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Influence of supercritical carbon dioxide on physicochemical, microbial and sensorial characteristics of sturgeon (*Acipenser* spp.) fish fillet during cool storage

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Sturgeon (*Acipenser* spp.) fish was one of the most valuable aquaculture species in the world owing to its excellent-quality muscle. This fish was highly susceptible to microbial contamination during processing and preservation. Supercritical carbon dioxide (SC-CO_2) was one of the non-thermal treatment effectively applied in meat and fishery industry for the goal of pasteurization. This research verified the effectiveness of supercritical carbon dioxide (SC-CO_2) at different conditions of pressure/temperature/time (control, 10/30/20, 12/35/15, 14/40/10, 16/45/5 MPa/°C/min) on the physicochemical, microbial and sensorial characteristics of sturgeon fish fillet during 28 days of cool storage. Supercritical carbon dioxide at 12/35/15 MPa/°C/min was optimal to maintain water holding capacity (93.02±0.27%), texture hardness (42.80±0.11 N), and overall acceptance (8.12±0.02 score) while minimizing the accumulation of total volatile base nitrogen (5.02±0.10 mg/100 g) and Thiobarbituric acid reactive substances (0.62±0.02 mg malonaldehyde/kg); proliferation of total plate count (3.69±0.20 log CFU/g), *Coliform* (1.42±0.00 log CFU/g) during cool storage. It was recommended that supercritical carbon dioxide would be considered as a promising non-thermal treatment to enhance physicochemical, microbial and sensorial properties of sturgeon fish fillet.

Keywords: Microbial, physicochemical, sensorial, sturgeon fish, supercritical carbon dioxide

INTRODUCTION

Supercritical carbon dioxide (SC-CO_2) was non-thermal treatment effectively applied in food industry for both liquid and solid product to inactivate spoilage and pathogen microorganisms (Erkmen, 2000; Ferrentino and Spilimbergo, 2011; Perrut, 2012). This method also maintained thermal-sensitive element, nutritional proximate and sensory characteristic with a better shelf-life (Norton and Sun, 2008). Main parameters of SC-CO_2 included pressure, temperature and duration directly affecting to microbial inactivation. The higher pressure, the more inactivation would be obtained. It was a result of better CO_2 solvating capacity inducing cellular exposure and acidification. High temperature also created better CO_2 diffusion leading to more fluidity (Wimmer and Zarevúcka, 2010). SC-CO_2 enhanced the exploitation of membrane elements like phospholipids (Budisa and Schulze-Makuch, 2014). Much more microbial inactivation would be greatly visible at higher pressure, temperature and longer duration compared to lower pressure, temperature and shorter time (Jung et al. 2009). Cell membrane of the microorganism was severely vulnerable under high pressure. This pressure modified the molecular structure of the peptide-lipid crosslink, disrupting the mechanism of membrane bilayer phosphatidic acid and losing its functionality (Patterson, 2005). SC-CO_2 was suggested on pork meat at pressure 7.4 and 15.2 MPa (Choi et al. 2008; Rawson et al. 2012; Choi et al. 2013).

Demand of sturgeon (*Acipenser* spp.) fish has increased dramatically in recent years due to its nutritional and markefigure values (Matteo et al. 2021; Samad et al. 2021). Sturgeon had a heterocercal caudal fin, a cartilaginous skeleton (Silas, 2017). It was mostly distributed in temperate marine and brackish water regions (Billard and Lecointre, 2001). This fish contained a rich source of soluble collagen, purine bases, vitamins and minerals (Badiani et al. 1996). Sturgeon muscle was also rich in monounsaturated and polyunsaturated fatty acids and low cholesterol content. Therefore sturgeon could be believed as a healthy seafood. The proportion of connective and muscular tissues was beneficial for the muscular one (Simeanu et al. 2015). Poor hygiene condition during processing of sturgeon created microbial cross-contamination (Heshmati et al. 2013). Purpose of our study investigated the the influence of supercritical carbon dioxide (SC-CO_2) at different conditions of pressure/temperature/time on the physicochemical (water holding capacity, texture hardness, total volatile base nitrogen, Thiobarbituric acid reactive substances), microbial (total plate count, *coliform*) and sensorial characteristics of sturgeon fish during 28 days of cool storage.

MATERIALS AND METHODS

2.1 Material

Raw fresh sturgeon fish was collected in Thu Duc market, Ho Chi Minh city, Vietnam. It was moved to laboratory quickly for experiment. Chemical reagents were all analytical grade originated from Fluka (Switzerland) and Sigma Aldrich (USA), purchased from Ho Chi Minh city, Vietnam. Petrifilm plates were supplied from 3M-Vietnam. Lab equipments and utensils included UV-Vis spectrophotometer, texture analyzer, stomacher, vortex mixer, weigh balance, incubator, colony counter, autoclave, test tube, and micropipette.

2.2 Researching method

Raw fresh fish was carefully gutted, dressed and filleted by sharp knife. These fillets were separated into 5 groups (G1, G2, G3, G4, G5) were kept aseptic plastic bags. Each bag was subjected to SC-CO_2 equipment at three different conditions: G1 (control), G2 (10/30/20 MPa/^{o}C/min), G3 (12/35/15 MPa/^{o}C/min), G4 (14/40/10 MPa/^{o}C/min), G5 (16/45/5 MPa/^{o}C/min). After treatment, samples were stored in cool storage at 4±0.5^{o}C for 28 days. In 7 day-interval, samples were taken to determine physicochemical properties (water holding capacity, texture hardness, Total volatile base nitrogen or TVB-N, Thiobarbituric acid reactive substances or TBARS), microbial loads (total plate count, *coliform*) and overall acceptance (sensory score).

Water holding capacity (%) was determined following protocol described by Nopianti et al. (2012). Texture hardness (N) was examined using a texture analyzer (Sfigure Microsystem Inc.) described by Nurkhoeriyati et al. (2012). Total volatile base nitrogen or TVB-N (mg/100 g) was determined following AOAC (1990). Thiobarbituric acid reactive substances or TBARS (mg malonaldehyde/kg) was determined following the 2-thiobarbituric acid spectrophotometric method (Anna *et al.* 2017). Total plate count (log CFU/g) and *Coliform* (log CFU/g) were enumerated by 3M-Petrifilm plates. These plates were incubated at 37°C for 24-48 h. Overall acceptance (sensory score) was determined by a group of specialists using 9-point Hedonic scale.

2.3 Statistical analysis

The experiments were run in triplicate with different groups of samples. The data were presented as mean±standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

RESULTS

3.1 Effect of supercritical carbon dioxide treatment to physicochemical characteristics of sturgeon fish fillet during cool storage

Water holding capacity expressed as the capacity of fish fillet to hold its inner moisture under gravitational force, cutting or squeezing (Yang and Park, 1998). It was a key variable of muscle protein gel because it not only affected the yield of production but also its physical property. In our research, significant difference in the water holding capacity was observed among treated samples. The initial water holding capacity of fish fillet was observed from 95.18±0.06 to 95.23±0.05%. There was a trend of gradual reduction of water holding capacity during cool storage in all samples. Fish fillet treated by SC-CO2 at G3 (12/35/15 MPa/^{o}C/min) maintained the highest water holding capacity (93.02±0.27%) while the control showed the lowest one (82.41±0.23 %) at the end of 28 days of cool storage. There was no significant difference of water holding capacity between the G2 (10/30/20 MPa/ºC/min) and G3 (12/35/15 MPa/ºC/min). Water holding capacity commonly represented for the degree of denaturation of the myofibril protein (Kristinsson and Liang, 2006). More releasable water was a signal of degradation of water holding capacity due to denaturising of protein (Suvanich et al. 2000). Water holding capacity decreased under SC-CO_2 treatment, it could be explained that the denaturation of the muscle protein happened as a result of the synergistic effect of temperature and high acidity (Jauhar et al. 2020b). Fresh chicken meat treated by supercritical carbon dioxide at 14 MPa and 45°C for 40 min had no significant difference of water holding capacity (Jauhar et al. 2020a). SC-CO_2 treatment at 7.4 and 15.2 MPa, 31.1 °C for 10 min caused no significant impact on the water holding capacity of porcine muscle (Choi et al. 2008). A slightly lower water holding capacity was noticed on breast fillets after SC-CO_2 treatment (Min and Ahn, 2012).

Hardness was defined as the optimal strength of the initial compacting power (Szczesniak, 1963). There was significant ($p \geq 0.05$) difference of hardness on the control and treated samples. The initial hardness of fish fillet was observed from 44.59±0.01 to 44.62±0.03 N. There was a gradual decrease of hardness during cool storage in all samples. There was no significant difference of hardness between the G2 (10/30/20 MPa/ºC/min) and G3 (12/35/15 MPa/ºC/min). Fish fillet treated by SC-CO2 at G3 (12/35/15 MPa/ºC/min) maintained the highest hardness (42.80±0.11 N) while the control showed the lowest one (31.92±0.13 N) at the end of 28 days of cool storage (figure 2). At increasing pressure (10-12 MPa), the hardness became higher due to the rising of muscle compactness presumed by the myofibrils disintegration (Ros-Polski et al. 2015). Consumers preferred to fresh meat in tenderness (Ma and Ledward, 2013), meanwhile fresh fish in firmness (Sriket, 2014). The texture firmness of bluefish muscle treated by SC-CO2 was better than the control (Ashie and Simpson, 1996). SC-CO_2 treatment at 7.4 and 15.2 MPa, 31.1 °C for 10 min caused no significant difference on the hardness of porcine muscle (Choi et al. 2008). Hardness of *Penaeus monodon* increased along with the accelerated pressure of SC-CO_2 (Kaur et al. 2016). High temperature and long exposure of SC-CO_2 could negatively affect the texture firmness. Fresh chicken meat treated by supercritical carbon dioxide at 14 MPa and 45°C for 40 min resulted to harder firmness (Jauhar et al. 2020a). Tilapia exposed with SC-CO_2 at 80 bar, 40 °C within 15 min maintained its texture firmness for 14 days of cool storage (Sugeng et al. 2020).

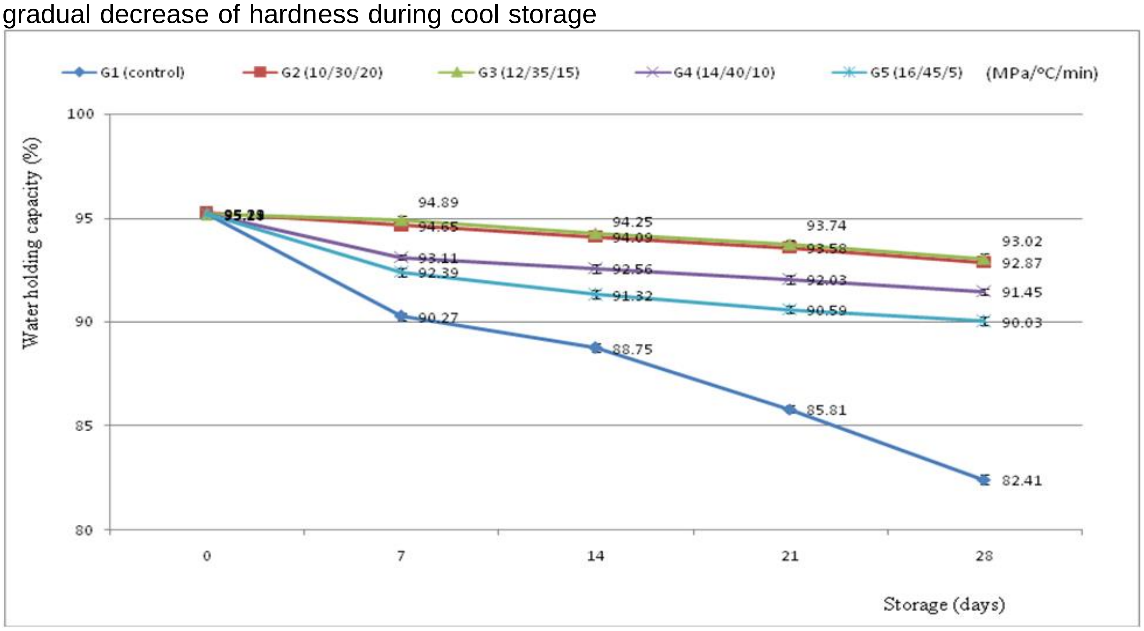

Figure 1: SC-CO_2 (MPa/ºC/min) to water holding capacity (%) of sturgeon fish fillet during cool storage

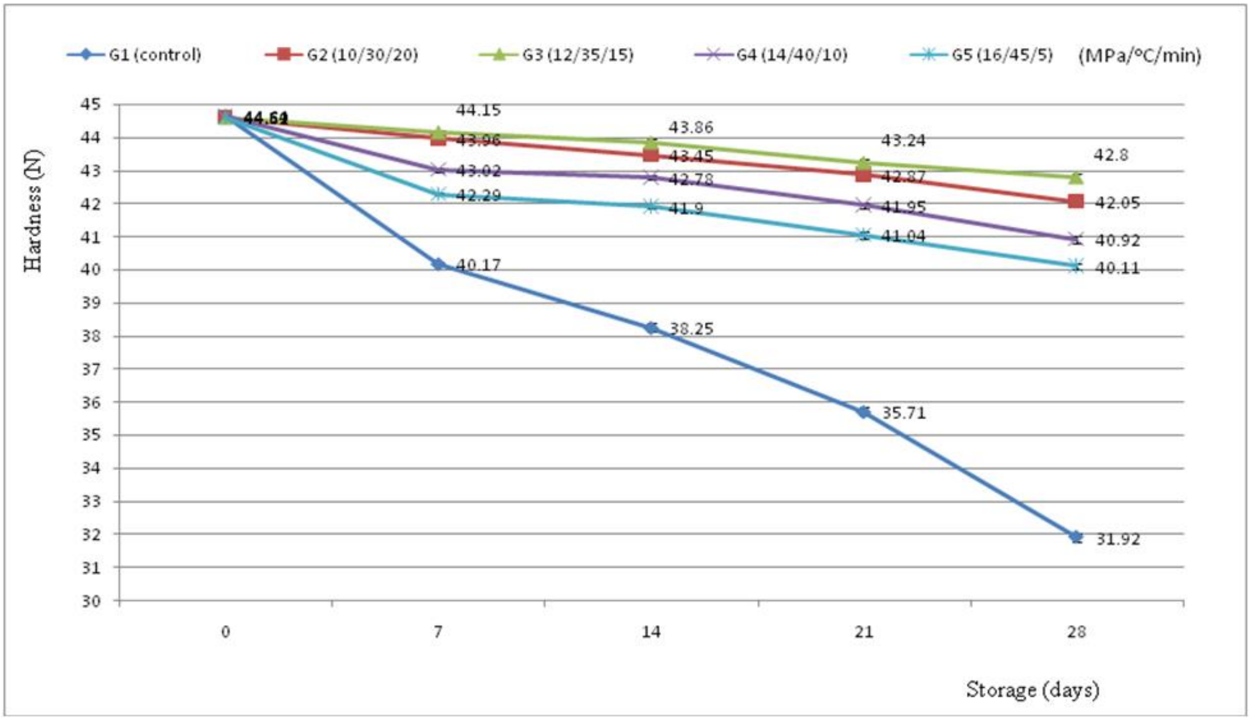

Figure 2: SC-CO_2 (MPa/ºC/min) to hardness (N) of sturgeon fish fillet during cool storage

TVB-N exerted detrimental effects for human health (Bekhit et al. 2021). TVB-N derived from the decomposition of proteins and amines and following production of trimethylamineN-oxide, trimethylamine, dimethylamine and formaldehyde, and the deamination of adenine nucleotides (Chen et al. 2019; Howgate, 2010; Servillo et al. 2018). Total volatile base nitrogen (TVB-N) was considered as one of the key biochemical parameters to estimate the microbial decomposition of fish (Wu and Bechtel, 2008). TVB-N content could be considered as an important index of meat freshness. Quality of fish was classified basing on TVB-N (mg/ 100 g) values as follows: 25 (very good), 30 (good), 30 - 35 (markefigure), over 35 (spoiled) (Duyar et al. 2019). In our research, the initial TVB-N value was recorded from 2.59±0.04 to 2.73±0.03 mg/100 g in fish fillet, following a gradual increase during preservation. The highest TVB-N was noticed at 28.07±0.10 mg/100 g on the 28th day of the control sample. Meanwhile, the most significant retardation of TVB-N was noticed at the sample treated by SC-CO_2 at 12/35/15 MPa/ºC/min (5.02±0.10 mg/100 g) (figure 3). TVB-N content in fish accumulated with storage duration reflecting microbial spoilage, formation of volatile substances, and degradation of overall acceptance (Li et al. 2019). TVB-N value was not affected by supercritical CO_2 treatment (Huang et al. 2017). TVB-N was mostly released from *Pseudomonas* sp. and *coliform*, subsequently causing adversely affect the fish quality (Sicuro 2019).

Fat in fish muscle was normally existed in form of triacyl glycerides, phospholipids, and sterols. These substances were unsfigure and quickly oxidized to release rancid smell, off-flavor, and went-down stability of fish. Iron composition, apportion of unsaturated fatty acids, acidity, and antioxidant degree significantly affected to the kinetic of lipid peroxidation (Falowo et al. 2014). Lipid oxidation commonly happened in fish products during processing and storage (Selami and Sadok, 2008). Thiobarbituric acid reactive substances (TBARS) was also one of the most important indicators to determine the oxidative rancidity of polyunsaturated fatty acids via formation of malonaldehyde facilitating to release ketones and aldehydes by peroxidase reaction (Bremner, 2002; Feliciano et al. 2010). TBARS should not over 2 mg malonaldehyde/kg fish to avoid bad smell and poor taste accumulation (Connell, 1990). In our research, the initial TBARS value was recorded from 0.16±0.00 to 0.19±0.01 mg malonaldehyde/kg in fish fillet. During storage, this variable greatly increased on the control sample (3.10±0.01 mg malonaldehyde/kg) while the lowest value was reported at sample treated by SC-CO2 at G3 (12/35/15 MPa/ºC/min) (0.62±0.02 mg malonaldehyde/kg) (figure 4). Oxidative rancidity directly affected to overall acceptance, leading the finished sturgeon unaccepfigure for consumption (Rostamzad et al. 2011). In another report, the fine pork meat had an accelerated value of TBARS after SC-CO_2 treatment (Huang et al. 2017). Supercritical carbon dioxide at 14 MPa and 45°C for 40 min significant retarded the lipid peroxidation in fresh chicken meat for 7 days of storage (Jauhar et al. 2020a

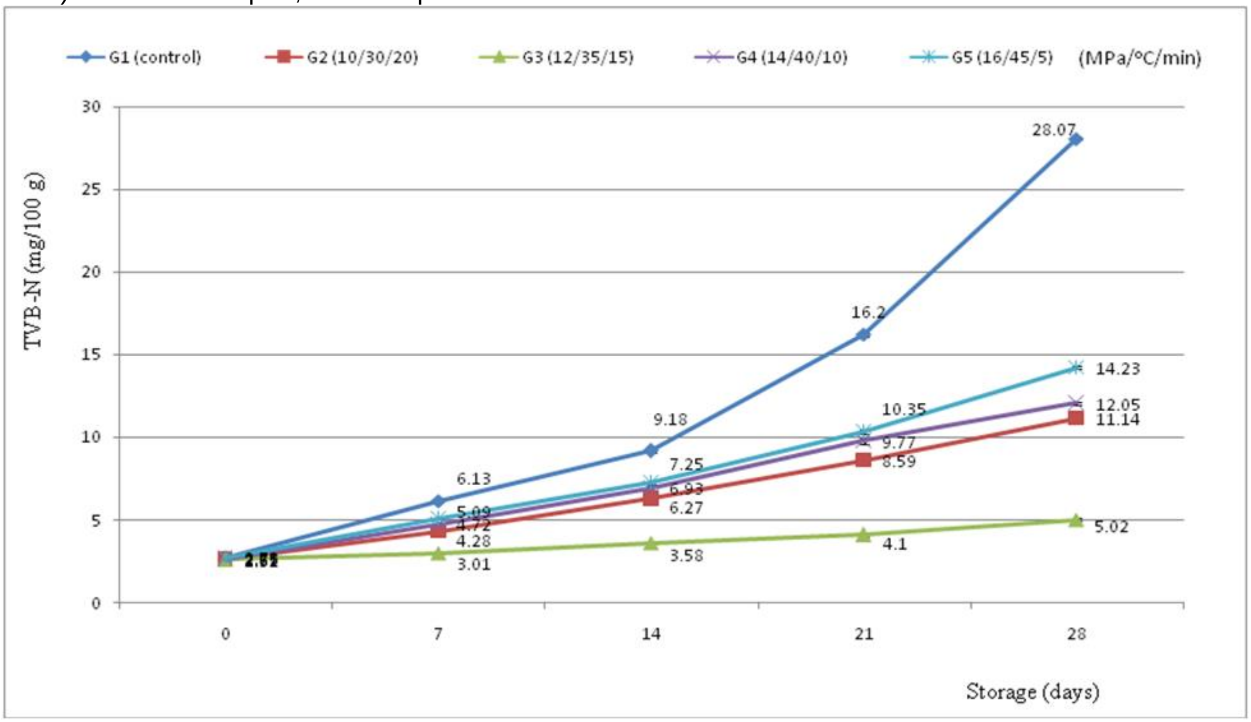

Figure 3: SC-CO_2 (MPa/ºC/min) to TVB-N (mg/100 g) of sturgeon fish fillet during cool storage

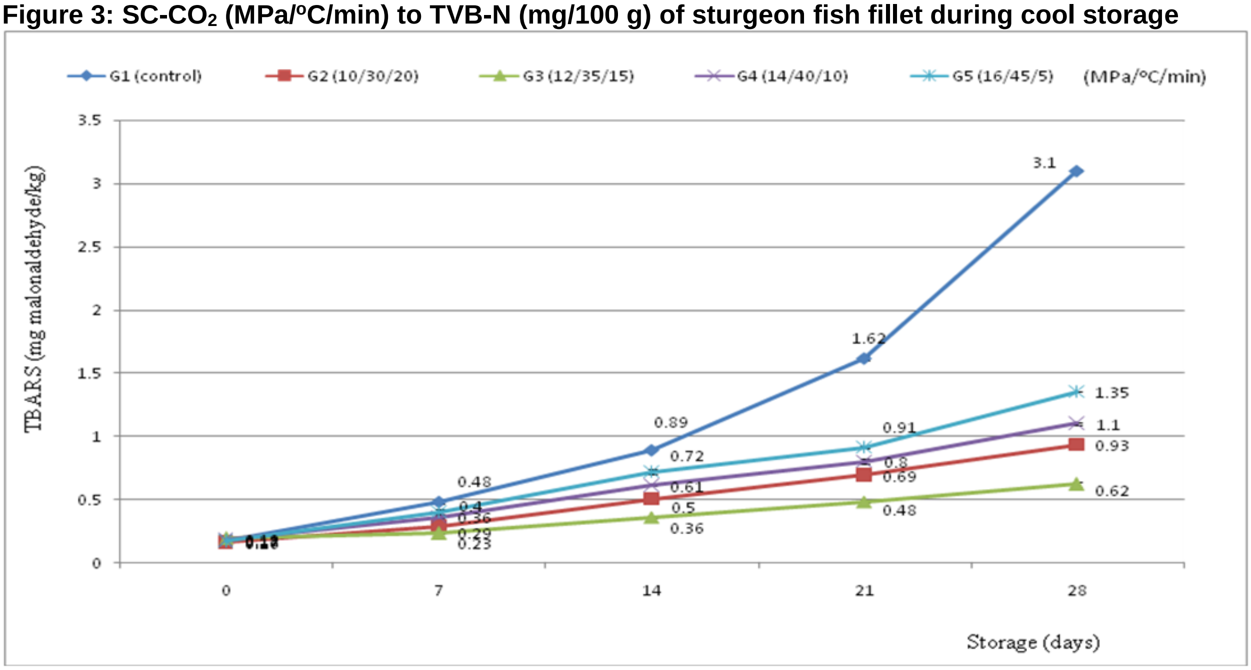

Figure 4: SC-CO_2 (MPa/ºC/min) to TBARS (mg malonaldehyde/kg) of sturgeon fish fillet during cool storage

3.2 Effect of supercritical carbon dioxide treatment to microbial characteristics of sturgeon fish fillet during cool storage

The initial total plate count (TPC) of fish fillet was 2.29±0.03 to 2.34±0.02 log CFU/g. During preservation, the TPC remarkably increased in the control (7.00±0.21 log CFU/g) while the lowest microbial proliferation was found at fish fillet treated by SC-CO2 at G3 (12/35/15 MPa/ºC/min) (3.69±0.20 CFU/g) (figure 5). *Coliform* was normally considered as an indicator of post-process contamination of fecal contamination (Verdos et al. 2019). The initial *Coliform* of fish fillet was from 0.21±0.03 to 0.27±0.02 log CFU/g. During preservation, the *Coliform* remarkably increased in the control (5.24±0.03 log CFU/g) while the lowest microbial proliferation was found at fish fillet treated with SC-CO2 at **G3** (12/35/15 MPa/ºC/min) (1.42±0.00 log CFU/g) (figure 6). Accepfigure limit of total plate count on fresh fish was 7 log CFU/g (ICMSF, 1986). High pressure require short exposure time to kill microbial cells (Lin et al. 1993). Pressure affected the gradient of CO_2 solubility directly facilitating the acidification of the internal cells and external environment. High CO_2 pressure contributed to a higher level of diffusivity. The inactivation efficiency increased with accelerating temperature by promoting the dispersion of CO_2, and the fluidity of the cell membrane to induce more penetration (Hong and Pyun, 1999). Microbial inactivation under SC-CO_2 could be explained as follows: CO_2 dispered in the cell membrane of microorganisms causing cell membrane alteration with a decrease in intracellular acidity (Giulitti et al. 2011). Enzymatic reaction and cellular biotransformation would be damaged by the alteration of the molecular metabolism (Spilimbergo et al. 2009). Finally, the intracellular electrolyte equilibrium would be collapsed and eventually essential constituents would be released from the cell (Garcia-Gonzalez et al. 2007). CO_2 pressure was applied to sterilize *Listeria monocytogenes* and *Salmonella* (Wei et al. 1991). A treatment of 15.2 MPa SC-CO_2 caused no significant difference of microbial load on marinades and marinated pork (Choi et al. 2009). SC-CO_2 at 100 bar, 37 °C for 30 minutes significantly decreased 2 and 3 logarithmic of total plate count on oysters (Meujo et al. 2010). 2.2 log reduction of *Salmonella* spp. was reported in ground pork (Bae et al. 2010). SC-CO2 was applied for microbial inactivation on shrimp (Ji et al. 2012). 3 log reduction in *Listeria monocytogenes* in dry cured ham was observed (Ferrentino et al. 2013). There was a closed correlation of the cell suspension and CO_2 proportion in microbial inactivation (Soares et al. 2013). A remarkable reduction of the total mesophilic count in raw pork meat was observed by SC-CO_2 (Cappelletti et al. 2015).

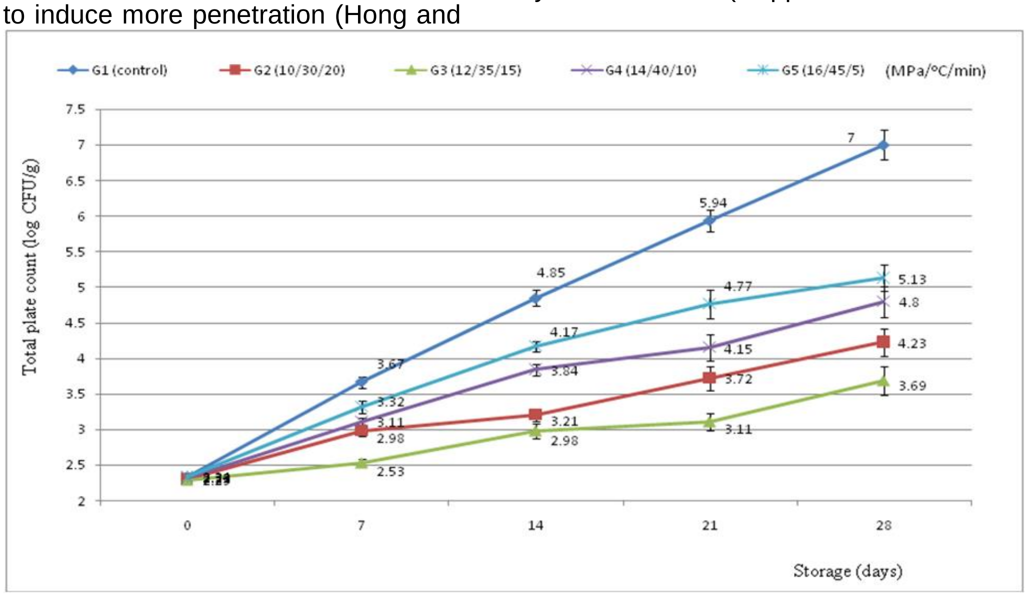

Figure 5: SC-CO_2 (MPa/ºC/min) to total plate count (log CFU/g) of sturgeon fish fillet during cool storage

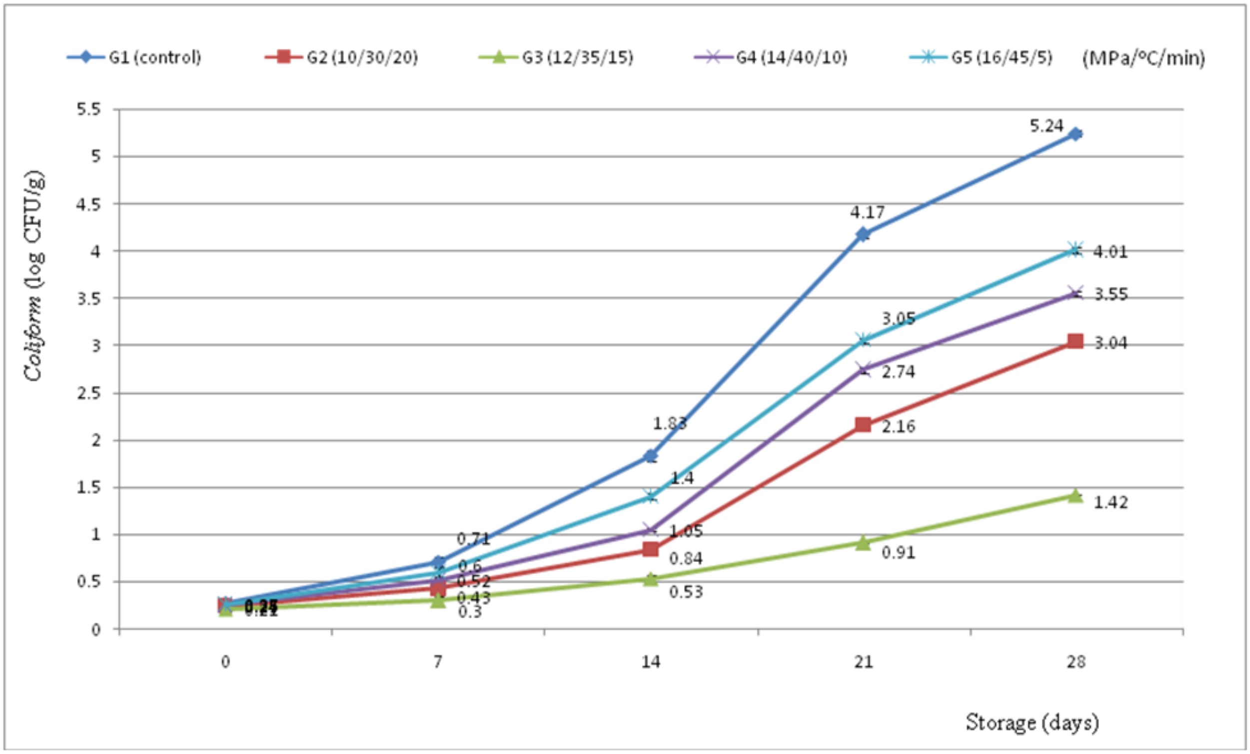

Figure 6: SC-CO_2 (MPa/ºC/min) to *Coliform* (log CFU/g) of sturgeon fish fillet during cool storage

***Staphylococcus aureus* in raw salmon was significantly inhibited by SC-CO_2 (Cuppini et al. 2016). SC-CO_2 was effective to inactivate Vibrio parahaemolyticus on oysters (de Matos et al. 2018). 2.5 log reduction of mesophilic microorganisms was observed after 15 min of SC-CO_2 (Morbiato et al. 2019). There was a reduction the total plate count (5.90 to 2.00 log CFU/g) on fresh chicken meat treated by supercritical carbon dioxide at 14 MPa and 45°C for 40 min (Jauhar et al. 2020a). Two logs reductions of microbial counts were noticed at SC-CO_2 at 80 bar, 40 °C within 15 min (Sugeng et al. 2020). E. coli inactivation had a great dependence on SC-CO_2 duration (1.4 log CFU/g reduction of after 15 min, and 5 log after 45 min) (Gonzalez-Alonso et al. 2020).**

3.3 Effect of supercritical carbon dioxide treatment to overall acceptance of sturgeon fish fillet during cool storage

Sensory score was also one of the key parameters influencing to acceptance of consumer in commerce. Figure 7 showed the influence of SC-CO_2 treatment on overall acceptance of sturgeon fish fillet during 28 days of cool storage. The control sample revealed the highest reduction of sensory score after 28 days of cool storage (8.88±0.02 down to 6.38±0.03); meanwhile the samples treated by SC-CO2 at G3 (12/35/15 MPa/ºC/min) showed the least reduction of overall acceptance (8.93±0.00 down to 8.12±0.02) after 28 days of cool storage. Results expressed that SC-CO_2 effectively contributed to the extended shelf-life of sturgeon fish fillet during storage. SC-CO_2 greatly changed the meat color of raw chicken, ground pork, chicken breast meat (Omana et al. 2011; Zhang et al. 2016; Huang et al. 2017). Discoloration was derived from globin denaturation and ferrous myoglobin oxidation to ferrous metmyoglobin (Choi et al. 2013). Low temperature and short SC-CO_2 treatments caused no significant difference on sensory value of the chicken meat (Jauhar et al. 2020b).

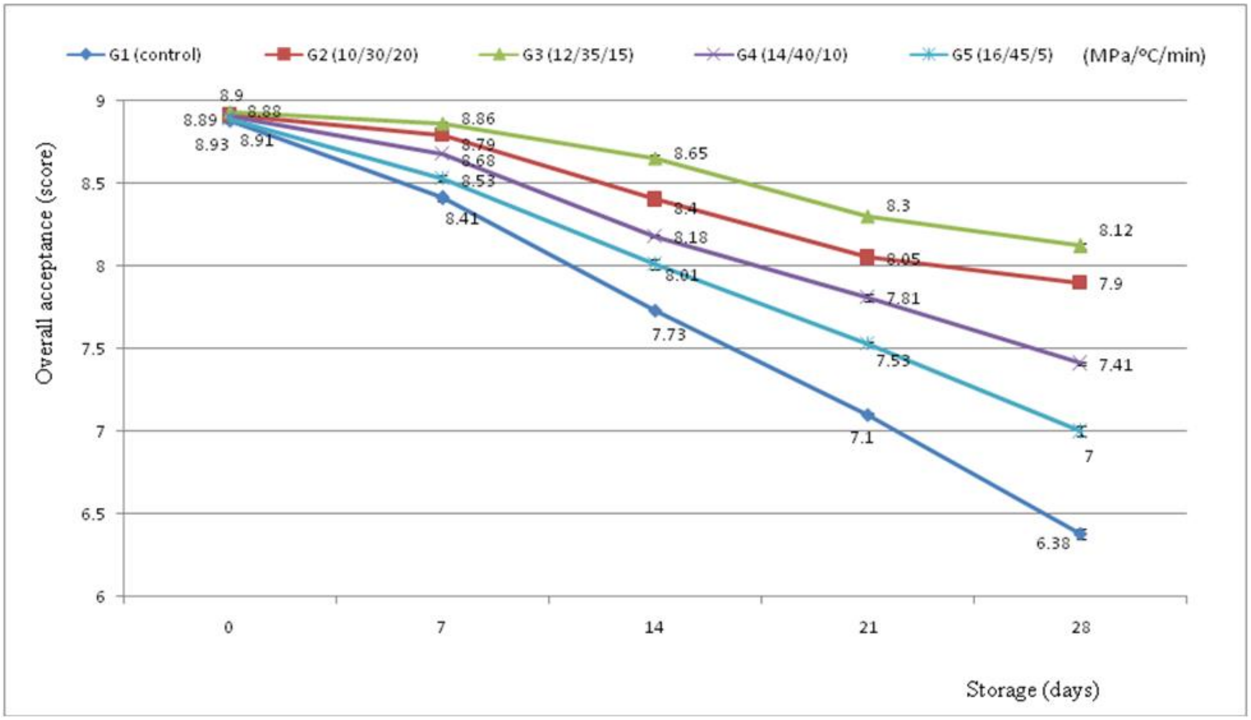

Figure 7: SC-CO_2 (MPa/ºC/min) to overall acceptance (score) of sturgeon fish fillet during cool storage

CONCLUSION

Sturgeon (Acipenser spp.) had high-quality meat with high commercial value. We have successfully verified the impact of SC-CO2 on the quality of sturgeon fish fillet during cool storage. Findings of this research suggested that SC-CO2 retained water holding capacity, texture hardness, and overall acceptance; minimized the production of total volatile base nitrogen and Thiobarbituric acid reactive substances, retarded the growth and proliferation of total plate count and Coliform of sturgeon fish fillet during 28 days of cool storage. SC-CO2 treatment would be potential candidate for preservation of fish with a low microbial load as well as a minimal modification of physicochemical and sensorial quality attributes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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