The Effects of Carbon Monoxide Treatment on the Physical and Chemical Qualities of Tuna Steak during Iced Storage

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Abstract

Injection or modification of the atmosphere in the meat packaging by carbon monoxide (CO) has been known to retain the color stability of red meat including those of tuna. The red color in tuna meat has been commonly used as a freshness indicator by consumers, especially those for raw consumption. However, other information on the freshness level in fish, in addition to color, is also important to assess in the food safety of marine and fisheries products. This study aims to evaluate the effects of CO on the chemical and physical properties of tuna steak during storage on ice. This study was conducted using bigeye tuna (*Thunnus obesus*) as the raw material. The tuna was cut into loins to form steaks and divided into two groups, one group without CO injection or control, and another group was injected with CO. Both CO-treated tuna steak and control were preserved in a cool-box filled with ice for 14 days. The observation was conducted every two days by determining color (chromameter method), sensory preference (hedonic method), and several chemical parameters, including total volatile base (TVB), K value, and histamine content that related to the spoilage process. Results showed that after 14 days of preservation in iced storage, the reddish color of CO-treated tuna steak was retained, whereas that of control turned brown. In the sensory tests, the panelists preferred the CO-treated tuna steak to control due to its reddish color. There were no significant differences between the content of TVB accumulation and the K value in CO-treated tuna steak and the control. Furthermore, the K value of CO-treated tuna steak and control reached the rejected level on day 14. The difference between CO-treated tuna steak and control was based on the content of histamine, where that of control was significantly higher than tuna steak treated with CO. Therefore, this research showed that the effects of CO treatment were only on the appearance of the steak; meanwhile, the deterioration process in fish is generally unaffected. Precautions are thus needed for consumers, since color may not be the only factor that indicates the freshness of tuna steak.

Keywords: tuna, carbon monoxide treatment, spoilage, total volatile base, K value, histamine

1. Introduction

Fresh tuna meat is one of the most popular raw foods, commonly consumed as sushi or sashimi. The freshness of raw fish as sushi or sashimi is influenced by post-harvest handling techniques, which is commonly indicated by color. Changes from reddish color to brown usually indicates a quality degradation in fish (Gómez-Sala et al., 2016; Smulevich et al., 2007). The reddish color of tuna fillet, which is aerobically packed and stored in refrigerators, is economically important since color is one of the parameters in determining the grade of the product. Tuna with bright red color has a higher grade and price compares to that of the brownish color (Bu et al., 2017; Nurani, Murdaniel, & Harahap, 2016). It is known that the red color of freshly cut tuna is due to the changing color of deoxymyoglobine to the bright red oxymyoglobine then to the brown metmyoglobine upon contact with oxygen during the natural deterioration process in fish (Ghaly, Dave, Budge & Brooks, 2010; Ottestad, Sørheim, Heia, Skaret & Wold, 2011; Zapata et al., 2011).

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Regarding the economic value of reddish meat, there has been a development in the post-harvesting technique to retain the stabilization of reddish color. Injection or modification of the packaging atmosphere by CO in meat has been known to improve the color of red meat (Neethling, Hoffman, & Britz, 2013). CO will substitute oxygen molecules in myoglobin compound and form CO-myoglobin, a bright red pigment much more stable than oxy-myoglobin (Bartolucci et al., 2010; Schubring, 2008).

Previous study has shown an insignificant color change in tuna meat treated with CO stored at ambient and freezing temperatures (Pivarnik et al., 2011). However, there has been a controversy in the application of CO treatment in meat (Van Rooyen, Allen, & O'Connor, 2017). The FDA states that the induction of CO is not a dangerous supplement, whereas, the EU and Japan reject CO-induced tuna because it belongs to fraud, disguising the decline in quality of tuna meat (Marrone et al., 2015; Smulevich et al., 2007; Wicklund et al., 2006; Zhou et al., 2012).

Some studies have published the effects of CO treatment on the quality of tuna flesh. Hygienic value, texture, water holding capacity, microbiological, sensory profiles, volatile ammonia base and other chemical parameters in fish spoilage, are variables that have been reported regarding the effects of CO treatment in flesh meat (Huang, Shiau, Hung, & Hwang, 2006; Kristinsson, Ludlow, Balaban, Otwell, & Welt, 2006; Neethling, Hoffman, & Britz, 2013; Pivarnik et al., 2011; Suryaningrum & Ikasari, 2019).Those studies usually observed specific variables in tuna meat that have been kept in frozen or refrigerated preservation. Our current study presents the comparison of tuna steak quality with and without CO treatment in iced storage, in order to obtain information on meat color retention as well as chemical changes during deterioration process. This is important from a food safety point of view, especially for raw fish products.

2. Material and Methods

2.1. Material

Two (2) frozen whole bigeye tuna (*T. obesus*), with a size of 48 – 52 kg were obtained from PT. Makmur Jaya Sentosa, Muara Baru, Jakarta. Other chemical materials used in this research were trichloroacetic acid (TCA) (Merck, Catalog: 100807), hydrochloric acid (HCl) (Merck, Catalog: 1.00317), potassium hydroxide (KOH) (Sigma-Aldrich, Catalog: 814353), dimethyl sulfoxide-d_6/DMSO-d_6 (Sigma-Aldrich, Catalog: 716731), and 1,2,4,5-tetrachloro-3-nitro-benzene (Sigma-Aldrich, Catalog: T7802).

2.2. Sample Preparation

The tuna sample was cut into loins to form steaks and subsequently divided into two groups, one of which was injected with CO, while the other was treated without CO injection as a control. Before treated with CO, the sample was packed in a plastic bag. CO injection was conducted with the pressure of 6 kg/cm² for 1-3 min, at 1.2-3.9 °C, stored in the refrigerator for 48 hours, and subsequently vacuum packed. The control was also refrigerated for 48 hours and vacuum packed. Both CO-treated tuna steak and control were transported to the Laboratory of Indonesian Research Centre for Marine and Fisheries Product Processing and Biotechnology (Balai Besar Riset Pengolahan Produk dan Bioteknologi Kelautan dan Perikanan/ BBRP2BKP) in insulated boxes filled with ice at the ratio of 1:3 (sample : ice). In the laboratory, insulated boxes filled with samples and ice were kept at room temperature for 14 days. Melted ice was replaced every 24 hours by maintaining the ratio of ice to sample. Observations of tuna deterioration processes were conducted every two days by analysing physical and chemical parameters. The measurements were performed in three replications. The nonparametric statistical Kruskall-Wallis was used to identify the difference between each parameter in control and tuna steak treated with CO. The statistical analyses were conducted using Past Statistical Software V3.08 (Hammer et al., 2001).

2.3. Physical Parameters

The physical analyses were conducted to quantify the level of color and to evaluate the sensory attributes of tuna steak. Quantification of tuna steak color was calculated following the method of Bjørlykke et al., (2011), using Chromameter Hunter Lab Colourflex Ez instrumentation. Steak sample was chopped and homogenized, placed in the sample container of a Chromamer, then subsequently measured for *L* (lightness), *a* (redness) and *b* (yellowness) parameters. The measurements (Hunter color scale) were repeated three times, and each time of analysis, the container head was rotated 90 degrees counterclockwise.

Sensory assessment using the hedonic method was also conducted referring to the SNI method (BSN, 2011) for fresh tuna. Measured attributes were color, odor, and texture using scales of 1-7 (1 very dislike and 7 very like) by seven trained panelists.

2.4. Chemical Parameters

Chemical analyses were focused on parameters related to the spoilage process in fish meat, e.g. TVB,

Figure 1. The color of tuna steak treated with CO (above) and without CO treatment /control (below) after 14 days of storage on ice
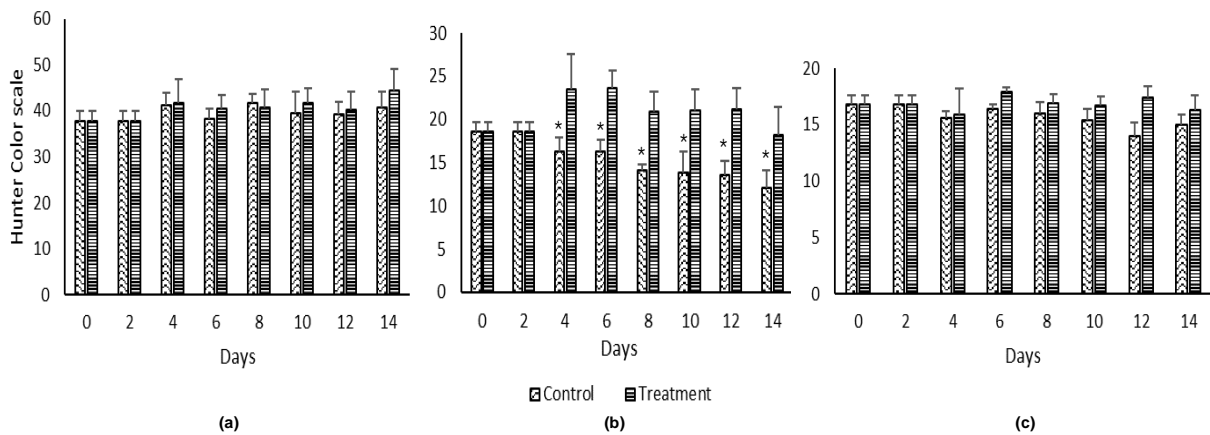

Figure 2. Quantification of color (a) lightness *L*, (b) redness *a*, and (c) yellowness *b* of CO-treated tuna steak vs control after 14 days storage on ice with (*) as $p < 0.05$

K value, and the ratio of histamine to histidine. TVB contents were analyzed by a micro-diffusion technique in Conway flask, according to Conway & O'Malley (1942). K value and histamine to histidine ratio were analyzed by extracting samples (10 g) with 30 mL TCA. The extract was filtered, and the pH was neutralized with 9 M KOH. Moreover, 0.2 mL of 76 mM 1,2,4,5-tetrachloro-3-nitrobenzene in DMSO-d_6 as internal standard was added, to quantify the compounds related to K value (adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine, hypoxanthine), histidine, and histamine in a proton NMR experiment. The experiment was conducted on a 400 Mhz Jeol NMR with 1024 scan. The identification of chemical shifts and quantification of each targeted compound was performed referring to Shumilina et al. (2015).

3. Results and Discussion

The physical analysis showed that tuna steak color treated with CO injection was still reddish, while the control had turned brown on the last day of observation

(Figure 1). These results were quantified by chromameter analysis, which found that redness (Hunter scale of *a* value) as the main difference between CO-treated tuna steak and control (Figure 2). The redness color (*a*) of CO-treated tuna steak and control showed significant difference ($p < 0.05$) starting on day 4 to day 14. The redness color (*a*) of control on day 14 decreased as much as 39%, whereas that of CO-treated tuna steak decreased by only 13%. These results indicated that the red color of tuna steak treated with CO was more stable compared to that of control. The process of CO injection into red meat has been known as an effective way to maintain redness. CO binding to myoglobin, forming CO-myoglobin, is more resistant to oxidation than oxy-myoglobin (Bernardi et al., 2008; Djenane & Roncalés, 2018; Sørheim, Aune, & Nesbakken, 1997; Pivarnik et al., 2011). Another study found that the CO treatment may retain the reddish color on meat after 32 days in freezing (-20°C) preservation (Neethling, Hoffman, & Britz, 2013).

The color differences were visually detected and may have affected panelist's reception in the sensory
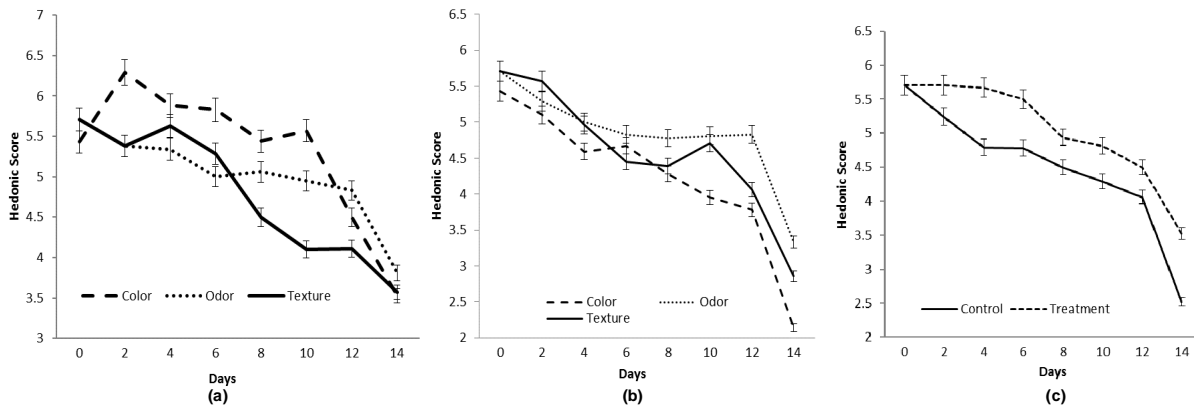
Figure 3. Hedonic scores of tuna steak stored on ice for 14 days : (a) CO-treated tuna steak, (b) control, and (c) overall acceptance
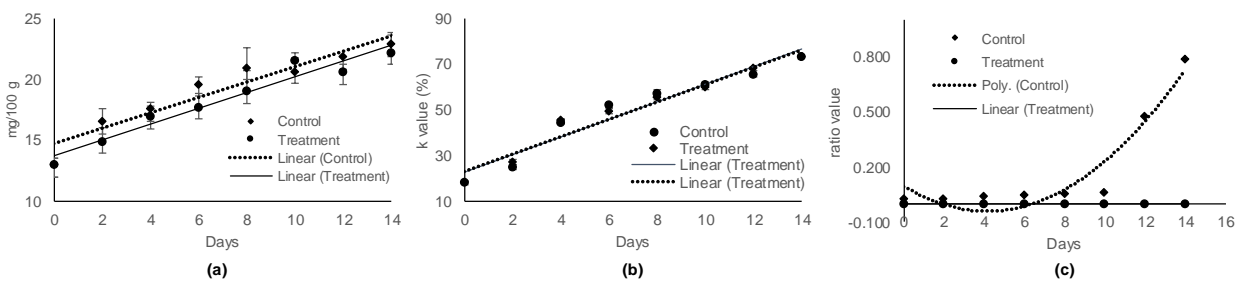

Figure 4. Chemical variables of tuna steak treated with CO and control in ice storage for 14 days: (a) TVB, (b) K value, and (c) ratio of histamine to histidine

test. There were no significant differences ($p > 0.05$) of the panelist's reception against the smell and texture of both CO-treated tuna steak and control. However, the color of CO-treated tuna steak was more favorable ($p < 0.05$) than that of control (Figure 3), after preservation on ice for 14 days.

It is shown in Figure 3 that color preservation in tuna steak treated with CO was a significant factor in determining panelist's reception in the sensory test. Our findings were consistent with those of previous studies, in which color was the main parameter affecting the panelists' reception rates of red meat quality in sensory tests (Gagaoua et al., 2015; Girolami et al., 2013; Kamruzzaman et al., 2012; Mancini & Hunt, 2005). Other studies also reported the preference of panelists toward the CO-treated tuna steak (Pivarnik et al., 2011) and CO-treated tilapia fillets (Chen, Chiou, Ding & Pan, 2015) over the control.

Different from physical parameters, the chemical parameters (TVB and K value) of tuna steak treated with CO did not show a significant difference compared to those of tuna steak control, except for the ratio of histamine to histidine (Figure 4). TVB and K value are important parameters associated with freshness and quality of fish. TVB is total volatile basic nitrogen formed during fish spoilage. K value is a percentage

of the transformation of ATP compound into IMP, which is subsequently degraded into inosine and hypoxanthine during the early stage of deterioration process (Heising et al., 2012; Ocaño-Higuera et al., 2011).

In this study, TVB and K values were found similar both in tuna steak control and tuna steak treated with CO. Pivarnik's findings also supported the results of TVB values in this study, describing that treated and untreated tuna with filtered smoke containing 16% CO showed similar TVB-N values (Pivarnik et al., 2011). In our study, the TVB values of both control and tuna steak treated with CO increased from 12.0 – 12.3 mg/100 g on day 1 to 22.5 – 23.0 mg/100 g on day 14. The increase of TVB values during storage indicated the occurence of deterioration process due to bacterial activities, resulting in protein degradation into volatile base compounds (Jinadasa, Galhena, & Liyanage, 2015; Pivarnik et al., 2011; Suryaningrum & Ikasari, 2019).

At the end of storage, TVB values reached 22.5 – 23 mg/100 g, still below the maximum limit commonly set for fresh fish. These results were consistent with those of previous study that reported the TVB values of yellowfin tuna stored in a vacuum and modified atmosphere packaging (70% CO_2, 30% O_2) in a cold

room (4-8 $^{\circ}$C) was still low at 13 days of storage (20.3 ± 2.0 mg/100 g). Furthermore, there were no significant difference of TVB values between the treated tuna and the untreated one (Adèle et al., 2016). The general guidelines of TVB maximum limit for fresh fish are 25 – 35 mg / 100 g (Debevere & Boskou, 1996; Etienne, Ifremer & Nantes, 2005; Howgate, 2010). The TVB values in fish can be varied depending on the species and method of handling and processing.

The analysis of K value in our study showed that there was no significant difference between CO-treated tuna steak and control. These results were in agreement with those reported for tuna (Huang, Shiau, Hung, & Hwang, 2006) and rainbow trout (Özogul & Özogul, 2000). These findings indicated that the CO treatment did not affect the biochemical process, involving the enzyme-assisted degradation of ATP, on the early stage of deterioration, but only affected the microbiological activity in the tuna steak (Andrade, Marsico, Godoy, Franco, & Conte, 2014; Ehira & Uchiyama, 1987; Huss, 1995; Massa, Palacios Paredi, & Crupkin, 2005; Özogul & Özogul, 2000). Ehira and Uchiyama (1987) proposed that a K value of 60% is the general freshness limit for most species fish being rejected, while Guizani, Busaidy, Belushi, Mothershaw, and Rahman (2005) suggested that the maximum limit of K value of yellowfin tuna is 68%. In this study, the K value of tuna steak at the beginning of storage was 18% and increased to 73% at the end of storage, passing the maximum limit for fresh fish. Widiastuti et al. (2013) reported that during chilled storage, the K value of tuna (*T. albacore*) steak increased linearly and reached 29.88% on day 15, much lower than that in this study. Variations in K values among fish species and individual within species were probably due to different rates of ATP degradation resulting from various fish maturity, type of muscle, stress during capture, season and storage conditions (Erickson, Beyer, & Sigholt,1997; Guizani, Al-Busaidy, Al-Belushi, Mothershaw, & Rahman, 2005; Hattula, 1997; Olafdottir et al., 1997)

Different from TVB and K value parameters, the ratio of histamine to histidine in tuna steak control was found higher than that of CO-treated tuna streak at the end of observation. Meanwhile, the ratio of histamine/histidine in tuna steak treated with CO remained steady. Histamine was not detected by qNMR analysis in tuna steak treated with CO throughout the observation. Capillas and Moral (2005) also found that histamine concentration in both control and tuna in Modified Atmosphere Packaging (MAP) remained constant in the beginning storage. After 11 days of storage, the histamine content in the control increased with a higher increment level than that in the sample. Previous research has shown that MAP

with 40% of CO gas suppressed histamine-forming microbial activity, leading to the negligible level of histamine during storage at 1°C storage for 28 days (Emborg, Laursen, & Dalgaard, 2005).

To summarize, it is shown in the current study that the injection of CO in tuna steak did not affect the biochemical process of deterioration in the sample, as indicated by the increasing rate of TVB and K values during storage. On the other hand, the CO treatment seemed to inhibit the production of histamine in tuna steak, which is commonly accelerated by the histamine-producing bacteria. Analysis of histamine-producing bacteria in the CO-treated tuna steak requires further study.

The CO gas injection in tuna steak, not only maintained the stability of meat color, but it also may have affected the microbial composition of the observed tuna steak. The activities of aerobic microbes that play an important role in the formation of histamine might have decreased. However, the microbial activity that plays a vital role in the degradation process of nitrogen compounds remained present. The degradation level of tuna steak control and tuna steak treated with CO was similar, although it had different physical attributes, as indicated by color quantification and sensory assessment.

4. Conclusion

Injection of CO in tuna steak maintained its reddish color, as well as prevented the increase of histamine to histidine ratio during storage on ice. However, there were no significant effects on the degradation process of nitrogen and phosphate (ATP) compounds as indicated by the increasing values of TVB and K value.

Acknowledgments

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