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ARTICLE

Effects of Supercritical CO_2 Treatment on Color, Lipid Oxidation, Heme Iron, Non-Heme Iron and Metmyoglobin Contents in Ground Pork

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Abstract The color, lipid oxidation, heme iron (HI) and non-heme iron (NHI) contents, metmyoglobin content and Soret band of myoglobin of ground pork subjected to supercritical CO_2 treatment under different conditions, or to heat treatment (40°C, 2 h) and subsequent storage at 4°C were evaluated during 9-day period. Supercritical CO_2 treatment significantly increased CIE L* and CIE b* values of ground pork during subsequent storage, while the HI content was slightly affected. In general, CIE a* value and metmyoglobin content were decreased. Supercritical CO_2 treatment for 2 h could increase the thiobarbituric acid-reactive substances (TBARS) value, while treatment for 1 h or less had no effect. The NHI content could be increased only after treatment at above 40°C or 17.2 MPa for 2 h. The Soret band of myoglobin was shifted to longer wavelength. Increasing treatment temperature from 35°C to 45°C could increase CIE L*, CIE a*, CIE b* and TBARS values, HI and NHI contents of the ground pork, while decreasing metmyoglobin content. As the treatment pressure increased from 13.8 MPa to 20.7 MPa, CIE b* and TBARS values were decreased, while the NHI and metmyoglobin contents were increased. However, the other parameters were unchanged. Extending exposure time from 0.5 h to 2 h could increase CIE L*, CIE b* and TBARS values, HI contents, while decreasing CIE a* value and metmyoglobin content. Correlation analysis showed that the TBARS value was significantly and negatively correlated with the HI content or metmyoglobin content in samples treated at 40°C or above for 2 h.

Keywords supercritical CO_2 treatment, ground pork, lipid oxidation, heme iron, metmyoglobin

Introduction

Ground meat is widely used in meat industry as a raw material for production of dried meat slices, sausage, meat stuffing, meat patties, meatball and other products. During grinding, the surface area of meat is greatly increased, leading to the spread of microorganisms on the meat surface (Bae et al., 2011b). Moreover, the ground meat is almost inevitably contaminated by microorganisms from the processing environment and equipment during the grinding process (Bae et al., 2011b). Therefore, ground meat

is more susceptible to spoilage than raw meat during storage and transportation. Meat spoilage will lead to great economic losses for producers and harm the health of consumers. Appropriate sterilization techniques should be applied to maintain the quality and safety of ground meat, which is also a major problem for meat industry.

In the past few decades, supercritical CO_2 sterilization technology has been regarded by the food industry as a feasible alternative to traditional heat sterilization technology (Ferrentino et al., 2012). This technology can inactivate the microorganisms and retain the original quality of food, but it does not damage the nutrients in food. Therefore, this technique is considered as a promising new non-thermal pasteurization technique. It is believed that this technology, when matures, will be the most promising non-thermal pasteurization technology for large-scale industrial application (Zeng et al., 2010).

Supercritical CO_2 sterilization technology is especially useful for ground meat due to the high penetration and diffusion rates of supercritical CO_2. Supercritical CO_2 can diffuse and penetrate deeply into the ground meat, helping to reduce the number of pathogenic bacteria inside the meat (Bae et al., 2011b). This technology can be applied at relatively mild conditions than heat treatment and has little effect on meat quality. Therefore, supercritical CO_2 is considered as a useful and novel tool to improve the microbiological safety of ground meat products (Bae et al., 2011b). The exact mechanism of microbial inactivation by supercritical CO_2 has not been clarified so far. Several mechanisms may be involved as reported in literature (Damar and Balaban, 2006). The bactericidal action of supercritical CO_2 may be associated with the extraction of cellular components from cell membranes and cytoplasm, key enzyme inactivation/cellular metabolism inhibition due to pH lowering, or cell rupture due to rapid depressurization and expansion of carbon dioxide within the cell.

In the process of food sterilization with supercritical CO_2, many studies have found that the efficiency of microbial inactivation was improved with increasing the treatment pressure, temperature and the exposure time (Bae et al., 2011a; Bae et al., 2011b). The effectiveness of supercritical CO_2 to inactivate microorganisms also depends largely on the type of food, including whether it is liquid or solid (Buszewski et al., 2022). Meat and meat products are solid foods, which cannot be stirred during supercritical CO_2 processing, the diffusion of CO_2 into meat and meat products is relatively limited. On the other hand, the proteins and fats present in meat and meat products may protect microorganisms from the bactericidal effects of CO_2 (Buszewski et al., 2022). Thus, it is more difficult to treat solid food with supercritical CO_2 than liquid food. In order to inactivate spoilage and pathogenic bacteria in meat and meat products, higher temperature, higher pressure and longer exposure time are needed (Garcia-Gonzalez et al., 2007). Sirisee et al. (1998) applied supercritical CO_2 treatment (42.5℃ and 31.03 MPa) to inactivate *Escherichia coli* and *Staphylococcus aureus* in ground beef and phosphate buffer, respectively, and found that 1 Log reduction in ground beef took 178 min, but only 1.7 min was needed in the liquid phosphate buffer. Wei et al. (1991) treated chicken meat strips with supercritical CO_2 at 13.7 MPa, 35℃ for 2 h, the inactivation rates of *Salmonella* and *Listeria* were only 94%–98% and 79%–84%, respectively. However, the quality of food may be affected under these conditions. Recently, we evaluated the inhibitory effects of the combined treatment of supercritical CO_2 and rosemary on ground pork, and found that supercritical CO_2 treatment at 35℃ and 13.8 MPa (2,000 psi) for 2 h can promote lipid oxidation in ground pork (Huang et al., 2017). Lipid oxidation is a major cause for quality deterioration of meat and meat products during storage, resulting in severe loss of flavour and nutritional value (mainly fatty acids and fat-soluble vitamins). Thus, when it comes to achieving the practical application of supercritical CO_2 in the meat industry and developing fresh, nutritious, safe and convenient meat products with supercritical CO_2, lipid oxidation should be taken into consideration. It is necessary to acquire the knowledge of effects of process parameters such as treatment pressure and temperature, exposure and storage time. However, there are few studies on the effect of supercritical CO_2 treatment on lipid oxidation in ground meat.

Many studies found that high pressure processing could lead to acceleration of lipid oxidation in meat and meat products under certain pressures. The reported reasons are varied. The release of iron ions during high pressure processing was thought to be a major cause. Myoglobin oxidation was believed to be another cause (Orlien et al., 2000). However, there is no report on the interrelationship between myoglobin oxidation, iron species and lipid oxidation of the ground pork treated with supercritical CO_2. Therefore, the purpose of this study is to investigate the effect of process parameters (treatment pressure, temperature and exposure time) on the lipid oxidation in treated ground meat during the subsequent 9 days of refrigerated storage. The relationship between myoglobin oxidation, iron release and lipid oxidation of the treated ground pork was also determined.

Materials and Methods

Chemicals

The carbon dioxide used (purity higher than 99.999%, v/v) was purchased from Guangdong Huate Gas (Foshan, China). Other chemicals were commercially available and analytical grade.

Sample preparation

Fresh pork (the *longissimus dorsi* muscle) was purchased from a local supermarket (in Xiangtan, China) after 24 h postmortem. After removing the visible fat and connective tissue, the pork was ground by using a meat grinder through a plate with Ø-6 mm holes. Then the ground pork samples (3 kg for each trial) were divided into nine batches (about 300 g each). Each batch was packed in low density polyethylene bag and frozen at −20℃ until processing.

Supercritical CO_2 treatment and heat treatment

The frozen ground pork samples were thawed at room temperature. Samples for supercritical CO_2 treatment were filled into the feed basket, and then placed in the cleaned and disinfected high-pressure vessel. The supercritical CO_2 treatment was performed by a batch type system under different conditions (Table 1). To investigate the effect of temperature, the supercritical CO_2 treatments were performed at temperatures of 35℃, 40℃ and 45℃ with a constant pressure of 17.2 MPa and exposure time of 2 h. To investigate the influence of pressure, the supercritical CO_2 treatments were performed in pressure ranging from 13.8 MPa to 20.7 MPa at a constant temperature of 40℃ and exposure time 2 h. To investigate the effect of exposure time, the supercritical CO_2 treatments were performed at a constant temperature of 40℃ and pressure of 17.2 MPa for 0.5, 1 and 2 h. Before each experimental run, the high-pressure vessel was pre-heated to the set temperature. After closing the lid, the vessel was purged with CO_2 for 1 min. Subsequently, liquid CO_2 was pumped into the vessel by using a constant flow/constant pressure dual piston pump (SFT-10, Supercritical Fluid Technologies, Newark, DE, USA). Once the set pressure is reached, the system is maintained at the pressure and temperature for the set time. Upon finishing the treatment, the vessel was decompressed and the sample was removed.

The effects of supercritical CO_2 treatment at 40℃ for 2 h were compared with heat treatment at the same temperature for the same exposure time. Samples for heat treatment were packed in sealable bags, and the packages were immersed in water bath at 40℃ for 2 h, then the samples were cooled with running tap water. Both supercritical CO_2 and heat treatments were performed in duplicate. After treatment, the sample was subdivided into five groups (each treatment×5 storage times) and each group was aerobically packaged in low density polyethylene bags together with untreated (UT) ground pork meat. All

Table 1. Process conditions of supercritical CO_2 treatment (SCT)

Treatment	Process conditions		
	Temperature (°C)	Pressure (MPa)	Exposure time (h)
SCT 1	35	17.2	2
SCT 2	40	17.2	2
SCT 3	45	17.2	2
SCT 4	40	17.2	0.5
SCT 5	40	17.2	1
SCT 6	40	13.8	2
SCT 7	40	20.7	2

groups were stored at 4±1°C for 9 days and one group was taken for analysis at days 1, 3, 5, 7 and 9.

Color measurement

Color values (CIE L*, CIE a*, and CIE b*) of ground pork were measured by using a Minolta chromameter (CR-400, Konica Minolta Sensing, Osaka, Japan). Before measurement, the instrument was calibrated with a white standard plate (CIE L*=95.60, CIE a*=−0.15, CIE b*=3.34). Each sample was mixed thoroughly and kept inside the Petri dishes. Five different locations across the sample surface were randomly selected for color measurement, the values of each measurement were recorded and the average was calculated.

Determination of thiobarbituric acid-reactive substances

The thiobarbituric acid-reactive substances (TBARS) value of the sample was determined according to the method previously reported (Huang et al., 2017). The result was expressed as mg of malondialdehyde per kilogram of meat. In brief, 10 g ground pork samples were homogenized with 50 mL 7.5% (w/v) trichloroacetic acid and filtered with double filter paper. Five millilitres 0.02 M TBA solution was added into 5 mL filtrate. The contents were vigorously shaken, and incubated in a water bath at 90°C for 40 min. After cooled to room temperature, the mixture was centrifuged at 8,525×g for 5 min. The supernatant was thoroughly mixed with 5 mL chloroform, then allowed to stand for separation. The resulting supernatant solution was measured for absorbance at 532 and 600 nm, respectively. The TBARS value was calculated by using the following formula:

$$\text{TBARS value (mg MDA/kg meat)} = (A_{532} - A_{600}) \times 1 \ / \ (1.56 \times 10^5) \times 72.06 \times 0.05 \ / \ 10 \times 10^6 \quad (1)$$

where A_{532}, A_{600} are the absorbance values at 532 and 600 nm, respectively; 1.56×10^5 is the extinction coefficient of malondialdehyde, $M^{-1}cm^{-1}$; 72.06 is the molar mass of malondialdehyde, g/mol; 0.05/10 is the number of filtrate volumes obtained per gram of sample, L/g; 10^6 is the number of milligrams per kilogram, mg/kg.

Determination of heme iron content

Heme iron (HI) content was determined according to the method reported by Wang et al. (2018). Five g of ground pork

sample was weighed in a test tube with lid and 25 mL of acidified acetone (45 mL of acetone, 4 mL of water and 1 mL of concentrated hydrochloric acid) was added. The mixture was homogenized for 30 s. Then the tube was covered with the lid and placed in the dark at room temperature for 1 h. Next, the mixture was centrifuged at 4℃ (160×g) for 10 min, and the absorbance of the supernatant was measured at 640 nm. The absorbance was multiplied by 6,800 and then divided by the sample weight to obtain the concentration of total pigments in the meat as μg hematin/g meat. The iron content was calculated with the factor of 0.0882 μg iron/μg hematin.

Determination of non-heme iron content

Non-heme iron (NHI) content was examined following the method described by Rhee and Ziprin (1987). Five grams of ground pork sample was weighed and mixed thoroughly with 0.2 mL 0.39% (w/v) $NaNO_2$ reagent. Then, 7.5 mL 6 M HCl and 7.5 mL 40% (w/v) trichloroacetic acid were added. The samples was incubated in a water bath at 65℃ for 20 h. After cooled to room temperature, 1 mL of the liquid above the meat residue was transferred to a centrifuge tube and 5 mL color reagent (Water:saturated sodium acetate solution:bathophenanthroline disulfonate reagent=20:20:1, by vol.) added. The mixture was centrifuged at 1,200×g for 5 min. The absorbance of the supernatant was read at 540 nm against the reagent blank (1 mL acid mixture+5 mL color reagent). The NHI content was calculated from an iron standard curve. The results were expressed as μg/g sample.

Determination of metmyoglobin content

The myoglobin in ground pork was extracted according to the method of Wang et al. (2018). The ground sample (5 g) was mixed with 25 mL phosphate buffer (0.04 M, pH 6.8) and then homogenized at 300×g for 30 s. The mixture was centrifuged at 4℃ at 1,200×g for 30 min and the supernatant was filtered. The filtrate sample was measured for absorbance at 503, 525, 582, and 557 nm. The proportion of metmyoglobin was calculated using the following equation according to the method of Tang et al. (2004).

$$[\text{metmyoglobin}] = -0.159R_1 - 0.085R_2 + 1.262R_3 - 0.520 \quad (2)$$

where $R_1=A_{582}/A_{525}$, $R_2=A_{557}/A_{525}$, $R_3=A_{503}/A_{525}$.

Determination of Soret peak in myoglobin

The absorption spectra of myoglobin solutions (obtained from the section of metmyoglobin content determination) in the range of 380 to 450 nm were measured to monitor the Soret peaks. CARY60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) was used to record the spectra, with a scanning speed of 1,000 nm/min. The phosphate buffer (40 mM, pH 6.8) was used as a blank.

Statistical analysis

The experimental data were analyzed by Excel 2010 and IBM SPSS Statistics Version 19 (IBM, Armonk, NY, USA), and the results were expressed as means±SD. The means were compared by Duncan's multiple range tests ($p<0.05$). A mixed-model ANOVA was used to analyzed the effects of the factors (treatment and storage time) on the variables (CIE L*, CIE a*, CIE b*, TBARS values, HI content, NHI content and metmyoglobin content).

Results and Discussion

Effects of supercritical CO_2 treatment on color values of ground pork

Table 2 showed the effects of treatment and storage time on color values (CIE L*, CIE a*, CIE b*), TBARS values, HI, NHI and metmyoglobin contents. It was found that the effects of treatment, storage time and their interaction were significant ($p<0.05$), which means that the effects were not independent (Beltran et al., 2004).

Table 3 showed the color values of ground pork by various treatments during 9 days of refrigerated storage. The CIE L*, CIE a* and CIE b* values were significantly affected by treatments, storage time and their interaction (Table 2). The CIE L* values of UT sample fluctuated throughout the storage, they were higher at days 5 or 9 than day 1 ($p<0.05$), while no differences were found between days 5 and 9 ($p>0.05$). HT, SCT 1–3 and SCT 5 samples had no significant changes in CIE L* value throughout the storage ($p>0.05$). SCT 4 sample had a higher CIE L* value at day 7 than day 5 ($p<0.05$), while no differences were found between the other days ($p>0.05$). The CIE L* values of SCT 6 sample were higher at days 1 and 3 compared to days 7 and 9 ($p<0.05$), and no differences were found between day 5 and the other days ($p>0.05$). SCT 7 sample had similar CIE L* values during storage, except that a lower value was found at day 9 ($p<0.05$).

The CIE L* values of all ground pork treated with supercritical CO_2 were significantly higher than those of control sample throughout the storage ($p<0.05$), indicating that CIE L* values increased significantly after supercritical CO_2 treatment. Similar results were obtained in our previous research (Huang et al., 2017). Choi et al. (2008) also found that the CIE L* values of porcine *longissimus dorsi* muscle were increased by supercritical CO_2 treatment, and attributed the increase to the sarcoplasmic protein denaturation. HT sample had higher CIE L* value than control sample at day 1 ($p<0.05$), thereafter, no notable difference was observed between both samples ($p>0.05$). These results showed that heat treatment at 40°C for 2 h had hardly any effect on the CIE L* value of ground pork during subsequent storage. This may be due to the small degree of denaturation of myoglobin at the treatment temperature (Thiansilakul et al., 2011).

To investigate the effect of treatment temperature on the color of ground pork during refrigerated storage, the instrumental color values of the samples treated at temperatures of 35°C, 40°C and 45°C under 17.2 MPa for 2 h were compared (SCT 1, 2 and 3). No remarkable differences in CIE L* value were observed between SCT 1 and 2 samples during the storage ($p>0.05$). SCT 3 had similar CIE L* values to SCT 1 and 2 samples during the first 3 days of storage ($p>0.05$), thereafter, it had higher CIE L* values than SCT 1 and 2 until the end of storage ($p<0.05$). The results showed that under supercritical CO_2 treatment at pressure of 17.2 MPa and exposure time of 2 h, increasing the treatment temperature from 35°C to 40°C had no effect on the brightness of the ground pork during subsequent refrigerated storage. However, as the treatment temperature increased further to 45°C, the brightness significantly increased ($p<0.05$). This could be due to the higher degree of the sarcoplasmic

Table 2. Effects of treatment and storage time on color values, TBARS value, heme iron content, non-heme iron content and metmyoglobin content of ground pork

Effects	Color values			TBARS value	Heme iron content	Non-heme iron content	Metmyoglobin content
	CIE L*	CIE a*	CIE b*				
Treatment (T)	**	**	**	**	**	**	**
Storage time (S)	**	**	**	**	**	**	**
T×S	**	**	**	**	**	*	**

* $p<0.05$, ** $p<0.01$.
TBARS, thiobarbituric acid-reactive substances.

Table 3. Effects of different treatments on the color values of ground pork during 9 days of refrigerated storage

Color value	Treatment	Storage time (days)				
		1	3	5	7	9
CIE L*	UT	43.26±0.92^{Be}	44.13±1.05^{ABd}	45.04±1.04^{Ad}	44.51±1.21^{ABd}	45.59±0.58^{Ad}
	HT	45.79±0.17^{Ad}	45.41±0.02^{Ad}	45.67±2.79^{Ad}	44.01±1.43^{Ad}	46.08±1.69^{Ad}
	SCT 1	53.73±1.88^{Aa}	53.90±0.99^{Aa}	53.78±0.82^{Ab}	51.85±1.55^{Abc}	51.35±2.54^{Abc}
	SCT 2	54.51±1.16^{Aa}	54.65±1.90^{Aa}	52.68±2.17^{Ab}	51.95±3.51^{Abc}	51.81±2.41^{Abc}
	SCT 3	55.80±1.1^{Aa}	55.54±1.65^{Aa}	56.70±1.59^{Aa}	55.60±0.29^{Aa}	55.71±0.75^{Aa}
	SCT 4	48.34±0.99^{ABc}	49.45±0.36^{ABc}	47.43±1.38^{Bd}	50.02±2.73^{Ac}	49.70±1.07^{ABc}
	SCT 5	51.46±2.37^{Ab}	51.61±0.33^{Ab}	50.06±1.83^{Ac}	51.85±1.34^{Abc}	49.79±2.09^{Abc}
	SCT 6	55.39±1.91^{Aa}	55.8±1.04^{Aa}	53.62±0.61^{ABb}	51.86±2.00^{Bbc}	52.52±1.01^{Bb}
	SCT 7	54.74±0.40^{Aa}	54.37±1.29^{Aa}	53.44±0.26^{Ab}	53.8±0.53^{Aab}	50.98±1.17^{Bbc}
CIE a*	UT	10.79±1.32^{Aa}	9.85±0.84^{Aab}	10.55±0.88^{Aa}	7.28±0.66^{Bb}	6.60±0.34^{Bab}
	HT	7.83±1.21^{Bc}	6.50±0.10^{Cc}	8.76±1.47^{Abcd}	8.83±0.90^{Aa}	6.78±0.08^{Cab}
	SCT 1	8.67±0.45^{Abc}	8.84±1.23^{Ab}	9.62±0.74^{Aabc}	5.93±0.72^{Bc}	5.34±1.06^{Bc}
	SCT 2	8.97±0.77^{Abc}	9.43±0.67^{Ab}	8.00±0.70^{Bcd}	5.87±0.36^{Bc}	5.49±0.32^{Cc}
	SCT 3	9.62±0.35^{Aab}	8.67±1.39^{Ab}	8.89±0.85^{Aabcd}	7.41±0.27^{Bb}	7.15±0.18^{Bab}
	SCT 4	9.43±0.43^{ABb}	8.58±0.15^{Bb}	9.94±0.41^{Aab}	6.98±1.55^{Cbc}	7.22±0.18^{Ca}
	SCT 5	9.44±0.33^{Ab}	9.59±1.11^{Aab}	8.61±2.45^{ABbcd}	7.00±1.43^{Bbc}	6.85±0.17^{Bab}
	SCT 6	9.26±0.61^{Ab}	9.49±0.22^{Aab}	7.18±0.25^{Bd}	5.65±0.66^{Cc}	5.58±1.13^{Cc}
	SCT 7	8.59±0.85^{Bbc}	10.78±0.62^{Aa}	7.15±0.32^{Cd}	6.16±0.41^{Dbc}	6.22±0.49^{Dc}
CIE b*	UT	6.83±0.87^{Ac}	4.43±0.26^{Bf}	3.70±0.49^{BCe}	2.96±0.44^{Cb}	2.97±0.37^{Cd}
	HT	8.07±0.70^{Abc}	8.39±0.04^{Acd}	3.40±0.55^{Be}	2.27±0.64^{Cb}	2.43±0.57^{Cd}
	SCT 1	9.09±0.71^{Aab}	9.16±1.53^{Aabc}	7.02±0.74^{Bb}	5.57±0.45^{Ca}	6.50±0.29^{BCbc}
	SCT 2	9.41±1.11^{Aab}	10.33±0.22^{Aa}	6.10±0.77^{Bcd}	6.40±0.77^{Ba}	6.33±1.38^{Bb}
	SCT 3	10.20±0.71^{Aa}	9.56±0.41^{Aabc}	8.31±0.63^{Ba}	6.08±0.41^{Ca}	6.51±1.11^{Cb}
	SCT 4	8.10±0.99^{Abc}	6.71±0.86^{Be}	4.20±0.22^{Ce}	4.86±0.35^{Cb}	4.46±0.47^{Cc}
	SCT 5	9.43±0.68^{Aab}	7.61±0.97^{Bde}	5.53±0.43^{Cd}	5.68±1.64^{Cab}	4.65±1.29^{Cbc}
	SCT 6	9.82±0.72^{Aa}	9.93±0.82^{Aab}	7.22±1.13^{Bb}	7.97±0.61^{Ba}	7.17±0.89^{Ba}
	SCT 7	9.70±0.92^{Aa}	8.72±1.04^{Bbcd}	6.69±0.20^{CDbc}	7.55±0.28^{Ca}	6.40±0.26^{Da}

UT: untreatment (control); HT: heat treatment (40°C for 2 h); SCT: supercritical CO_2 treatment (1, 35°C/17.2 MPa/2 h; 2, 40°C/17.2 MPa/2 h; 3, 45°C/17.2 MPa/2 h; 4, 40°C/17.2 MPa/0.5 h; 5, 40°C/17.2 MPa/1 h; 6, 40°C/13.8 MPa/2 h; 7, 40°C/20.7 MPa/2 h).
$^{A–D}$ Different capital letters on the same row indicate significant differences between storage time for the same treatment ($p<0.05$).
$^{a–f}$ Different lowercase letters in the same column indicate significant differences between treatments on the same storage time ($p<0.05$).

protein denaturation in ground pork treated with supercritical CO_2 at 45°C. Similarly, Bak et al. (2012) reported that the brightness of pork *longissimus dorsi* slightly increased as the high-pressure treatment temperature was increased from 5°C to 20°C.

To investigate the effect of treatment pressure on the color of ground pork during refrigerated storage, the instrumental color values of the samples treated under pressures of 13.8, 17.2 and 20.7 MPa at 40°C for 2 h were compared (SCT 6, 2 and 7). Throughout the storage period, SCT 2, 6 and 7 samples have the same CIE L* values ($p>0.05$), but they have higher CIE L* values than HT sample treated at the same temperature ($p<0.05$). These results showed that compared with heat treatment

at the same temperature, supercritical CO_2 treatment at 40℃ for 2 h could significantly increase the brightness of ground pork during the subsequent storage. However, there was no significant change in brightness as the treatment pressure increased from 13.8 MPa to 20.7 MPa ($p>0.05$). Similar results were obtained by Jauhar et al. (2020a) who treated raw chicken meat with different pressures (7.4, 11.4 and 15.4 MPa) of supercritical CO_2 at a low temperature (31℃) for a short duration (10 min) and then stored at 4℃ for seven days.

To investigate the effect of treatment time on the color of ground pork during refrigerated storage, the instrumental color values of the samples treated under 17.2 MPa at 40℃ for 0.5, 1 and 2 h were compared (SCT 4, 5 and 2). SCT 2, 4 and 5 samples had CIE L* values in the following order within the first 5 days of storage: SCT 2>SCT 5>SCT 4. Thereafter, they had similar CIE L* values ($p>0.05$). These results indicated that under supercritical CO_2 treatment at pressure of 17.2 MPa and temperature of 40℃, extending the exposure time from 0.5 h to 2 h could increase the CIE L* value of ground pork during subsequent storage. Thiansilakul et al. (2011) reported that with increasing temperature and incubation time, oxymyoglobin was susceptible to oxidation and conformational changes.

The CIE a* value generally showed a decreasing trend for ground pork throughout the storage ($p<0.05$). The decrease in CIE a* value indicated the loss of CIE a*. This was most likely due to the oxidation of oxymyoglobin or deoxymyoglobin into metmyoglobin, as well as to the denaturation of myoglobin (Bak et al., 2019). SCT 1, 2, 6 and 7 samples generally had lower CIE a* values than UT sample during storage ($p<0.05$). No remarkable differences in CIE a* values were observed between SCT 3 and UT samples throughout the storage ($p>0.05$). SCT 4 sample displayed a lower CIE a* value than UT sample at day 1 ($p<0.05$), thereafter no differences were found between them ($p>0.05$). SCT 5 and HT samples had lower CIE a* values than UT sample within the first 5 days ($p<0.05$). Thereafter, no differences were found ($p>0.05$). These results suggested that except for SCT 3 sample, the other supercritical CO_2 treated samples had some decreased CIE a* values. During supercritical CO_2 treatment, oxidation of oxymyoglobin or deoxymyoglobin to metmyoglobin could occur. Meanwhile, some of the formed metmyoglobin could be reduced back to its ferrous form. It was reported that the amount of reduced metmyoglobin increased with the treated pressure and temperature (Chun et al., 2014). It would be expected that samples treated at higher temperature would have a higher reduction. Thus, SCT 3 sample has a relatively lower metmyoglobin content and higher CIE a* value ($p<0.05$).

There are no remarkable differences in CIE a* value between SCT 1 and 2 samples during the storage ($p>0.05$). SCT 3 had CIE a* values similar to those of SCT 1 and 2 samples during the first 5 days of storage ($p>0.05$), thereafter, a higher CIE a* value was observed ($p<0.05$). These results showed that under supercritical CO_2 treatment at pressure of 17.2 MPa and exposure time of 2 h, increasing the treatment temperature from 35℃ to 40℃ had no effect on the CIE a* of the ground pork during subsequent refrigerated storage. However, as the treatment temperature increased further to 45℃, the CIE a* increased to some extent. It appears that supercritical CO_2 treatment at 45℃ could maintain the CIE a* of the ground pork during subsequent refrigerated storage. Higher treatment temperatures increased the amount of reduced metmyoglobin (Chun et al., 2014), resulting in more retention of CIE a* values.

No notable differences in CIE a* values were displayed between SCT 4 and 5 samples throughout the storage ($p>0.05$). Similar CIE a* values were observed between SCT 2 and 5 samples during 7 days of storage ($p>0.05$), while a higher CIE a* value was found at day 9 for SCT 5 sample ($p<0.05$). No remarkable differences in CIE a* values were observed between SCT 2 and 4 samples during the first 3 days ($p>0.05$), thereafter a higher CIE a* value was found in SCT 4 sample until the end of storage ($p<0.05$). The results suggested that as the treatment time of supercritical CO_2 was extended from 0.5 h to 2 h, the CIE a* value of the ground pork decreased to some extent during subsequent storage. This was likely due to more

denaturation of myoglobin by longer treatment time (Thiansilakul et al., 2011).

During storage, the CIE a* values of SCT 2, 6 and 7 samples were similar ($p>0.05$). Three samples had higher CIE a* values than HT sample during the first three days ($p<0.05$), similar CIE a* values at day 5 ($p>0.05$), and lower CIE a* values during 7–9 days of storage ($p<0.05$). These results indicate that the CIE a* values of ground pork treated with supercritical CO_2 decreases faster than that of HT sample treated at the same temperature during subsequent storage. The CIE a* value was not changed as the treatment pressure increased from 13.8 MPa to 20.7 MPa. These result are consistent with the findings of Jauhar et al. (2020a), who treated raw chicken meat with different pressures (7.4, 11.4 and 15.4 MPa) of supercritical CO_2 at 31℃ for 10 min and then stored at 4℃ for 7 days.

The CIE b* values of all the samples gradually reduced with increasing storage time. This results agree with the findings of Villamonte et al. (2017) who observed that the CIE b* of the UT pork batters decreased with refrigerated storage. Similarly, de Alba et al. (2012) found that CIE b* values decreased during storage in sliced dry-cured ham treated at 400, 500 and 600 MPa for 5 min at 12℃ and then stored at 8℃ during 60 d. They attributed the change in CIE b* values to an altered chemical state of myoglobin. All samples treated with supercritical CO_2 had significantly higher CIE b* values than the control sample (UT) during storage ($p<0.05$), except that SCT 4 sample had higher CIE b* values than UT sample at days 3 and 9 ($p<0.05$), and similar values at the other days ($p>0.05$). It appears that after supercritical CO_2 treatment under different conditions, the ground pork had increased CIE b* values during subsequent storage. Jauhar et al. (2020b) found that after treated with supercritical CO_2 at 14 MPa and 45℃ for 40 min, the fresh chicken meat exhibited higher CIE L* and CIE b*, and lower CIE a* during 7 days of refrigerated storage.

During storage, similar CIE b* values were observed among SCT 1, 2 and 3 samples ($p>0.05$), except for day 5, in which SCT 3 exhibited higher CIE b* values than SCT 1 and 2 samples ($p<0.05$). These results showed that under the pressure of 17.2 MPa and exposure time of 2 h, increasing the treatment temperature from 35℃ to 45℃ could increase the CIE b* of ground pork to some extent during subsequent storage. On the contrary, McArdle et al. (2010) reported that bovine *M. pectoralis profundus* HP pressurised at 40℃ had lower CIE b* values than that processing at 20℃, regardless of the pressure. The inconsistency in CIE b* values may stems primarily from the original form of myoglobin (Bolumar et al., 2021). Since the ground pork used in our study was subjected to a freeze-thaw cycle before supercritical CO_2 treatment, metmyoglobin would be the most abundant form in the treated ground pork (Coria-Hernández et al., 2020).

SCT 2 had significantly higher CIE b* value than SCT 4 throughout the storage ($p<0.05$). Similar CIE b* values were observed between SCT 4 and 5 samples during storage ($p>0.05$), except that a higher CIE b* value was found in SCT 5 sample at day 5 ($p<0.05$). SCT 2 had a higher CIE b* value than SCT 5 at day 3 ($p<0.05$). No significant difference was found between the two samples at the other days ($p>0.05$). These results indicated that under supercritical CO_2 treatment at pressure of 17.2 MPa and temperature of 40℃, extending the exposure time from 0.5 h to 2 h could increase the CIE b* of ground pork to some extent during subsequent storage. Increase in the CIE b* may be related to the oxidation of metmyoglobin. The oxidation is favoured as time increase (Domínguez et al., 2019).

Throughout the storage, SCT 2, 6 and 7 samples have significantly higher CIE b* values than HT sample treated at the same temperature ($p<0.05$), indicating that the CIE b* of meat samples increased after supercritical CO_2 treatment at 40℃ under different pressure for 2 h. No remarkable differences in CIE b* values were displayed between SCT 6 and 7 samples throughout the storage ($p>0.05$). Similar CIE b* values were observed between SCT 2 and 7 samples during storage ($p>0.05$), except that a higher CIE b* value was found in SCT 7 sample at day 9 ($p<0.05$). SCT 6 sample had higher CIE b* values than SCT 2 sample at days 5 and 9 ($p<0.05$), while no notable differences were observed between both samples at the other days

($p>0.05$). These results indicated that under supercritical CO_2 treatment at 40°C for 2 h, increasing treatment pressure from 13.8 MPa to 17.2 MPa could decrease the CIE b* of ground pork to some extent during the subsequent storage. As the treatment pressure increased further to 20.7 MPa, the CIE b* was almost unchanged. Our results are in agreement with those of Jauhar et al. (2020a), who observed that minimal changes in the CIE b* between chicken meat samples treated with three different pressures.

Effects of supercritical CO_2 treatment on lipid oxidation in ground pork

Table 4 displayed the TBARS values of ground pork with various treatments during 9 days of refrigerated storage. TBARS was often used to measure lipid oxidation secondary products, and to indicate the degree of lipid oxidation. The TBARS values of UT sample gradually increased during the first 3 days of storage, thereafter, the values were kept unchanged until the end of storage period. Gradual increase in TBARS value was also found in SCT 2, 6 and 7 samples with increasing storage time up to 5 days. Thereafter, no change was observed. For HT, SCT 1 and SCT 3–5 samples, TBARS value gradually increased to the maximum and then decreased with the increase of storage time. HT and SCT 1 samples had the maximum values on day 7, while SCT 3–5 samples reached the values on day 5. The decrease in TBARS value indicates the decomposition of secondary lipid oxidation products (Bolumar et al., 2016).

No remarkable differences in TBARS value were observed between SCT 4, 5 and UT ($p>0.05$) while SCT 3 had a higher value than UT throughout the storage ($p<0.05$). The UT, HT and SCT 1 samples had similar TBARS values during storage ($p>0.05$), except that the UT sample had a lower TBARS value on day 7 ($p<0.05$). TBARS values were not significantly different between SCT 2, 6, 7 and UT during the first 3 days of storage ($p>0.05$) and significant differences were found thereafter with UT having a lower value ($p<0.05$). These results showed that supercritical CO_2 treatment at 17.2 MPa, 40°C for 0.5 h or 1 h had no effect on lipid oxidation of ground pork during subsequent storage. Supercritical CO_2 treatment at 17.2 MPa, 35°C or 40°C for 2 h, and 13.8 MPa or 20.7 MPa, 40°C for 2 h had some accelerated effect on lipid oxidation. The most damaging supercritical CO_2 treatment for lipid oxidation is the treatment at 17.2 MPa, 45°C for 2 h. Supercritical CO_2

Table 4. Effects of different treatments on TBARS values (mg malondialdehyde/kg) of ground pork during 9 days of refrigerated storage

Treatment	Storage time (days)				
	1	3	5	7	9
UT	0.14 ± 0.02^{Bb}	0.23 ± 0.02^{Ab}	0.24 ± 0.02^{Ad}	0.23 ± 0.02^{Ac}	0.24 ± 0.04^{Acd}
HT	0.13 ± 0.02^{Db}	0.20 ± 0.01^{Cb}	0.25 ± 0.02^{Bd}	0.34 ± 0.04^{Ab}	0.23 ± 0.01^{Ccd}
SCT 1	0.13 ± 0.02^{Cb}	0.22 ± 0.03^{BCb}	0.29 ± 0.11^{Bd}	0.39 ± 0.03^{Ab}	0.28 ± 0.07^{Bc}
SCT 2	0.18 ± 0.02^{Ba}	0.25 ± 0.07^{Bb}	0.39 ± 0.08^{Ac}	0.36 ± 0.05^{Ab}	0.38 ± 0.02^{Ab}
SCT 3	0.18 ± 0.04^{Ca}	0.35 ± 0.05^{Ba}	0.61 ± 0.04^{Aa}	0.58 ± 0.12^{Aa}	0.38 ± 0.02^{Bb}
SCT 4	0.12 ± 0.02^{Cb}	0.22 ± 0.03^{Bb}	0.27 ± 0.02^{Ad}	0.23 ± 0.04^{ABc}	0.22 ± 0.02^{Bcd}
SCT 5	0.12 ± 0.02^{Cb}	0.23 ± 0.01^{Bb}	0.29 ± 0.05^{Ad}	0.21 ± 0.03^{Bc}	0.19 ± 0.03^{Bd}
SCT 6	0.14 ± 0.01^{Cb}	0.26 ± 0.08^{Bb}	0.47 ± 0.08^{Ab}	0.50 ± 0.13^{Aa}	0.54 ± 0.00^{Aa}
SCT 7	0.13 ± 0.03^{Cb}	0.25 ± 0.04^{Bb}	0.42 ± 0.03^{Abc}	0.38 ± 0.02^{Ab}	0.39 ± 0.06^{Ab}

UT: untreatment (control); HT: heat treatment (40°C for 2 h); SCT: supercritical CO_2 treatment (1, 35°C/17.2 MPa/2 h; 2, 40°C/17.2 MPa/2 h; 3, 45°C/17.2 MPa/2 h; 4, 40°C/17.2 MPa/0.5 h; 5, 40°C/17.2 MPa/1 h; 6, 40°C/13.8 MPa/2 h; 7, 40°C/20.7 MPa/2 h).
$^{A–D}$ Different capital letters on the same row indicate significant differences between storage time for the same treatment ($p<0.05$).
$^{a–d}$ Different lowercase letters in the same column indicate significant differences between treatments on the same storage time ($p<0.05$).
TBARS, thiobarbituric acid-reactive substances.

treatment was found to accelerate lipid oxidation of ground pork during subsequent refrigerated storage under some combinations of treatment pressure, temperature and time. Lipid oxidation promoted by supercritical CO_2 treatment varied primarily with treatment temperature and time, and to a lesser degree with treatment pressure.

SCT 3 had higher TBARS values than SCT 1 throughout the storage and than SCT 2 during 3–7 days of storage ($p<0.05$). No remarkable difference was found between SCT 3 and SCT 2 at the other days ($p>0.05$). SCT 2 had higher TBARS values than SCT 1 at days 5 and 9 ($p<0.05$), and similar values were observed at the other days ($p>0.05$). These results indicated that under supercritical CO_2 treatment at pressure of 17.2 MPa and exposure time of 2 h, increasing the treatment temperature from 35℃ to 45℃ could promote lipid oxidation of ground pork during subsequent storage. Similar results were obtained by Ma et al. (2006) who treated beef with high pressure at different temperatures. Since lipid oxidation is a temperature-dependent reaction, it would be expected that higher temperatures would lead to faster oxidation rates (Huang et al., 2019).

SCT 4 and SCT 5 had similar TBARS values during the whole storage ($p>0.05$). They had significantly lower TBARS values than SCT 2 during 5–9 days of storage ($p<0.05$), while similar TBARS values were found at the other days ($p>0.05$). These results showed that under supercritical CO_2 treatment at pressure of 17.2 MPa and temperature of 40℃, extending the treatment time from 0.5 h to 1 h had no effect on lipid oxidation during subsequent storage. As the treatment time increased further to 2 h, the lipid oxidation was accelerated to some extent. Jauhar et al. (2020a) also found that treating raw chicken meat with supercritical CO_2 at 31℃ for a short duration (10 min) had no significant effect on lipid peroxidation, regardless of the treatment pressure.

No remarkable differences in TBARS value were observed between SCT 6 and HT samples during the first 3 days of storage ($p>0.05$), thereafter SCT 6 had a higher value until the end of storage ($p<0.05$). Similar TBARS values were found between SCT 2 and HT samples at days 3 and 7 ($p>0.05$), while higher values were found for SCT 2 at the other days ($p<0.05$). SCT 7 had higher TBARS values than HT at days 5 and 9 ($p<0.05$). No remarkable difference was observed between both samples at the other days ($p>0.05$). SCT 2 sample had a higher TBARS value than SCT 6 sample at day 1 ($p<0.05$), no remarkable differences at day 3 ($p>0.05$), and significantly lower values until the end of storage ($p<0.05$). SCT 2 and 7 samples had similar TBARS values during storage ($p>0.05$), except that SCT 2 had a higher TBARS value on day 1 ($p<0.05$). The TBARS values of SCT 6 and 7 samples did not differ significantly over the 5-day storage ($p>0.05$). Thereafter, SCT 6 had significantly higher values until the end of storage ($p<0.05$). These results showed that compared with heat treatment at the same temperature, supercritical CO_2 treatment at 40℃ for 2 h could promote the lipid oxidation of ground pork to some extent during the subsequent storage. Increasing the treatment pressure from 13.8 MPa to 20.7 MPa could retard the lipid oxidation to some extent. Ma et al. (2007) studied lipid oxidation in beef treated with high hydrostatic pressure (0.1–800 MPa) at different temperatures (20℃–70℃) for 20 min during subsequent storage at 4℃ for 7 days. They found that after treatment at 60℃ and 70℃, lipid oxidation appeared to be reduced as the pressure rose from 600 MPa to 800 MPa. Jauhar et al. (2020a) processed raw chicken meat with supercritical CO_2 at 7.4–15.4 MPa, 31℃ for 10 min and then stored at 4℃ for seven days, they found that the treatment did not change the TBARS values of the meat during the subsequent storage, regardless of the treatment pressure. They attributed the lack of changes in lipid peroxidation to the removal of visible fat from the chicken samples, thereby limiting the oxidation process.

Effects of supercritical CO_2 treatment on heme iron content of ground pork

Table 5 showed the HI contents in ground pork of various treatments during subsequent refrigerated storage. After treatment, the HI contents of the samples varied between 14.12±2.49 and 20.60±1.11 μg/g sample. In general, HI content

Table 5. Effects of different treatments on the heme iron content (μg/g) of ground pork during 9 days of refrigerated storage

Treatment	Storage time (days)				
	1	3	5	7	9
UT	17.17±2.08^{Aabc}	13.55±2.37^{Babc}	14.21±2.59^{ABa}	11.69±1.95^{Bab}	6.13±2.25^{Cc}
HT	16.04±1.49^{Abc}	14.33±1.42^{Babc}	13.35±1.15^{Bab}	13.34±1.98^{Ba}	11.34±1.57^{Cb}
SCT 1	14.12±2.49^{Ac}	10.80±4.67^{ABbc}	9.36±2.75^{BCb}	6.19±0.71^{Cd}	6.73±0.19^{BCc}
SCT 2	17.56±2.89^{Aabc}	13.15±3.03^{ABabc}	14.07±2.33^{ABa}	11.26±3.64^{Babc}	9.96±3.06^{Bb}
SCT 3	15.07±3.06^{ABc}	17.89±7.62^{Aa}	11.03±3.30^{Bab}	10.49±0.19^{Babc}	15.23±2.32^{ABa}
SCT 4	19.65±2.79^{Aab}	13.27±0.87^{Babc}	9.37±1.16^{Cb}	9.18±1.54^{Cbc}	10.29±2.16^{Cb}
SCT 5	20.60±1.11^{Aa}	10.42±1.84^{BCc}	13.53±5.36^{Bab}	8.53±2.08^{Ccd}	10.74±2.07^{BCb}
SCT 6	15.82±1.60^{Ac}	15.65±0.40^{Aab}	12.30±3.09^{Bab}	9.96±1.30^{Bbc}	10.09±2.52^{Bb}
SCT 7	19.73±2.93^{Aa}	13.67±1.06^{Babc}	14.60±3.49^{Ba}	9.52±2.25^{Cbc}	12.22±1.41^{BCb}

UT: untreatment (control); HT: heat treatment (40°C for 2 h); SCT: supercritical CO_2 treatment (1, 35°C/17.2 MPa/2 h; 2, 40°C/17.2 MPa/2 h; 3, 45°C/17.2 MPa/2 h; 4, 40°C/17.2 MPa/0.5 h; 5, 40°C/17.2 MPa/1 h; 6, 40°C/13.8 MPa/2 h; 7, 40°C/20.7 MPa/2 h).
$^{A–C}$ Different capital letters on the same row indicate significant differences between storage time for the same treatment ($p<0.05$).
$^{a–d}$ Different lowercase letters in the same column indicate significant differences between treatments on the same storage time ($p<0.05$).

gradually decreased with the increase of storage time. This may be due to the release of free iron from heme or the interaction between heme pigments and muscle components, e.g., myofibrillar proteins and/or cellular membranes (Zareian et al., 2019).

During storage, HT, SCT 2–3 and SCT 6–7 samples had similar HI contents as UT sample ($p>0.05$), except for day 9, in which a lower content was found in UT sample ($p<0.05$). Compared to UT sample, significantly lower HI contents were observed at day 5 for SCT 4, at day 7 for SCT 5 and at days 5 and 7 for SCT 1 ($p<0.05$). However, significantly higher contents were found at day 9 for SCT 4 and 5 samples ($p<0.05$). No significant differences were observed among these samples at the other days ($p>0.05$). It seems that supercritical CO_2 treatment at 40°C or above for 2 h could protect heme molecules from degradation to some extent, regardless of treatment pressure. It was reported that oxymyoglobin was more prone to pressure-induced denaturation than deoxymyoglobin in aqueous solution (Ogunmola et al., 1977). Therefore, it is reasonable to assume that the deoxymyoglobin percentage would be higher in supercritical CO_2 treated ground pork than in control sample. The HI in deoxymyoglobin was tightly wrapped in the protein. No ligand was bound at the sixth coordination bond of porphyrin iron, and therefore there was no pull of ligand, causing the near side histidine pulled the iron ions out of the porphyrin ring (Zhang et al., 2021). As a result, the hydrophobic pocket structure of protein was maintained (Das et al., 2020), and the heme was protected from supercritical CO_2 treatment.

No significant difference in the HI content was found between SCT 1 and 2 within the first 3 days of storage ($p>0.05$). However, SCT 2 had a higher content throughout the subsequent storage period ($p<0.05$). There was no significant difference in HI content between SCT 1 and 3 on the first day of storage ($p>0.05$). Thereafter, SCT 3 had the higher content ($p<0.05$). During storage, similar HI contents were observed between SCT 2 and 3 samples ($p>0.05$), except for day 9, in which a higher content was found in SCT 3 sample ($p<0.05$). The results suggested that under the pressure of 17.2 MPa and exposure time of 2 h, increasing the treatment temperature from 35°C to 45°C could increase the HI content of ground pork to some extent during subsequent storage. This may be explained by the increased percentage of deoxymyoglobin in the ground pork due to the increased treatment temperature (Zhang et al., 2021).

Compared with HT samples, the HI content of SCT 2 was not significantly different throughout the storage period ($p>0.05$), while SCT 6 and 7 had significantly lower contents at day 7, SCT 7 had significantly higher content at day 1

($p<0.05$). SCT 2 had a HI content similar to that of SCT 6 or 7 throughout the storage period ($p>0.05$). SCT 7 had a higher HI content than SCT 6 at day 1 ($p<0.05$). Thereafter, there are no significant differences between both samples ($p>0.05$). These results showed that compared with heat treatment at the same temperature, supercritical CO_2 treatment at 40°C for 2 h had slight effect on the HI content of ground pork during the subsequent storage. The HI content was almost unchanged as the treatment pressure increased from 13.8 MPa to 20.7 MPa. It is possible that the degree of myoglobin denaturation did not change as the treatment pressure increased from 13.8 MPa to 20.7 MPa. Choi et al. (2008) found that the extent of sarcoplasmic protein denaturation was similar in 7.4 and 15.2 MPa treated pork *longissimus dorsi* muscle.

The HI contents of SCT 5 were not significantly different from those of SCT 2 and 4 throughout the storage period ($p>0.05$), while a significantly higher content was found at day 5 for SCT 2 compared to SCT 4 ($p<0.05$). These results indicated that under the pressure of 17.2 MPa and temperature of 40°C, extending exposure time from 0.5 h to 2 h could increase the HI content of ground pork to a certain extent during subsequent storage. It is possible that the longer the exposure time, the greater the conformational change of myoglobin, leading to the release of heme (Thiansilakul et al., 2011).

Effects of supercritical CO_2 treatment on non-heme iron content of ground pork

Table 6 showed the NHI contents in ground pork with different treatments during refrigerated storage. For HT and SCT 1–3 samples, the NHI contents decreased gradually with the increase of storage time. Slight but not significant increases in the NHI content were observed for UT and SCT 4–7 samples as the storage time increased from day 1 to day 3 ($p>0.05$), followed by a gradual decrease thereafter. A decrease in NHI content was also found by Schiell et al. (2023) in iron-rich 3D-printed hybrid food products (composed mainly of pork and chicken liver and red lentils) baked and packed under two different modified atmospheres during 21 days of storage at 4°C. They speculated that the 21-day follow-up period may not have been sufficient to observe the increase in NHI content.

No significant differences in the NHI content were observed between SCT 4, 6 and UT samples throughout the storage ($p>0.05$). However, compared to UT sample, higher contents were detected in SCT 1 at day 1, SCT 5 at day 5, SCT 3 and 7

Table 6. Effects of different treatments on the non-heme iron content (μg/g) of ground pork during 9 days of refrigerated storage

Treatment	Storage time (days)				
	1	3	5	7	9
UT	1.21±0.14^{ABde}	1.41±0.07^{Aab}	1.03±0.09^{Bcd}	1.13±0.17^{ABbc}	1.30±0.33^{ABa}
HT	1.13±0.07^{Ae}	1.11±0.08^{Ac}	1.02±0.00^{ABd}	1.09±0.14^{Abc}	0.95±0.25^{Bb}
SCT 1	1.40±0.13^{Aab}	1.25±0.18^{ABbc}	1.07±0.12^{BCbcd}	1.12±0.06^{BCbc}	0.96±0.29^{Cb}
SCT 2	1.37±0.11^{Aabc}	1.36±0.09^{ABab}	1.20±0.03^{Babc}	1.31±0.12^{ABa}	1.23±0.10^{ABab}
SCT 3	1.43±0.08^{Aa}	1.40±0.05^{Aab}	1.27±0.09^{ABa}	1.12±0.13^{Bbc}	1.11±0.29^{Bab}
SCT 4	1.27±0.07^{ABbcde}	1.31±0.09^{Aab}	1.17±0.06^{ABCabcd}	1.11±0.07^{BCbc}	1.02±0.19^{Cab}
SCT 5	1.29±0.02^{ABbcd}	1.35±0.07^{Aab}	1.24±0.31^{ABab}	1.04±0.10^{Bc}	1.25±0.17^{ABab}
SCT 6	1.26±0.07^{ABcde}	1.38±0.19^{Aab}	1.14±0.03^{BCabcd}	1.07±0.10^{Cbc}	1.08±0.09^{Cab}
SCT 7	1.37±0.07^{ABabc}	1.48±0.17^{Aa}	1.29±0.12^{ABa}	1.26±0.15^{Bab}	1.23±0.10^{Bab}

UT: untreatment (control); HT: heat treatment (40°C for 2 h); SCT: supercritical CO_2 treatment (1, 35°C/17.2 MPa/2 h; 2, 40°C/17.2 MPa/2 h; 3, 45°C/17.2 MPa/2 h; 4, 40°C/17.2 MPa/0.5 h; 5, 40°C/17.2 MPa/1 h; 6, 40°C/13.8 MPa/2 h; 7, 40°C/20.7 MPa/2 h).
$^{A–C}$ Different capital letters on the same row indicate significant differences between storage time for the same treatment ($p<0.05$).
$^{a–e}$ Different lowercase letters in the same column indicate significant differences between treatments on the same storage time ($p<0.05$).

samples at day 1 and 5, and SCT 2 at day 1 and 7; while lower contents were found in HT at day 3 and 9 ($p<0.05$). These results suggested that supercritical CO_2 treatment under certain conditions could promote the release of NHI. Under these conditions, the denaturation of myoglobin may occur (Choi et al., 2008), causing the release of free iron called "non-heme iron" (Wang et al., 2023). The released amount varies with the degree of denaturation.

SCT 1 sample had a lower NHI content than SCT 3 sample at day 5 ($p<0.05$). SCT 2 sample had a higher content than SCT 1 and 3 samples at day 7 ($p<0.05$). No significant differences were displayed among these samples at the other days ($p>0.05$). These results showed that under the pressure of 17.2 MPa and exposure time of 2 h, treatment at 40℃ appeared to increase the NHI content of ground pork more than treatment at 35℃ or 45℃ during subsequent storage. As mentioned above, elevated temperature could facilitate the denaturation of myoglobin. However, the thermal denaturation would be suppressed by pressure at the unfolding temperatures of myoglobin (Fernández-Martín et al., 1997). Therefore, samples treated at 40℃ had a relatively higher NHI content than those treated at 45℃ during subsequent storage.

Similar NHI contents were found among SCT 2, 4 and 5 samples during the storage ($p>0.05$), except for day 7, in which a higher content was detected in SCT 2 sample ($p<0.05$). These results indicated that under supercritical CO_2 treatment at pressure of 17.2 MPa and temperature of 40℃, extending the exposure time from 0.5 h to 1 h had no effect on the NHI content of ground pork during subsequent storage. As the exposure time increased further to 2 h, the NHI content was increased to some extent. Jalarama Reddy et al. (2015) treated chevon meat piece with high hydrostatic pressure at 300 and 600 MPa for 5 and 10 min at 28±2℃, and observed that processing time did not impart any significant ($p>0.05$) changes in NHI.

During storage, SCT 2 and 7 samples had higher NHI contents than HT sample ($p<0.05$), except for day 9, in which a similar content was found among these samples ($p>0.05$). SCT 6 had a higher NHI content than HT at day 3 ($p<0.05$). No significant difference was observed between the two samples at the other days ($p>0.05$). SCT 6 had a lower content than SCT 2 and 7 at day 7 ($p<0.05$), while no significant differences were observed among these samples at the other days ($p>0.05$). These results showed that compared with heat treatment at the same temperature, supercritical CO_2 treatment at 40℃ for 2 h could increase the NHI content of ground pork to some extent during the subsequent storage. The treatment pressure exerted an additional effect, increasing the pressure from 13.8 MPa to 20.7 MPa could increase the NHI content to a certain extent. Jalarama Reddy et al. (2015) found that the NHI in chevon meat increased significantly when the treatment pressure increased from 300 MPa to 600 MPa.

Effects of supercritical CO_2 treatment on metmyoglobin content of ground pork

Metmyoglobin in meat results from the oxidation of ferrous myoglobin (deoxymyoglobin and oxymyoglobin). The metmyoglobin can be further oxidized to hypervalent myoglobin species (such as perferrylmyoglobin and ferrylmyoglobin) in the presence of hydrogen peroxide or hydroperoxide (Wongwichian et al., 2015), which can promote lipid oxidation (Chaijan, 2008). In addition, the metmyoglobin can also be reduced to deoxymyoglobin and oxymyoglobin in the presence of metmyoglobin-reducing system (Alonso et al., 2016).

Table 7 showed the metmyoglobin contents in ground pork by various treatments during refrigerated storage. In general, the metmyoglobin content showed a decreasing trend for ground pork from different treatments over the storage period, indicating the metmyoglobin may be further oxidized or reduced back to deoxymyoglobin and oxymyoglobin. No significant differences in metmyoglobin content were observed between SCT 1, HT and UT throughout the storage ($p<0.05$). SCT 3 had a lower content than UT during the storage ($p<0.05$), except for day 9, in which a similar content was found ($p>0.05$). SCT 2,

Table 7. Effects of different treatments on the metmyoglobin content (%, w/w) of ground pork during 9 days of refrigerated storage

Treatment	Storage time (days)				
	1	3	5	7	9
UT	62.27±1.33^{Aab}	59.86±0.71^{Aabc}	60.23±0.77^{Aa}	57.83±3.18^{Aa}	53.18±5.30^{Ba}
HT	61.51±0.71^{Aab}	58.12±0.28^{Bcd}	58.15±0.83^{Ba}	56.81±0.99^{Cab}	54.86±0.94^{Da}
SCT 1	61.15±1.40^{Aabc}	60.32±2.32^{Aab}	57.45±0.79^{Bab}	55.35±2.17^{BCab}	53.68±1.37^{Ca}
SCT 2	60.76±1.43^{Abc}	58.58±0.57^{Abcd}	48.14±2.14^{Bd}	49.96±3.66^{Bcd}	50.04±4.84^{Ba}
SCT 3	59.15±3.22^{Ac}	53.68±0.69^{ABe}	42.54±5.79^{Ce}	47.86±5.00^{BCd}	52.37±6.58^{ABa}
SCT 4	63.26±0.49^{Aa}	60.65±0.43^{Aa}	51.50±1.26^{Bcd}	50.43±2.40^{Bcd}	50.95±3.12^{Ba}
SCT 5	62.12±0.43^{Aab}	58.91±2.31^{Aabc}	50.87±0.96^{Bd}	49.68±3.16^{Bcd}	49.76±3.51^{Ba}
SCT 6	63.18±0.21^{Aa}	56.97±0.50^{Bd}	54.30±1.37^{Cbc}	50.52±2.72^{Dcd}	51.22±2.25^{Da}
SCT 7	61.53±1.32^{Aab}	59.63±0.34^{Babc}	52.38±0.58^{Dc}	53.02±2.33^{Dbc}	55.93±0.50^{Ca}

UT: untreatment (control); HT: heat treatment (40°C for 2 h); SCT: supercritical CO_2 treatment (1, 35°C/17.2 MPa/2 h; 2, 40°C/17.2 MPa/2 h; 3, 45°C/17.2 MPa/2 h; 4, 40°C/17.2 MPa/0.5 h; 5, 40°C/17.2 MPa/1 h; 6, 40°C/13.8 MPa/2 h; 7, 40°C/20.7 MPa/2 h).
$^{A–D}$ Different capital letters on the same row indicate significant differences between storage time for the same treatment ($p<0.05$).
$^{a–e}$ Different lowercase letters in the same column indicate significant differences between treatments on the same storage time ($p<0.05$).

4, 5 and 7 samples had lower metmyoglobin contents than UT sample at days 5 and 7 ($p<0.05$), and similar contents were found at the other days ($p>0.05$). There are no significant differences in metmyoglobin contents between SCT 6 and UT samples at days 1 and 9 ($p>0.05$), while lower contents were found for SCT 6 at the other days ($p<0.05$). These results showed that supercritical CO_2 treatment at 40°C or above reduced the metmyoglobin content of in ground pork. Supercritical CO_2 treatment at 40°C or above accelerated lipid oxidation in ground pork, and the produced hydroperoxides caused metmyoglobin to be further oxidized (Wongwichian et al., 2015).

No significant differences in metmyoglobin content were observed between SCT 1, 2 and 3 samples at days 1 and 9 ($p>0.05$). SCT 1 and 2 samples had higher metmyoglobin contents than SCT 3 sample during 3–7 days of storage ($p<0.05$). The metmyoglobin contents of SCT 1 sample were higher than those of SCT 2 sample at days 5 and 7 ($p<0.05$), and no significant differences were observed at the other days ($p>0.05$). These results showed that supercrirical CO_2 treatment at different temperatures with a constant pressure of 17.2 MPa and exposure time of 2 h had some effect on the metmyoglobin content of ground pork during subsequent storage. It appears that higher treatment temperatures favor the oxidation of metmyoglobin during subsequent storage. This is often seen in oxidation reactions, since oxidation is favoured as temperature increase (Domínguez et al., 2019).

SCT 5 sample has similar metmyoglobin content with SCT 2 and 4 samples within the first 3 days of storage ($p>0.05$), whereas SCT 2 has a lower content than SCT 4 ($p<0.05$). Thereafter, similar metmyoglobin contents were observed among these samples ($p>0.05$). The results indicated that treatments with supercritical CO_2 at 17.2 MPa and 40°C for different time had some effects on the metmyoglobin content of ground pork during subsequent storage. Treatment for 2 h could enhance the oxidation of metmyoglobin during subsequent storage.

No significant differences in metmyoglobin content were observed between SCT 2, 6, 7, and HT within the first 3 days of storage ($p>0.05$) and significant differences were found at days 5 and 7, with HT having a higher value ($p<0.05$). SCT 6 sample had higher metmyoglobin contents than SCT 2 at days 1 and 5, and had a lower content than SCT 7 at day 3 ($p<0.05$). Whereas, SCT 2 had a lower metmyoglobin content than SCT 7 at day 5 ($p<0.05$). These results showed that compared with heat treatment at the same temperature, supercritical CO_2 treatment at 40°C for 2 h could promote the oxidation of

metmyoglobin in ground pork to some extent during the subsequent storage. The promotion effect seems to be stronger at the treatment pressure of 17.2 MPa. Supercritical CO_2 could penetrate and then accumulate in ground meat. The solubilization rate and total solubility of CO_2 are governed by pressure, higher pressures enhance CO_2 solubilization and solubility (Ferrentino et al., 2013). The dissolved CO_2 could prevent the easily oxidized components of the meat from oxidation to a certain extent during storage. On the other hand, as mentioned above, supercritical CO_2 could cause metmyoglobin to be oxidized. It is possible that the combination of these two effects results in greater oxidation of metmyoglobin at 17.2 MPa.

Effects of supercritical CO_2 treatment on Soret peak of myoglobin from ground pork

A Soret band reflects the interaction of the haem moiety with apomyoglobin and can be applied to detect the unfolding of haem proteins (Benjakul and Bauer, 2001). Changes in wavelengths of Soret peak of myoglobin solutions from ground pork by different treatments during refrigerated storage are shown in Table 8. At day 1, HT, SCT 1, 3 and 4 samples had the Soret peaks at wavelengths of 415, 412, 411 and 407 nm, respectively. Whereas, SCT 2, 5, 6, and 7 samples had the same Soret peaks as UT sample. When the storage time was increased to day 9, the wavelengths of the Soret peaks for SCT 2, 5, 6, 7 and UT samples gradually increased from 410 nm to 419 nm, 417 nm, 416 nm, 420 nm and 415 nm, respectively. While the wavelengths for SCT 3 and 4 samples gradually increased from 411 and 407 nm to 418 nm, respectively. However, the wavelengths for SCT 1 and HT samples increased gradually only up to the fifth day of storage, thereafter decreased until the end of storage.

It was reported that the Soret peaks for deoxymyoglobin, oxymyoglobin and metmyoglobin in meat were at 434, 416 and 410 nm, respectively (Swatland, 1989). Ferrylmyoglobin had a Soret peak at 424 nm (Baron et al., 2000). Changes in the Soret wavelength to a higher number (410 to 420 nm) for the treated sample suggested that metmyoglobin may be gradually converted to ferrylmyoglobin (Thiansilakul et al., 2012b).

In general, the intense absorption peak gradually decreased for all samples with storage time (data not shown). This indicated that the heme protein may be disrupted or the porphyrin was detached from globin (Wongwichian et al., 2015). During storage, radicals produced by lipid oxidation can denature haem proteins to release the haem group. The released haem was readily localized in phospholipid membrane, promoting lipid oxidation (Thiansilakul et al., 2012a).

Table 8. Effects of different treatments on the Soret peak (nm) of myoglobin from ground pork during 9 days of refrigerated storage

Treatment	Storage time (days)				
	1	3	5	7	9
UT	410	411	414	412	415
HT	415	413	415	414	413
SCT 1	412	416	416	415	414
SCT 2	410	415	417	418	419
SCT 3	411	410	417	418	418
SCT 4	407	412	417	417	418
SCT 5	410	413	417	416	417
SCT 6	410	413	416	416	416
SCT 7	410	410	418	421	420

UT: untreatment (control); HT: heat treatment (40°C for 2 h); SCT: supercritical CO_2 treatment (1, 35°C/17.2 MPa/2 h; 2, 40°C/17.2 MPa/2 h; 3, 45°C/17.2 MPa/2 h; 4, 40°C/17.2 MPa/0.5 h; 5, 40°C/17.2 MPa/1 h; 6, 40°C/13.8 MPa/2 h; 7, 40°C/20.7 MPa/2 h).

Relationship between the variables

Table 9 shows the pearson's coefficients between CIE L* value, CIE a* value, CIE b* value, TBARS value, HI content, NHI content and metmyoglobin content in different treatment samples. In SCT 1, 2 and 6 samples, the CIE L* value was significantly and positively correlated with the CIE a* value, while significant and negative correlation was found in SCT 4 sample ($p<0.05$). CIE L* value was significantly and positively correlated with CIE b* value in SCT 2 sample ($p<0.05$).

Changes in CIE a* and CIE b* values caused by pressure usually have the same mechanism, and are related to changes in the chemical state of myoglobin (Bak et al., 2019). Thus, positive correlations would be expected between CIE a* and CIE b*

Table 9. Pearson's coefficients of the studied variables in different treated samples

Variable	CT	HT	SCT 1	SCT 2	SCT 3	SCT 4	SCT 5	SCT 6	SCT 7
CIE L* value									
CIE a* value	–0.629	–0.533	0.980**	0.947*	0.354	–0.938*	0.334	0.988**	0.618
CIE b* value	–0.873	0.287	0.785	0.949*	0.100	–0.069	0.612	0.841	0.814
TBARS value	0.845	–0.755	–0.623	–0.917*	0.478	0.031	–0.342	–0.898*	–0.642
Heme iron content	–0.839	–0.053	0.811	0.756	–0.519	–0.232	0.017	0.987**	0.447
Non-heme iron content	–0.119	–0.385	0.693	0.766	0.042	–0.333	–0.191	0.950*	0.688
Metmyoglobin content	–0.820	0.118	0.900*	0.916*	–0.660	–0.187	0.503	0.861	0.404
CIE a* value									
CIE b* value	0.741	–0.457	0.677	0.815	0.933*	0.342	0.816	0.882*	0.703
TBARS value	–0.508	0.508	–0.540	–0.760	–0.443	–0.181	–0.071	–0.932*	–0.669
Heme iron content	0.900*	0.129	0.733	0.786	0.217	0.435	0.589	0.997**	0.456
Non-heme iron content	–0.167	0.129	0.562	0.570	0.923*	0.631	0.732	0.948*	0.993**
Metmyoglobin content	0.915*	0.194	0.801	0.743	0.305	0.480	0.864	0.882*	0.723
CIE b* value									
TBARS value	–0.932*	–0.791	–0.917*	–0.92*	–0.641	–0.879*	–0.591	–0.930*	–0.960**
Heme iron content	0.782	0.807	0.907*	0.553	0.549	0.953*	0.737	0.882*	0.649
Non-heme iron content	0.130	0.726	0.785	0.861	0.992**	0.782	0.465	0.890*	0.719
Metmyoglobin content	0.758	0.747	0.909*	0.953*	0.514	0.969**	0.970**	0.786	0.838
TBARS value									
Heme iron content	–0.661	–0.571	–0.918*	–0.752	–0.770	–0.950*	–0.556	–0.949*	–0.765
Non-heme iron content	–0.015	–0.299	–0.707	–0.881*	–0.610	–0.415	–0.079	–0.831	–0.653
Metmyoglobin content	–0.615	–0.650	–0.775	–0.992**	–0.970**	–0.786	–0.552	–0.958*	–0.938*
Heme iron content									
Non-heme iron content	–0.270	0.920*	0.884*	0.504	0.536	0.656	0.415	0.930*	0.418
Metmyoglobin content	0.997**	0.967**	0.932*	0.669	0.729	0.920*	0.694	0.912*	0.695
Non-heme iron content									
Metmyoglobin content	–0.249	0.795	0.925*	0.892*	0.506	0.865	0.655	0.709	0.676

UT: untreatment (control); HT: heat treatment (40°C for 2 h); SCT: supercritical CO_2 treatment (1, 35°C/17.2 MPa/2 h; 2, 40°C/17.2 MPa/2 h; 3, 45°C/17.2 MPa/2 h; 4, 40°C/17.2 MPa/0.5 h; 5, 40°C/17.2 MPa/1 h; 6, 40°C/13.8 MPa/2 h; 7, 40°C/20.7 MPa/2 h).
* $p<0.05$; ** $p<0.01$.
TBARS, thiobarbituric acid-reactive substances.

values and their correlations with the other parameters would be relatively consistent. The CIE b* value was positively correlated with CIE a* value and NHI content, and the correlations were significant in SCT 3 and 6 samples ($p<0.05$). The CIE a* value was positively correlated with HI content and metmyoglobin content, and their correlations were significant in CT and SCT 6 samples ($p<0.05$). The CIE b* value was also positively correlated with HI content and the correlation was significant in SCT 1, 4 and 6 samples ($p<0.05$). A significant and positive correlation was observed between CIE b* value and metmyoglobin content in SCT 1, 2, 4 and 5 samples ($p<0.05$). For SCT 3, 6 and 7 samples, a significant and positive correlation was displayed between CIE a* value and NHI content ($p<0.05$).

The HI content was significantly and positively correlated with NHI content in HT, SCT 1 and 6 samples ($p<0.05$), and with metmyoglobin content in CT, HT, SCT 1, 4 and 6 samples ($p<0.05$). The metmyoglobin content was positively and significantly correlated with NHI content and CIE L* value in SCT 1 and 2 samples ($p<0.05$). The CIE L* value was positively and significantly correlated with HI and NHI content in SCT 6 sample ($p<0.05$).

A significant and negative correlation between CIE L* value and TBARS value was found in SCT 2 and 6 samples ($p<0.05$). The CIE b* value was significantly and negatively correlated with the TBARS value in CT, SCT 1, 2, 4, 6 and 7 samples ($p<0.05$). The TBARS value was significantly and negatively correlated with the CIE a* value in SCT 6 sample ($p<0.05$). Similar results were obtained by Wang et al. (2021) in beef patties with or without dielectric barrier discharge cold plasma treatment.

The HI content was negatively correlated with TBARS value in SCT 1, 4 and 6 samples ($p<0.05$). The TBARS value was significantly and negatively correlated with the metmyoglobin content in SCT 2, 3, 6 and 7 samples ($p<0.05$). A significant correlation between TBARS value and NHI content was observed only in SCT 2 sample ($p<0.05$). These results indicated that lipid oxidation in supercritical CO_2 treated samples was mainly related to the HI content and metmyoglobin content, with little correlation to the NHI content. Supercritical CO_2 treatment could denature heme proteins, leading to release of heme, which accelerated lipid oxidation. Richards et al. (2005) also reported that lipid oxidation was associated with heme loss from myoglonn and hemoglobin in washed trout muscle at pH 6.3. They suggested that heme dissociation from heme proteins played a major role in promotion of lipid oxidation. Shang et al. (2020) also found there is a negative correlation between TBARS and HI in Cantonese sausage with different D-sodium erythorbate during storage, and a positive correlation between metmyoglobin content and HI content. It was reported that heme was more effective in catalyzing lipid peroxidation than NHI in red blood cell membranes (Chiu et al., 1996). Orlien et al. (2000) found that the increased lipid oxidation in high pressure-treated chicken breast muscle was not caused by the release of iron ions.

Many studies have reported a good positive correlation between lipid and myoglobin oxidation reactions in muscle foods (Wang et al., 2018; Wongwichian et al., 2015). In this study, a good negative correlation between metmyoglobin and lipid oxidations were observed in SCT 2, 3, 6 and 7 samples ($p<0.05$). The possible reasons are as follows. The ground pork used in this study was subjected to a freeze-thaw cycle before treatment. Metmyoglobin would become the most abundant form in the processed ground pork (Coria-Hernández et al., 2020). During the subsequent storage of the ground pork, the metmyoglobin could be further oxidized, and the oxidation products accelerated lipid oxidation (Chaijan, 2008).

Conclusion

Supercritical CO_2 treatment under the studied process conditions could increase the CIE L* and CIE b*, while decreasing the CIE a* of ground pork during subsequent storage. Supercritical CO_2 treatment for 2 h could increase lipid oxidation,

regardless of the treatment pressure or temperature. The enhanced effect on lipid oxidation by supercritical CO_2 treatment did not primarily come from the release of free iron during the treatment. The promotion of lipid oxidation is probably the result of heme release from myoglobin and metmyoglobin oxidation. Our results provided theoretical guidance for reasonable selection of supercritical CO_2 treatment conditions that can maintain meat quality to a greater extent.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Huang S. Data curation: Tang M, Zhao S. Formal analysis: Chen F, Chen D. Validation: Zhao S, Chen D. Investigation: Tang M, Chen F. Writing - original draft: Huang S. Writing - review & editing: Huang S, Tang M, Chen F, Zhao S, Chen D.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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