65 ± 0.49c	4.0 ± 0.92a	20.2 ± 2.05a
900/5	51.65 ± 0.77a	5.55 ± 1.62bc	7.6 ± 1.55e	7.5 ± 0.85c	45.7 ± 0.78cd
900/10	54.15 ± 1.10ab	5.15 ± 0.49ab	4.5 ± 0.71c	9.0 ± 1.48e	45.6 ± 1.2cd
900/15	59.1 ± 2.96cd	5.25 ± 2.05b	8.0 ± 2.12ef	8.8 ± 1.48de	45.8 ± 11.8cd
950/5	53.75 ± 0.71ab	5.0 ± 0.71ab	9.55 ± 0.21g	8.9 ± 0.07de	45.2 ± 8.91cd
950/10	52.05 ± 1.34a	4.05 ± 0.07a	2.05 ± 0.35a	4.7 ± 0.28ab	36.4 ± 4.31b
950/15	54.9 ± 6.64ab	5.6 ± 0.14bc	8.75 ± 0.21f	9.1 ± 0.35e	56.8 ± 0.49d
1100/5	59.75 ± 4.45c	5.25 ± 0.91b	3.85 ± 1.34b	6.5 ± 0.28b	43.2 ± 2.19c
1100/10	56.75 ± 3.88bc	5.85 ± 1.20c	6.65 ± 1.76d	6.6 ± 0.35b	45.1 ± 3.61cd
1100/15	61.35 ± 0.21c	6.1 ± 0.71c	7.75 ± 2.19ef	8.1 ± 0.64d	48.1 ± 2.47cd

Different letters in the same column indicate significance differences ($p \leq 0.05$) between the treatments. Results represent an average of 3 replications and standard deviation.

Table 3: Effects of various pressures and holding times on cholesterol, total carotene content, and microbial reduction in white shrimps (*Litopenaeus vannamei*).

Pressure (psi)/holding time (min)	Cholesterol (mg/g)	Total carotene (mg/g)	Microbial reduction (log N/No)
Control	1.63 ± 0.34f	4.24 ± 0.33a	
900/5	1.39 ± 0.28cd	3.80 ± 1.09b	0.983 ± 0.00a
900/10	1.27 ± 0.32a	3.78 ± 0.09bc	0.979 ± 0.00ab
900/15	1.46 ± 0.29de	3.79 ± 0.64bc	0.974 ± 0.00bc
950/5	1.47 ± 0.32de	3.77 ± 2.02c	0.974 ± 0.00bc
950/10	1.39 ± 0.34de	3.76 ± 0.37c	0.969 ± 0.00c
950/15	1.36 ± 0.34bc	3.69 ± 0.55c	0.952 ± 0.00d
1100/5	1.46 ± 0.33de	2.51 ± 0.29d	0.803 ± .00e
1100/10	1.48 ± 0.31e	2.37 ± 0.06d	0.805 ± 0.00e
1100/15	1.34 ± 0.29ab	2.29 ± 0.12d	0.793 ± 0.00f

Means with the same superscript letter in the same column were not significant different ($p \leq 0.05$).



(a) (b) (c) (d) (e)

Figure 2: SEM images of microstructures of white shrimp with high-pressure CO_2 treatments. (a) Raw shrimp muscle at transversal section showing a well-organized structure. (b) Raw shrimp muscle at longitudinal section. (c) Swollen muscle fiber due to protein denaturation identified in treated shrimps at 900 psi for 10 min. (d) Denser muscle fiber and large sarcomeric space were formed in treated shrimps at 950 psi for 10 min. (e) Shrinkage of sarcomere and broken muscle fiber identified in treated shrimps at 1100 psi for 10 min.

between fat solubility and HPCD treatment at various pressures and holding time. The data showed that the fat content in untreated shrimps was 0.53%, while, in treated shrimps, it ranged from 0.23% to 0.44%. A similar finding reported that the fat content of *Nila tilapia* was not affected by $scCO_2$ from 80 to 90 bars at 40°C [29]. On the contrary, fat content in tempeh reduced at 1100 psi at 31°C [30]. Fat in foods is lipophilic and soluble in supercritical CO_2; therefore, it decreased during HPCD processing. The explanation was related to the presence of protein, cholesterol, and other organic compounds in shrimps, which could act as a barrier effect on the solubility of fat-$scCO_2$ and the lower moisture content (74%), which reduced the amount of CO_2 able to dissolve in the food matrix; consequently, fat loss was not significant. Another study reported the protective effect of carbohydrate in foods on the inactivation of *Brocothrix thermosphacta* inoculated on minced and skinned beef treated with HPCD at 6.1 MPa, 45°C for 150 min [31].

High-pressure CO_2 did not have a significant effect ($p \geq 0.05$) on the moisture of shrimps, although there was a decrease in moisture of up to 74% in shrimps treated with 1100 psi for 15 min. There is no removal of water from the shrimps, but rather the pressured CO_2 attracts water molecules by binding to the water and forming HCO_3^- and CO_3^- ions and consequently reducing the water content. Increasing pressure and longer treatment time increased the solubility and mass transfer of CO_2, thereby enhancing the decrease in water content as what happened in shrimps

Table 4: Effects of high-pressure CO_2 at various pressures and holding times on the proximate and microbial reduction.

Pressure (psi)/holding time (min)	Moisture (%)	Crude fat (%)	Crude protein (%)	Ash (%)
900/5	79.99 ± 0.71a	0.36 ± 0.005a	16.04 ± 0.08f	0.70 ± 0.03a
900/10	79.84 ± 0.14a	0.38 ± 0.01a	18.11 ± 0.13hi	0.99 ± 0.1ab
900/15	79.97 ± 0.1a	0.28 ± 0.03a	16.75 ± 0.12a	0.99 ± 0.1b
950/5	77.21 ± 1.48a	0.28 ± 0.007a	18.20 ± 0.14hi	1.06 ± 0.3c
950/10	78.48 ± 1.06a	0.24 ± 0.42a	18.15 ± 0.18bcd	1.54 ± 0.3cd
950/15	78.29 ± 1.84ab	0.60 ± 0.45a	16.78 ± 0.34ab	0.81 ± 0.12cd
1100/5	77.9 ± 1.27ab	0.78 ± 0.45a	18.45 ± 0.28de	1.28 ± 0.78cd
1100/10	74.96 ± 0.6ab	0.78 ± 0.53a	18.94 ± 0.13fg	1.52 ± 0.5cd
1100/15	74.38 ± 1.42bc	0.77 ± 0.046a	18.21 ± 0.14bc	1.03 ± 0.04d
Untreated	79.08 ± 1.36c	0.29 ± 0.021a	18.09 ± 0.68h	0.96 ± 0.1e

Means with the same superscript letter in the same column were not significant different ($p \leq 0.05$).

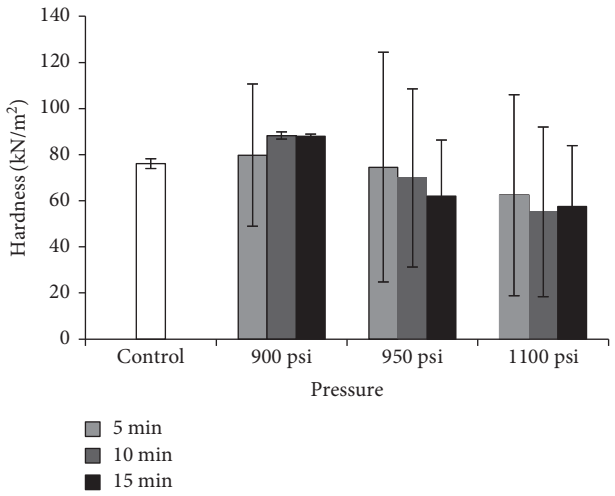


Figure 3: Texture of untreated and treated shrimps with HPCD at various pressures and holding times. The results represented the mean values obtained from 3 different samples and two replications.

treated with 1100 psi for 15 min. Ash content of the shrimps treated at 1100 psi significantly increased ($p \leq 0.05$) compared with the other samples. A decrease in moisture content can cause a relative increase in protein, fat, and ash [7].

3.3. Microbiological Analysis. Table 3 displays the reduction number of aerobic bacteria in treated shrimp with HPCD. The high-pressure treatment significantly destroyed bacteria ($p \leq 0.05$) at 1100 psi. The number of aerobic bacteria in fresh shrimp (before treatment) and in treated shrimp at 1100 psi was 4.5×10^5 CFU/g and 3.5×10^4 CFU/g, respectively. In contrast, the number of microbes in tilapia decreased by 2 log cycles at a pressure treatment of 80 bar reported by Sugiharto et al. [29]. Meanwhile, Kustyawati et al. [30] found that tempeh treated with 900 psi significantly ($p \leq 0.05$) reduced the number of microbes by 2 logs. The type and chemical contents of products processed with supercritical CO_2 could be the reason for inhibiting microbiological inactivation in treated shrimps in this study. Shrimps are a marine product that contains high protein, fat, minerals, and many bioactive compounds in their matrix, which can inhibit penetration of CO_2 into the bacterial cells and prevent them from dying. Moreover, lower moisture (74%) content due to the dissolution of CO_2 into liquid shrimps, which dissociates and forms HCO_3^-, CO_3^- and H^+ ions, could increase the bactericidal effect of high-pressure CO_2 treatment. Erkem [31] showed that the bactericidal action of HPCD was more effective in a liquid substrate, where *Brochothrix thermosphacta* cells inoculated in brain–heart infusion broth were completely inactivated under 6.1 MPa after 30 min at 45°C, while it was reduced one log cycle, when the cells were inoculated in minced beef treated with HPCD at 6.1 MPa and 45°C for 150 min.

3.4. Effect of High-Pressure Treatment on Cholesterol and Total Carotene. Shrimps are rich in cholesterol even though they contain high nutrient quality of polyunsaturated fatty acids [32, 33]. In this study, the effects of HPCD processing on cholesterol and total carotene in shrimp were measured and displayed in Table 3. It showed that HPCD processing significantly decreased ($p \leq 0.05$) cholesterol and total carotene content in shrimps. The initial cholesterol content in untreated shrimps was 1.63 mg/g. Cholesterol levels in treated shrimps fluctuated between 1.27 and 1.48 mg/g, and the lowest level was reached at 900 psi for 10 min of holding time. Compared with the study conducted by Higuera-Ciapara et al. [5], which found that cholesterol was extracted in supercritical CO_2 (310 bar; 37°C), our findings suggest that cholesterol was extracted in both subcritical CO_2 (900 psi) and supercritical CO_2 (1100 psi). This can be explained by considering the molecular polarity and solubility of CO_2. In the supercritical region, supercritical CO_2 ($scCO_2$) possesses high solubility, where the density of $scCO_2$ increased with increasing pressure, so that more cholesterol was extracted. In the subcritical region slightly below the critical point, CO_2 can be a liquid, which is more polar, and cholesterol can most likely be extracted in this CO_2 phase [34]. Below the critical point and above the triple point of −56.6°C and 0.52 MPa, CO_2 exists as a liquid. At and above the critical temperature of 31°C and critical pressure of 7.35 MPa (critical point), CO_2 exists as a gas and liquid in equilibrium; increasing temperature or pressure above this critical point does not result in any further phase changes. Cholesterol ($C_{27}H_{46}O$) is mostly a nonpolar hydrocarbon compound [33], but cholesterol is water-soluble because it

has a polar OH functional group, so more cholesterol is extracted at 900 psi. Cholesterol levels of all processed shrimps in our study were in accordance with the recommended daily intake (<300 mg/day) [35]. However, one needs to be cautious about including shrimps in diet, because the normal total cholesterol in human blood is 160–200 mg/100 g. The higher the cholesterol level in the blood, the greater the risk of atherosclerosis, which is the thickening and hardening of the artery wall due to cholesterol build-up [36].

The effect of HPCD processing on total carotenoid content in shrimps is displayed in Table 4. It showed that HPCD processing significantly reduced ($p \leq 0.05$) total carotenoid in shrimps. Fresh shrimps contained 4.53 mg/g of total carotenoid. With increasing pressure, more carotenoid was extracted, as in this study the lowest carotenoid content was 2.29 mg/g in shrimps treated at 1100 psi for 10 minutes. A similar finding reported by Mezzomo and Ferreira [37] and Radzali et al. [38] was that β-carotene in shrimps was highly extracted and pressure-dependent. A study on the effect of HPCD on the degradation of anthocyanin showed that there was no reduction after 30 MPa, 45°C, but when pressure was increased to 60 MPa, 55°C, for 30 min, anthocyanin decreased [39]. Another study reported that β-carotene in the *Dunaliella salina* microalgae was extracted at a higher pressure (400 bar and 65°C) [40]. In this our study, solubility of CO_2 and food matrix may influence reduction of carotenoid in treated shrimps. As the pressure increased, the density increased, enhancing the solubility of CO_2, and therefore, more carotenoid was extracted. Carotenoid is a nonpolar molecule of fat-soluble pigment, which easily interacts with nonpolar CO_2, resulting in reduction of carotenoid. However, high diffusion of CO_2 can alter the conformation of the protein, leading to denaturation, and result in carotenoid extraction.

3.5. Storage Study. The storage study aimed to evaluate the effectiveness of high-pressure CO_2 treatment on the shelf life of shrimps. According to the study, the best treatment was at the pressure of 1100 psi and holding time of 10 minutes (Table 5). Shrimps were then treated with the best treatment and stored at 4°C for 10 days. The changes in quality parameters, including microbial count, lipid, PV, pH, and formation of total volatile base nitrogen (TVB-N), were monitored (Figures 4–8). TMA level was not monitored in this study due to the limited availability of the chemical during COVID-19. No significant changes were found ($p \leq 0.05$) in PV and lipid in treated and $scCO_2$-treated shrimps, but $scCO_2$ treatment significantly affected pH, TVBN, and microbial count during storage. The initial TVB-N value of untreated and treated shrimps was 9.33 and 9.95 mg/100g, respectively. The TVB-N scales of acceptability for raw shrimps are <12 mg N/100 g for fresh, 12–20 for edible but slightly decomposed, 20–25 for borderline, and >25 mg N/100 g for inedible and decomposed [7]. All shrimps showed an increase of TVB-N during 10 days of storage. It sharply increased in fresh shrimp and reached 32.59 mg/100g at day 10 of storage, whereas it flatly increased in treated shrimps with $scCO_2$ and ended at 30.21 mg/100g at day 10 of storage. Our results indicated that the TVB-N (24.21 mg/g) of treated shrimps with $scCO_2$was not acceptable after day 8 of storage. Most likely, the spoilage of shrimp was caused by degradation of nitrogenous compounds as a result of the activity of endogenous enzymes [41].

Figure 5 shows that the initial microbiological count of fresh and treated shrimp was 4.4×10^5 and 3.9×10^4 CFU/g, respectively. For treated shrimps, the microbiological count started to increase at day 4 and flatly grew until at day 10 of storage (5.1×10^6 CFU/g). The increased counts were likely associated with the adaptability of the bacteria, which were resistant and adaptable to refrigeration temperature. Some aerobic mesophilic bacteria, including spore producing bacteria, may be present in the treated shrimps. Even though psychrotrophs are able to grow at refrigeration temperature [42], these bacteria are easily destroyed by HPCD treatment [43].

The change of pH in shrimp meat could be one of the indications of its freshness during storage [2]. The initial pH value of treated shrimps was 7.7 and gradually declined to 6.6 and was flat after day 6 of storage (Figure 6). Jin et al. [44] found that the initial pH of marine-trawling shrimps was 7.02, and no significant differences were found during deep-frozen temperature storage. Dai et al. [45] reported that the decrease in pH could result from glycogen glycolysis of shrimp for an hour after death, while the increase in pH is related to the accumulation of alkaline molecules, such as ammonia and amines, produced from the metabolism of amino acids, peptides, and protein. In this present study, the decline of the pH value suggested that the $scCO_2$ treatment could have an inhibitory effect that slows down the pH of the shrimps during storage. New macromolecule conformations as a result of protein denaturation and ionic formation of HCO_3^- and CO_3^- in the matrix of shrimp meat due to diffusivity of CO_2 treatment could influence pH changes during storage at 4°C. Moreover, there is no protein buffering effect in this case because the denaturation or conformation of the unfolded protein as a result of the $scCO_2$ treatment changes its function. In general, pH 7.7 or below is the scope for good freshness of the shrimps [46]. Illera et al. [6] reported that HPCD treatment at 12 MPa, 40°C deactivated PPO in shrimps and produced good visual aspects for 5 days of storage at 4°C.

Sensory analysis of treated and untreated shrimps during storage at 4°C included Duo-Trio test and Hedonic test. Duo-Trio test was where sensory panelists were asked to distinguish the sample that had slight differences in aroma, color, and texture (Table 6). Twenty panelists were involved. So, there must be 18 panelists stating that the sample differs from the standard sample at the 5% level. It was shown that sensory panelists did not recognize odor differences between $scCO_2$-treated shrimps and untreated shrimps during the storage. All of the 20 sensory panelists stated that the $ScCO_2$-treated sample (code 983) had a significant difference in terms of color compared with the other samples at eight-day storage, while 16 sensory panelists stated that the untreated sample (code 611) had a texture different from that of the

Table 5: Recapitulation of quality parameter values in white shrimp (*Litopenaeus vannamei*) treated with high-pressure CO_2.

Quality parameter	Treatment (pressure (psi)/holding time (min))									
	900/5	900/10	900/15	950/5	950/10	950/15	1100/5	1100/10	1100/15	Untreated
Moisture (%)	79.99	79.84	79.97	77.21	78.48	78.29	77.9	74.96*	74.38*	79.08
Fat (%)	0.35	0.38	0.28	0.275	0.235	0.6*	0.77	0.78*	0.77	0.28
Protein (%)	16.04*	18.11	16.75*	18.21	18.15*	16.78*	18.45	18.94	18.21	18,09
Hardness (kN/m^2)	79.8	58.3	27.9	74.6*	69.9*	62	62.4	55.2	57.6	46.1
ΔL^* value	51.65	54.15	59.1	53.75	52.05	54.9	59.75	56.75	61.35*	51.55
a^* value	5.55	5.15	5.25	5	4.05	5.6	5.25	5.85*	7.1	4.15
Cholesterol (mg/g)	1.39*	1.27	1.46	1.47	1.39	1.36*	1.46	1.48*	1.34*	1.63
Total carotene (mg/g)	3.8	3.78	3.79	3.77	3.76	3.69	2.51	2.37*	2.29*	4.24
Microbial reduction (log N/No)	0.98	0.979	0.974	0.974	0.969	0.952	0.803	0.805	0.793	

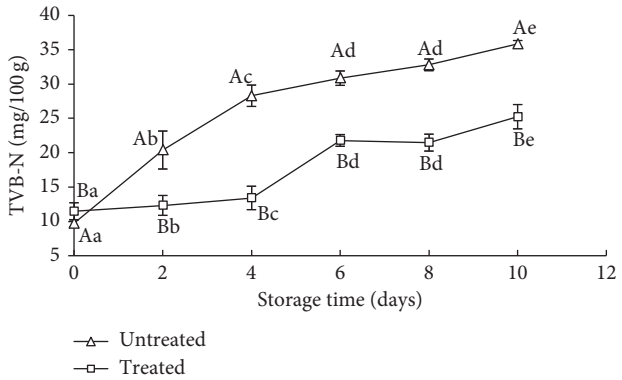


Figure 4: Changes of TVB-N during storage of untreated and $scCO_2$-treated shrimp at 4°C for 10 days. Different capital letters indicate that the treatment had significant effect on TVB-N value ($p < 0.05$). The different lowercase letters indicate that storage time had a significant effect on TVN value ($p < 0.05$).

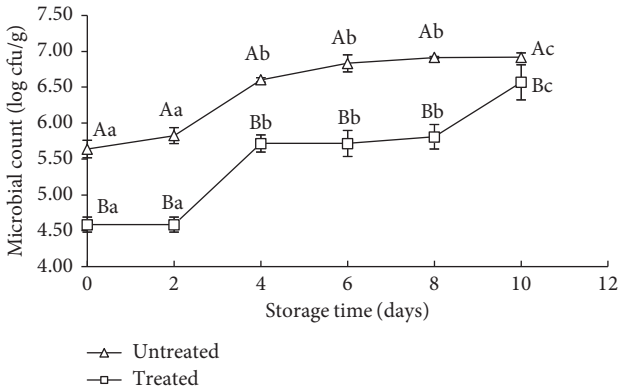


Figure 5: Changes of microbial count during storage of untreated and $scCO_2$-treated shrimps at 4°C for 10 days. Different capital letters indicate that the treatment had significant effect on microbial count ($p < 0.05$). The different lowercase letters indicate that storage time had significant effect on microbial count ($p < 0.05$).

other samples at day 6 of storage. Similarly, Kincal et al. [47] showed that the sensory panelists could not detect any significant aroma differences between the control frozen fresh orange juice and HPCD-treated orange juice after a two-week storage at 17°C. Some researchers showed that HPCD slightly reduced volatile compounds [48, 49]. Chen et al. [48] found no change in ester composition and slight change in alcohol and aldehydes in Hami melon juice after treatment with HPCD at 35 MPa and 55°C for 60 min and four-week storage. Zhou et al. [50] found that low threshold was responsible for the changes in the odor/flavor of foods. The aroma of foods mostly generated from volatile compounds. In shrimps, volatile compounds can be come from chemical reactions and enzymatic reactions, involving amino acids side chain of protein, carbohydrate, and lipid. When $scCO_2$ was used, lipid and nonpolar amino acids were oxidized or extracted, enzymatic activation was changed, and new volatile compounds in the food matrix can be produced. However, the volatile compound may be stripped off during depressurized CO_2, thereby having no effect on food odor. This was why the sensory panelists could not differentiate between the treated and untreated shrimps. Kleekayai et al. [51] found that N- and S-containing compounds with major volatile compounds, trimethylamine, 2,5-dimethylpyrazine, and dimethyl trisulfide, and nonpolar compounds were responsible for the aroma of shrimps. In addition, aldehydes, ketones, and alcohols mostly produced from oxidative cleavages of lipids, and degradation of amino acids and saccharides were also responsible for the shrimp odor.

Consumer acceptance test was performed by 20 students from the departments. A 5-point hedonic scale was used to evaluate overall acceptance and color, texture, and odor acceptance (Figure 9). Any changes in the compounds responsible for the color, texture, and odor can lead to their acceptance. It can be concluded that, among the samples, there was no hedonic difference in odor, color, and texture, as well as overall acceptance ($p < 0.05$). The score of overall acceptance was moderate (score of 3.8) for treated shrimp at eight-day storage. Sensory panelist acceptance to the sense of color was moderate (score of 3.8) for treated shrimp at eight-day storage. However, acceptance of sensory panelists to odor was neither like or dislike (score of 3.0) for treated shrimp during storage at 4°C. The score of the sense of texture given by sensory panelists was neither like or dislike (3.1) during storage at 4°C, but panelists accepted the sample more (score of 3.8, moderate) at eight-day storage.

Figure 7 shows the lipid changes of $scCO_2$-treated and untreated shrimps during storage. The result of analysis variance showed that both the $scCO_2$ treatment and storage

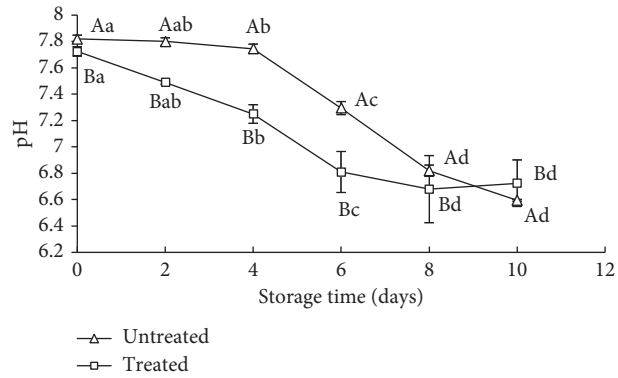


Figure 6: Changes of pH during storage of untreated and $scCO_2$-treated shrimps at 4°C for 10 days. Different capital letters indicate that the treatment had significant effect on pH value ($p < 0.05$). The different lowercase letters indicate that storage time had significant effect on pH value ($p < 0.05$).

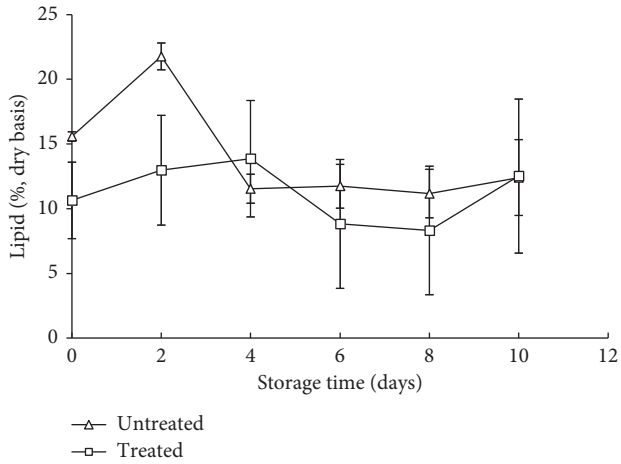


Figure 7: Changes of total lipid during storage of untreated and $scCO_2$-treated shrimps at 4°C for 10 days.

time did not significantly affect the total lipid during storage ($p > 0.05$). The same results were reported by Senapati et al. [52], which showed that the crude fat content of white-leg shrimps (*Litopenaeus vannamei*) did not significantly change during 14 days of chilled storage. In this study, supercritical CO_2 treatment and low storage temperature could protect shrimp fat from hydrolysis; therefore, no significant fat changes were found during storage at 4°C. Shrimp lipids, a nonpolar biomolecule, are reduced when the shrimp is treated with $scCO_2$, thereby preventing possible hydrolysis during storage. Supercritical CO_2 dissolves nonpolar or slightly polar compounds [4]. Additionally, lipid hydrolysis occurred rapidly at higher storage temperatures, which increased free fatty acids, resulting in the shrimps' off odor [53, 54].

Figure 8 shows the peroxide value (PV) changes in $scCO_2$-treated and untreated shrimps during storage. The result of analysis of variance showed that $scCO_2$ treatment and storage time did not significantly affect the PV during storage ($p > 0.05$). Similar results were reported by Minh et al. [55] that showed that the PV of black tiger shrimp (*Penaeus monodon*) control samples did not significantly change during 12 months of storage. In this study, $scCO_2$ treatment could potentially slow down the mechanisms responsible for lipid damage in shrimps during storage at 4°C. Peroxide value is a product of lipid autoxidation, which indicates the oxidation deterioration level of lipid. Lipid deterioration is influenced by the oxygen presence, temperature, and light during the storage of product [56–58]. Additionally, temperature affected PV value, which significantly increased when shrimps were kept under high temperature and in the presence of sunlight [59].Light catalyzes the oxidation of lipid under photo-oxidation process. During photo-oxidation, the energy of light is absorbed and turns the free radical group, triplet oxygen into singlet oxygen, which easily reacts with unsaturated fatty acids and generates hydrogen peroxide [60].

It could be said that $scCO_2$-treated shrimps were accepted by panelists until eight-day storage at 4°C and found no significant changes in quality degradation parameters responsible for shrimp deterioration.

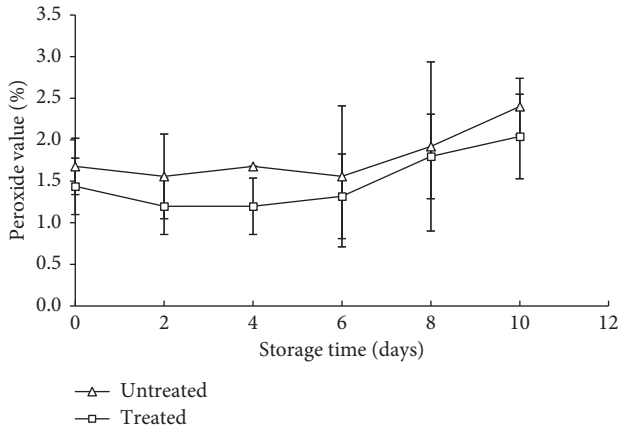


Figure 8: Changes of peroxide value during storage of untreated and $scCO_2$-treated shrimps at 4°C for 10 days.

Table 6: Duo-Trio test of untreated and treated shrimps with supercritical CO_2 during storage at 4°C.

Parameters	Day 0		Day 2		Day 4		Day 6		Day 8		Day 10	
	463	198	257	671	553	879	611	537	227	983	265	871
Odor	13	10	10	13	12	11	10	13	11	12	12	11
Color	10	13	10	13	9	14	15	8	3	20*	11	12
Texture	15	8	11	12	13	10	16*	7	15	8	13	10

The numbers followed by an asterisk (*) were significantly different among other samples; code of untreated samples: 463, 257, 553, 611, 227, and 265; code of samples treated with supercritical CO_2: 198, 671, 879, 537, 983, and 871.

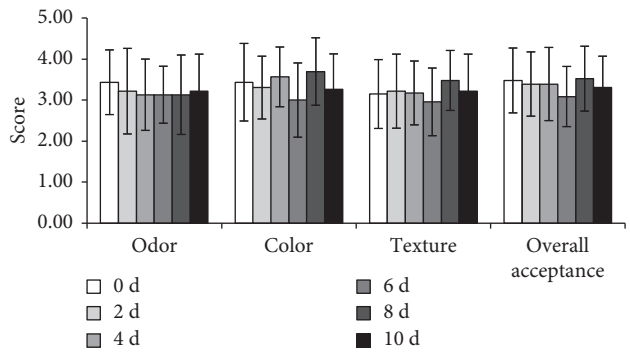


Figure 9: Consumer acceptance test of $scCO_2$-treated shrimps during storage at 4°C by using a 5-point hedonic scale.

4. Conclusions

Subcritical and supercritical CO_2 did not affect the hardness and lightness of shrimps, indicating that the quality of freshness of shrimps is maintained. However, cholesterol reduction occurred at either the subcritical or supercritical treatment. Supercritical CO_2 treatment at 1100 psi for 10 min was the best treatment recommended for processing white shrimps. A storage study was performed to see the effectiveness of $scCO_2$ treatment at 1100 psi for 10 min during storage. No significant changes were found in PV and lipid in untreated and $scCO_2$-treated shrimps, but the treatment significantly affected pH, TVBN, and microbial counts during storage. Among the samples, there was no hedonic difference in odor, color, texture, and overall acceptance during storage. Moreover, untreated shrimps possessed a texture significantly different from that of the other samples at six-day storage, while $scCO_2$-treated shrimps possessed a very significant color difference compared with the other samples at eight-day storage.

It was found that $scCO_2$-treated shrimps, at 1100 psi for 10 min, were accepted by panelists when stored up to eight days. The results of this study provided useful information about the possibility of applying supercritical CO_2 at 1100 psi for 10 min as an alternative method for preservation of shrimps.

Data Availability

All data generated or analyzed during this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest

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References

[1] K. Manheem, S. Benjakul, K. Kijroongrojana, N. Faithong, and W. Visessanguan, "Effect of pre-cooking times on enzymes, properties, and melanosis of Pacific white shrimp during refrigerated storage," *International Aquatic Research*, vol. 5, no. 1, pp. 1–11, 2013.

[2] N. Xu, W. Shi, X. Wang, and Z. Wang, "Effect of ice water pretreatment on the quality of Pacific White Shrimps (*Litopenaeus vannamei*)," *Food Science & Nutrition*, vol. 7, no. 2, pp. 645–655, 2019.

[3] L. Laboureur, M. Ollero, and D. Touboul, "Lipidomics by supercritical fluid chromatography," *International Journal of Molecular Sciences*, vol. 16, no. 12, pp. 13868–13884, 2015.

[4] H. Monhemi and S. Dolatabadi, "Molecular dynamics simulation of high-pressure CO_2 pasteurization reveals the interfacial denaturation of proteins at CO_2/water interface," *Journal of CO2 Utilization*, vol. 35, pp. 256–264, 2020.

[5] I. Higuera-Ciapara, A. R. Toledo-Guillen, L. Noriega-Orozco, K. G. Martinez-Robinson, and M. C. Esquade-Valle, "Prodution of a low-cholesterol shrimp using supercritical extraction," *Journal of Food Process Engineering*, vol. 28, no. 5, pp. 526–538, 2005.

[6] A. E. Illera, M. T. Sanz, S. Beltrán, and O. Benito-Román, "Effect of high pressure carbon dioxide on polyphenoloxidase from *Litopenaeus vannamei*," *LWT*, vol. 109, pp. 359–365, 2019.

[7] B. Zhang, L. K. Ma, S. G. Deng, C. Xie, and X. H. Qiu, "Shelf-life of pacific white shrimp (*Litopenaeus vannamei*) as affected by weakly acidic electrolyzed water ice-glazing and modified atmosphere packaging," *Food Control*, vol. 51, pp. 114–121, 2015.

[8] J. Li, W. Sun, H. S. Ramaswamy et al., "High pressure extraction of astaxanthin from shrimp waste (*Penaeus Vannamei* Boone): effect on yield and antioxidant activity," *Journal of Food Process Engineering*, vol. 40, no. 2, p. e12353, 2017.

[9] T. Jantakoson, K. Kijroongrojana, and S. Benjakul, "Effect of high pressure and heat treatments on black tiger shrimp (*Penaeus monodon* Fabricius) muscle protein," *International Aquatic Research*, vol. 4, no. 1, pp. 1–12, 2012.

[10] M. McHugh and V. Krukonis, *Supercritical Fluid Extraction*, H. Brenner, Ed., Butterworth-Heinemann, UK, 2nd edition, 2013.

[11] B. Werner and J. Hotchkiss, "Modified atmosphere packaging," in *Microbiology of Fruits and Vegetables*, M. G. Sapers, J. R. Gorny, and A. E. Yousef, Eds., CRC Press, Boca Raton, FL, USA, 1st edition, 2006.

[12] D. Saputra, "Puffing dehydrated vegetable with carbon dioxide," in *Jurnal Keteknikan Pertanian*, pp. 157–165, 2006.

[13] M. Plascencia-Jatomea, G. Viniegra, R. Olayo, M. M. Castillo-Ortega, and K. Shirai, "Effect of chitosan and temperature on spore germination of *Aspergillus Niger*," *Macromolecular Bioscience*, vol. 3, no. 10, pp. 582–586, 2003.

[14] T. Saldanha, M. T. Benassi, and N. Bragagnolo, "Fatty acid contents evolution and cholesterol oxides formation in Brazilian sardines (*Sardinella brasiliensis*) as a result of frozen storage followed by grilling," *LWT-Food Science and Technology*, vol. 41, no. 7, pp. 1301–1309, 2008.

[15] H. D. de Holanda and F. M. Netto, "Recovery of components from shrimp (*Xiphopenaeus kroyeri*) processing waste by enzymatic hydrolysis," *Journal of Food Science*, vol. 71, no. 5, pp. C298–C303, 2006.

[16] G. Latimer, *Official Methods of Analysis of AOAC International*, AOAC International, Rockville, MD, USA, 21st edition, 2019.

[17] National Standardization Body, *SNI 2354.8-2009: Chemical Test Method-Part 8: Determination of Total Volatile Base Nitrogen (TVB-N) and Trimethyl Amin Nitrogen (TMA-N) Levels in Fishery Products*, National Standardization Body, Geneva, Switzerland, 2009.

[18] IFRA, *Determination of the Peroxide Value*, The International Fragrance Association, Geneva, Switzerland, 2019.

[19] J. A. Dorado-Rodelo, J. M. Ezquerra-Brauer, and H. Soto-Valdez, "Effect of ovenproof plastic films on the quality of spotted rose snapper (*Lutjanus guttatus*) fillets during frozen storage," *Packaging Technology and Science*, vol. 20, no. 5, pp. 301–307, 2007.

[20] M. Meilgaard, G. V. Civille, and B. T. Carr, *Sensory Evaluation Techniques*, CRC Press, Boca Raton, FL, USA, 4th edition, 2007.

[21] H. S. Youn, M. K. Roh, A. Weber, G. T. Wilkinson, and B. S. Chun, "Solubility of astaxanthin in supercritical carbon dioxide," *Korean Journal of Chemical Engineering*, vol. 24, no. 5, pp. 831–834, 2007.

[22] A. P. Sánchez-Camargo, H. A. Martinez-Correa, L. C. Paviani, and F. A. Cabral, "Supercritical CO_2 extraction of lipids and astaxanthin from Brazilian redspotted shrimp waste (*Farfantepenaeus paulensis*)," *Journal of Supercritical Fluids*, vol. 56, no. 2, pp. 164–173, 2011.

[23] F. J. Pashkow, D. G. Watumull, and C. L. Campbell, "Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease," *American Journal of Cardiology*, vol. 101, no. 10 Suppl, pp. S58–S68, 2008.

[24] J. Li, C. Guo, and J. Wu, "Astaxanthin in liver health and disease: a potential therapeutic agent," *Drug Design, Development and Therapy*, vol. 14, pp. 2275–2285, 2020.

[25] W. Yan, J. Cui, R. Dai, H. Wang, and X. Li, "Effects of dense phase carbon dioxide on quality and physical-chemical properties of chilled pork," *Nongye Gongcheng Xuebao/ Transactions of the Chinese Society of Agricultural Engineering*, vol. 26, no. 7, pp. 346–350, 2010.

[26] N. S. Tabitha and T. Anand, "Nutritive value of cultured white leg shrimp *Litopenaeus vannamei*," *International Journal of Fisheries and Aquaculture*, vol. 5, no. 7, pp. 166–171, 2013.

[27] V. Venugopal, "Nutritional value of shrimp the popular shellfish," *EC Nutrition*, vol. 02, pp. 1–9, 2020.

[28] C. Da Porto and A. Natolino, "Supercritical fluid extraction of polyphenols from grape seed (*Vitis vinifera*): study on process variables and kinetics," *Journal of Supercritical Fluids*, vol. 130, pp. 239–245, 2017.

[29] S. Sugiharto, N. Bintoro, J. Karyadi, and Y. Pranoto, "Supercritical carbon dioxide pasteurization to reduce the activity of muscle protease and its impact on physicochemical properties of *Nile tilapia*," *Research Ideas and Outcomes*, vol. 6, p. 56887, 2020.

[30] M. E. Kustyawati, F. Pratama, D. Saputra, and A. Wijaya, "Viability of molds and bacteria in tempeh processed with supercritical carbon dioxides during storage," *International Journal of Food Science*, vol. 2018, 2018.

[31] O. Erkmen, "Antimicrobial effects of pressurised carbon dioxide on *Brochothrix thermosphacta* in broth and foods," *Journal of the Science of Food and Agriculture*, vol. 80, no. 9, pp. 1365–1370, 2000.

[32] D. R. Pires, D. Morais, A. C. N. Coelho, A. F. Góes, L. C. D. S. A. Ferreira, and T. Saldanha, "Nutritional

composition, fatty acids and cholesterol levels in Atlantic white shrimp (*Litopenaeus schimitti*)," *International Food Research Journal*, vol. 25, no. 1, pp. 151–157, 2018.

[33] S. M. Pourmortazavi, Z. Saghafi, A. Ehsani, and M. Yousefi, "Application of supercritical fluids in cholesterol extraction from foodstuffs: a review," *Journal of Food Science and Technology*, vol. 55, no. 8, pp. 2813–2823, 2018.

[34] O. H. Nautiyal, "Review article food processing by supercritical carbon dioxide-review," *EC Chemistry*, vol. 2, no. 1, pp. 111–135, 2016.

[35] D. Kartono, J. A. B. Hardiyansyah, and A. Sulaeman, "Summary of recommended nutritional adequacy for Indonesians," in *Proceedings of the Widyakarya Nasional Pangan Dan Gizi (WNPG) X*, pp. 1–18, Jakarta, Indonesia, April 2017.

[36] R. K. Eldiaz, A. Agustono, and K. T. Pursetyo, "The content of low density lipoprotein, high density lipoproteins, cholesterol on pen shells (*Atrina pectinata*) fish catch in kenjeran surabaya," *Journal of Marine and Coastal Science*, vol. 7, no. 2, p. 51, 2020.

[37] N. Mezzomo and S. R. S. Ferreira, "Carotenoids functionality, sources, and processing by supercritical technology: a review," *Journal of Chemistry*, vol. 2016, Article ID 3164312, 17 pages, 2016.

[38] S. A. Radzali, B. S. Baharin, and R. A. Rahman, "Optimisation of supercritical fluid extraction of astaxanthin from *Penaeus monodon* waste using ethanol-modified carbon dioxide," *Journal of Engineering Science and Technology*, vol. 11, no. 5, pp. 722–736, 2016.

[39] K. Marszałek, Ł. Woźniak, B. Kruszewski, and S. Skapska, "The effect of high pressure techniques on the stability of anthocyanins in fruit and vegetables," *International Journal of Molecular Sciences*, vol. 18, no. 2, 2017.

[40] A. Molino, V. Larocca, G. Di Sanzo et al., "Extraction of bioactive compounds using supercritical carbon dioxide," *Molecules*, vol. 24, no. 4, 2019.

[41] C. Niamnuy, S. Devahastin, and S. Soponronnarit, "Quality changes of shrimp during boiling in salt solution," *Journal of Food Science*, vol. 72, no. 5, pp. S289–S297, 2007.

[42] J. M. Jay, M. J. Loessner, and D. A. Golden, *Modern Food Microbiology*, Springer US, New York, NY, USA, 7th edition, 2005.

[43] T. Yu, L. Niu, and H. Iwahashi, "High-pressure carbon dioxide used for pasteurization in food industry," in *Food Engineering Reviews*, vol. 12, no. 3, pp. 364–380, 2020.

[44] L. Jin, G. Ding, P. Li, J. Gu, and X. Zhang, "Changes in quality attributes of marine-trawling shrimp (*Solenocera crassicornis*) during storage under different deep-frozen temperatures," *Journal of Food Science and Technology*, vol. 55, no. 8, pp. 2890–2898, 2018.

[45] X. Y. Dai, M. X. Zhang, X. Y. Wei, R. C. Hider, and T. Zhou, "Novel multifunctional hydroxypyridinone derivatives as potential shrimp preservatives," *Food and Bioprocess Technology*, vol. 9, no. 7, pp. 1079–1088, 2016.

[46] H. Mu, H. Chen, X. Fang, J. Mao, and H. Gao, "Effect of cinnamaldehyde on melanosis and spoilage of Pacific white shrimp (*Litopenaeus vannamei*) during storage," *Journal of the Science of Food and Agriculture*, vol. 92, no. 10, pp. 2177–2182, 2012.

[47] D. Kincal, W. S. Hill, M. Balaban et al., "A continuous high-pressure carbon dioxide system for cloud and quality retention in orange juice," *Journal of Food Science*, vol. 71, no. 6, pp. C338–C344, 2006.

[48] J. L. Chen, J. Zhang, L. Song, Y. Jiang, J. Wu, and X. S. Hu, "Changes in microorganism, enzyme, aroma of hami melon (*Cucumis melo* L.) juice treated with dense phase carbon dioxide and stored at 4°C," *Innovative Food Science and Emerging Technologies*, vol. 11, no. 4, pp. 623–629, 2010.

[49] L. Niu, X. Hu, J. Wu et al., "Effect of dense phase carbon dioxide process on physicochemical properties and flavor compounds of orange juice," *Journal of Food Processing and Preservation*, vol. 34, no. Suppl. 2, pp. 530–548, 2010.

[50] L. Zhou, X. Bi, Z. Xu, Y. Yang, and X. Liao, "Effects of high-pressure CO_2 processing on flavor, texture, and color of foods," *Critical Reviews in Food Science and Nutrition*, vol. 55, no. 6, pp. 750–768, 2015.

[51] T. Kleekayai, S. Pinitklang, N. Laohakunjit, and W. Suntornsuk, "Volatile components and sensory characteristics of Thai traditional fermented shrimp pastes during fermentation periods," *Journal of Food Science and Technology*, vol. 53, no. 3, pp. 1399–1410, 2016.

[52] S. R. Senapati, G. P. Kumar, C. B. Singh et al., "Melanosis and quality attributes of chill stored farm raised whiteleg shrimp (*Litopenaeus vannamei*)," *Journal of Applied and Natural Science*, vol. 9, no. 1, pp. 626–631, 2017.

[53] J. Lu, M. Langton, S. Sampels, and J. Pickova, "Lipolysis and oxidation in ultra-high temperature milk depend on sampling month, storage duration, and temperature," *Journal of Food Science*, vol. 84, no. 5, pp. 1045–1053, 2019.

[54] C. Rivera-Pérez, F. L. García-Carreño, and R. Saborowski, "Purification and biochemical characterization of digestive lipase in whiteleg shrimp," *Marine Biotechnology*, vol. 13, no. 2, pp. 284–295, 2011.

[55] N. P. Minh, T. Thi, Y. Nhi et al., "Quality of frozen black tiger shrimp (*Penaeus monodon*) under a low-density polyethylene bag coextruded with butylated hydroxyanisole," vol. 11, no. 4, pp. 1483–1486, 2019.

[56] M. H. Gordon, "Factors affecting lipid oxidation," in *Understanding and Measuring the Shelf-Life of Food*, Woodhead Publishing Limited, Sawston, UK, 2004.

[57] D. R. Johnson and E. A. Decker, "The role of oxygen in lipid oxidation reactions: a review," *Annual Review of Food Science and Technology*, vol. 6, pp. 171–190, 2015.

[58] F. Kong and R. P. Singh, "Advances in instrumental methods to determine food quality deterioration," in *Food and Beverage Stability and Shelf Life*, Woodhead Publishing Limited, Sawston, UK, 2011.

[59] D. Y. Li, Z. Yuan, Z. Q. Liu et al., "Effect of oxidation and maillard reaction on color deterioration of ready-to-eat shrimps during storage," *Lwt*, vol. 131, Article ID 109696, 2020.

[60] H. H. Chang, *Quantitative Changes of Volatile Compound in Soybean and Algal Oil and Effects of Antioxidants on the Oxidative Stability of Algal Oil under Light Storage*, The Ohio State University, Columbus, OH, USA, 2011.