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E-mail: Agrointek@trunojoyo.ac.id
EXPLORATORY STUDY OF NEAR AND SUPERCRITICAL CARBON DIOXIDE WARM PASTEURIZATION ON NILE TILAPIA

Sugeng Pondang Sugiharto¹,²*, Nursigit Bintoro², Joko Nugroho Wahyu Karyadi², Yudi Pranoto²

¹Center for Agroindustrial Technology, Agency for Assessment and Application of Technology, Jakarta, Indonesia
²Agricultural Engineering Faculty, Gadjah Mada University, Yogyakarta, Indonesia

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ABSTRACT
Effects of both near critical and supercritical CO₂ pasteurization on inactivation of muscle protease and lipase of Nile tilapia (Oreochromis niloticus) were investigated along with its impact on microbial reduction and physical appearance. The near critical phase was designed at 70 bar, and supercritical phases were designed at 80 and 90 bar. It was found that CO₂ pressure had greater effect on protease inactivation than on lipase inactivation. Temperature increase did not improve both protease and lipase inactivation while fillet texture was adversely affected. The fillet color experienced color changes significantly.

Keyword
Supercritical; carbon dioxide; tilapia; pasteurization

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* Corresponding author
Email sugeng.pondang@bppt.go.id
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INTRODUCTION

During storage, the fish will undergo both biochemical and microbiological changes that result product deterioration. The microbiological related deterioration will increase along with temperature increase. For chilled and frozen storage, enzymatic changes will dominate the deterioration process over microbiological changes. Under ice-cold storage, fish are prone to a problem which is well known as “softening” or “mushiness”. This problem is caused by protease activity, a post-mortem activity which autolytic protease degrade and scavenge muscle tissue (Sriket, 2014). Cathepsin D, one of proteases, is found highly active in fish muscle under frozen storage(Cropotova et al., 2020). Another autolytic enzyme, lipase, is responsible for degradation of muscle fat; which eventually causes food rancidity and unpleasant odor.

Dense phase carbon dioxide (DCPD) is non-thermal technology which utilize the phase change behavior of CO₂ for food processing purposes. DCPD has been known to have bactericidal and fungicidal effect as well as inhibitory effect on food automatically enzymes (Damar and Balaban, 2006). However, since food processing that employ DPCD also involve high pressure, it also affecting physicochemical properties of subjected food (Ashie and Simpson, 2002).

The objective of this study is to explore the suitable DPCD variables for pasteurizing Nile tilapia (Oreochromis niloticus). Pasteurization is intended to reduce microbial loads and enzymatic activity; and therefore, extend shelf life of perishable food for few days during chilled storage or few months under frozen storage (Fellows, 2012). Since supercritical CO₂ (scCO₂) is achieved at warm temperature or more (≥ 31.1°C), pasteurization with CO₂ can be expected at much lower temperatures than that of conventional pasteurization.

Though reports of inactivation of isolated enzymes are available, studies of the effect of DPCD on muscle enzymes are hardly found. Compared to liquid foods, the DPCD process applied to solid foods has been under-researched due to the complexity of the biological matrices, which could make CO₂ bactericidal action arduous, and the lack of information about the inactivation mechanism which is almost obscure and scarcely studied (Ferrentino and Spilimbergo, 2011). This study was aimed to fill that void and provide more knowledge about the effect of DPCD pasteurization on fish muscle protease and lipase.

Nile tilapia was chosen since it is the second most cultivated freshwater fish after carp (Miao, 2015), and Indonesia is the second largest producer of this cichlid fish (Mapfumo, 2018). Furthermore, due to vast culture of seafood culinary, tilapia could be served not only cooked but also served as raw delicacy. Therefore, keeping it near raw condition during storage will provide the cooks with wider option to serve their costumer.

METHOD

Preparation of tilapia

Tilapia was purchased alive from the local market, killed by electric stunning, eviscerated, beheaded, and cleaned by tap water. Prior to pasteurization, fishes were cut into three pieces, placed in polyethylene plastic and inserted into the pressure vessel.

Pressure vessel and CO₂ pressurization

A pressure vessel with capacity of 2000 mL was developed in Faculty of Agriculture Engineering, Gadjah Mada University. The lid of pressure vessel was equipped with an analogue 160 bar pressure gauge. To obtain near critical and supercritical CO₂ (scCO₂), food grade
liquid CO$_2$ was supplied into pressure vessel by inverting 25 kg CO$_2$ cylinder and then, pressure vessel temperature was raised to experimental temperature. Excess CO$_2$ was released through exhaust outlet before experimental pressure achieved. Barrel temperature was controlled by a calibrated digital thermostat (Elitech STC-3000). Heat was supplied by a pair of 700 watts U-heater and cooled by 17 watts 6 inches deskfan. The schematic diagram of the pressure vessel is shown in Fig. 1.

**Crude enzyme extraction**

Crude enzymes was extracted by following the method of Lakshmanan et al. (2005). Ten grams of samples were homogenized by IKA Turax T25 S5 (Janke and Kunkel Gmbh) homogenizer in 4°C mixture of deionized water and ice for 2 min. Homogenate was allowed to stand for 30 min in ice with occasional stirring. After that, the homogenate was centrifuged at 14,600×g and 4°C for 20 min in refrigerated centrifuge (Beckman Alegra X30R). The supernatant was filtered by 0.45 μm syringe filter (Merck Millipore) and stored into a deep freezer at -80°C prior to further analysis.

**Residual protease activity assay**

Protease assay followed the manual of Amano Enzyme Inc. (2016). One mL supernatant was added into 5 mL 0.2% casein solution in 0.02 M pH 8.0 phosphate buffer. The mixture was incubated at 37 ± 0.5°C for 10 min and shaken at 300 rpm. Protease digestion was terminated by addition of 5 mL trichloro acetic acid solution and incubated for 10 min at 37 ± 0.5°C and shaken at 300 rpm. Reaction solution was centrifuged at 10,000×g at 25°C for 10 min. One mL of supernatant was added to 5 mL mixture of 3-folds folin reagent (1 part) and of 0.55 M Na$_2$CO$_3$ (5 parts). The reaction solution was vortexed and allowed to stand at 37 ± 0.5°C for 30 min. Two hundred mL of final solution was pipetted into 96 wells microplate, and its absorbance was read at 660 nM in ELISA reader (Bio-Tek μQuant). The residual activity (A/A$_0$) is defined as a percentage of protease activity of treated samples (A) to the protease activity of untreated samples (A$_0$).

**Residual lipase activity assay**

Lipase assay followed the manual of Asahi Kasei Enzymes (2018) with slight modification. The emulsifier, Adekatol (Adeka, SO–120), was replaced by Polyvinil alcohol (Merck, CAS-No 9002-89-5). The substrate mixture was prepared from 25% consumer grade olive oil, 1.5% PVA , and deionized water; boiled and mixed rigorously. The substrate must be cooled adequately before further usage. Five mL of the substrate was added with 4 mL of 0.05 M buffer tris HCl (pH 7.7). One mL of crude lipase extract was added into the substrate and incubated at 37 ± 0.5 °C for 20 min and shaken at 150 rpm. The enzymatic digestion was ended by addition of 5 mL mixture of 50% ethanol and 50% acetone. Two drops of phenolphthalein indicator was added into the mixture and shaken carefully. Eventually, the mixture was titrated with 0.05 M NaOH until flash pink color appeared. The residual activity (A/A$_0$) of lipase is defined as a percentage of activity of treated samples (A) to the activity of untreated samples (A$_0$).
Aerobic Total Plate Count

Ten grams of samples in 90 mL of 0.85% NaCl was homogenized in a bag stomacher (Interscience BagMixer 400P) for 5 min. Serial dilution of untreated samples was performed to find appropriate dilution level. One mL of diluted samples was mixed with melted plate count agar (44 – 46°C) and incubated at 35°C for 48 hrs. After incubation, the petri dishes of incubated samples were placed on top of backlight source and digitally photographed with resolution of 10 million pixels. All images were processed, cropped, and scaled at 2772 pixels horizontally. OpenCFU software was used to enumerate the colony forming unit (CFU) (Geissmann, 2013). The minimum diameter of enumerated colony was set at 8 pixels which was equal to 0.25 mm spot diameter. Total Plate Count (TPC) of aerobic microbial is stated as CFU.g⁻¹ sample. Detection limit was set at 25-250 CFU/plate (Maturin and Peeler, 2001). The reduction of microbial loads is stated as \( \log_{10} \) of initial TPC (\( \log_{10}(N_0) \)) of raw fish subtracted by \( \log_{10} \) of pasteurized fish TPC (\( \log_{10}(N) \)).

Fillet texture recording

TA.XT plus texture analyzer (Stable Microsystem Inc.) with 50 kg load-cell was used to measure textural property of fish chunks. Fish chunks with 40 mm width, were compressed with 36 mm diameter cylindrical flat probe. The compression speed was 2 mm/s. The readings were recorded with an interval of 0.005 s. The compression travel was half of the original thickness of fish chunk. The hardness of fillet, which is maximum force recorded during compression, represented textural property of fillet. The unit of hardness was kg.

Fillet color measurement

Tilapia was filleted, and the inner part surface color was read. Subcutaneous or skin color could not be used for color readings due to uneven pigmentation. Reflectance colorimeter (Color muse, Variable Inc.) was placed on top of the fillet to record the colors. The colorimeter had 4 mm reading diameter area, 45° illumination angle, 0° reading angle, and 2° standard observer.
Before color reading, colorimeter had to be calibrated against supplied standard white. Each fillet was read on three different spots. Color was expressed in CIE L* (whiteness or brightness), a* (redness/greenness) and b* (yellowness/blueness) coordinates. Total Color differences (ΔE00) were calculated according to CIEDE-2000 standard (Sharma et al., 2005).

**Fillet temperature datalogging**

Thermocouple K was inserted ±1 cm into fish chunks and connected to Owon B35T multimeter. Realtime data were sent to android phone through bluetooth connection and recorded as CSV(Comma Separated Values) file.

**Statistical analysis**

Results were expressed as mean values ± standard deviation of triplicates. Three ways analysis of variance was carried out to examine the significance of treatments (p < 0.05). After analysis of variance, New Duncan Multiple Range Test was carried out to perform pairwise comparisons. All statistical procedures were performed with R statistical computing program (R Core Team, 2000).

**RESULT AND DISCUSSION**

**Effect of CO₂ pasteurization on residual protease and lipase activity**

As shown in Table 1, the effect of CO₂ pressure on protease inactivation was much more significant than that of temperature and holding time. Increasing time and temperature did not have significant impact on protease inactivation when applied pressure was at 90 bar. The longer holding time was found to improve protease inactivation when applied pressure was 80 bar or lower. When the experimental temperature was 50°C and holding time was 30 min, the effect of pressure was negligible, indicated the effect of temperature and holding time overrode the effect of CO₂ pressure. It could be determined that with 90 bar application, scCO₂ pasteurization was capable to reduce roughly three quarters of tilapia muscle protease.

Meanwhile, the effect of CO₂ pasteurization on lipase inactivation was mild. As shown in Table 2, the lowest residual activity was 74.38%; or roughly three quarters of initial lipase activity was still retained after CO₂ pasteurization. The effect of CO₂ pasteurization on lipase inactivation which was too low in comparison to protease inactivation is in agreement to the reports of several investigators.

Ishikawa et al. (1995) reported complete inactivation of alkaline protease and lipase after solution of alkaline protease and lipase exposed to 150 bar and 35°C micro-bubbling scCO₂. The application of micro-bubble scCO₂ was reported increased enzyme inactivation three folds than that of without micro-bubble scCO₂. The pH lowering effect of dissolved scCO₂ was reported affected alkaline protease inactivation while the same effect did not work on acid protease and lipase inactivation. Acid protease needed higher temperature to be inactivated, while lipase needed more pressure to get the same inactivation rate of alkaline protease. Therefore, the ratio between alkaline and acid protease would determine total inactivation of protease and the pressure in this study was insufficient to inactivate lipase at significant level.
Table 1. Effect of DPCD pasteurization on residual activity of muscle protease

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>t (min)</th>
<th>Residual protease activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>70 bar</td>
</tr>
<tr>
<td>40</td>
<td>15</td>
<td>71.4±15.04&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>59.65±8.52&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>80±7.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>39.82±13.83&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated fish</td>
<td></td>
<td>100±13.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 2. Effect of DPCD pasteurization on residual activity of muscle lipase

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>Residual lipase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>70 bar</td>
</tr>
<tr>
<td>40</td>
<td>15</td>
<td>90.29±3.41&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>96.69±1.24&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>92.56±1.43&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>86.78±2.15&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated fish</td>
<td></td>
<td>100±7.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 3. Effect of DPCD pasteurization on reduction of microbial load

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>t (min)</th>
<th>Microbial reduction (Log&lt;sub&gt;10&lt;/sub&gt;(N&lt;sub&gt;0&lt;/sub&gt;)-Log&lt;sub&gt;10&lt;/sub&gt;(N))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>70 bar</td>
</tr>
<tr>
<td>40</td>
<td>15</td>
<td>1.02±0.59&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.41±0.16&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>1.92±0.81&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.08±0.55&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated fish</td>
<td></td>
<td>0.53±0.23&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Fadıloğlu and Erkmen (2002) found that reducing initial pH of enzyme solution prior to CO<sub>2</sub> pressurization, could inactivate lipase completely under atmospheric CO<sub>2</sub> pressure (1 atm). However, adding acidifier would result altered sensory properties, since acidifier remains intact after pasteurization. The advantage of pH lowering effect of scCO<sub>2</sub> application is dissolved CO<sub>2</sub> would be immediately evaporated after pressure dropped. Therefore, the final pH should be near to its initial pH. It could be expected that pH lowering effect of scCO<sub>2</sub> does not alter the sensory properties of pasteurized food.

The inactivation of an enzyme could be temporary or permanent. When environmental condition supports, certain enzyme could be reactivated (Airas, 1972).
Pasteurization is intended to reduce enzymes activity permanently to maintain food quality during prolonged storage. Effect of scCO$_2$ pasteurization on long term inactivation of enzyme was investigated by Ishikawa et al. (2000). It was found that micro-bubble scCO$_2$ scavenge α-helix structure of enzyme, and therefore, disable its activity permanently.

Since micro-bubble scCO$_2$ became key in enzyme inactivation in the aqueous system, the absence of micro-bubble producing apparatus in this work and still achieved good inactivation rate of muscle protease; might be related to the presence of fish muscle as biological matrix of intracellular and tissue enzymes. The cell size of eukaryotes is within 10-100 μm (Campbell and Reece, 2005), and the cell membrane pores are much smaller, 110 ± 40 nm (Zhou et al., 2009). Therefore, the muscle tissue could be a substitution for micro-filter that was used in Ishikawa et al. (1995).

**Effect of CO$_2$ pasteurization on microbial loads**

One of the objectives of pasteurization is to reduce microbial presence in foodstuff, particularly scavenging and pathogen microbes. As shown in Table 3, scCO$_2$ pasteurization at 90 bar was able to reduce microbial loads more than 4-logs reduction. Since the regulation of food safety mandates maximum $5 \times 10^5$ cfu/g (BSN, 2009 and ICSMF, 1986), initial total plate count at max $10^5$ cfu/g must be ensured prior to scCO$_2$ pasteurization at 90 bar. Besides the effect of scCO$_2$ pasteurization on intracellular enzyme, penetration of scCO$_2$ into cell interior also induced cell lysis (Hong and Pyun, 2001). The bactericidal effect of CO$_2$ was obvious when compared to pasteurization attempt with N$_2$ (Hong and Pyun, 1999).

Another method to increase the killing rate on microbial loads is using explosive decompression (Foster et al., 1962 and Fraser, 1951). As shown in Table 3, the holding time did not affect microbial loads reduction; this was the indication that single compression and decompression cycle affected microbial reduction more significant than that of pasteurization holding time.

However, burst decompression will explode the cell and cause cell lysis. This method is not applicable for solid food since it would blast the food as well and damage the food texture. This study found that rapid decompression caused clogged exhaust outlet and the fillet became slurry.

**Effect of CO$_2$ pasteurization on physical appearance of fillet**

As reported by Canto et al. (2012), the drawbacks of high-pressure application are changes of texture and color. There was no exception for scCO$_2$ pasteurization when the pressure exceed tolerable limit for tilapia fillet. In this work, all 50°C pasteurization produced unacceptable soft fillet. As shown in Table 4, the fillet was capable to withstand under 80 bar scCO$_2$ pressure at 40°C. However, at 40°C, the pressure increase did not related to the level of softening. The fillet that pasteurized under subcritical pressure (70 bar) for 15 min was softer than that of supercritical 80 bar for 15 min.

When the holding time was increased, the fillet was going to be softer. Therefore, the optimum of scCO$_2$ pasteurization should be around 80 bar and at 40°C or lower temperatures. Fig 2. shows the integrity of fish chunk to support its own weight.
Table 4. Effect of DPCD pasteurization on the texture of fillet

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>t (min)</th>
<th>70 bar</th>
<th>80 bar</th>
<th>90 bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>15</td>
<td>10.426±2.947&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>13.8±4.56&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.19±0.609&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>7.445±2.789&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.369±1.160&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.678±0.961&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>2.656±0.452&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.410±1.178&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.724±0.919&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.384±1.019&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.905±0.737&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.192±0.140&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated fish</td>
<td></td>
<td></td>
<td>15.617±6.543&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Effect of DPCD pasteurization on the color of fillet

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>t (min)</th>
<th>70 bar</th>
<th>80 bar</th>
<th>90 bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>15</td>
<td>80.90±3.57&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.67±2.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.97±1.92&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>75.70±4.40&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.09±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.46±0.45&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>75.91±0.94&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.02±1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.21±1.59&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>75.85±3.99&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.43±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.06±0.10&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>80</td>
<td>15</td>
<td>75.85±1.75&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.53±2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.51±2.53&lt;sup&gt;bcd&lt;/sup&gt;</td>
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<td></td>
<td>30</td>
<td>70.29±3.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.82±2.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.36±2.15&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>15</td>
<td>81.17±5.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.78±5.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.71±4.43&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>30</td>
<td>82.37±3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.94±2.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.81±3.44&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td>Untreated fish</td>
<td></td>
<td></td>
<td>64.92±2.38&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.48±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Under 80 bar scCO2 pasteurization, only at 40°C for 15 min pasteurization, the fish chunks could support its own weight without suffering deformation.

Similar to that of high-pressure processing (HPP) application on fish (Truong et al., 2015), the drawback of HPP is adverse effect on fillet color. DPCD pasteurization also suffers color changes that similar to color of cooked fillet. As shown in Table 5, all fillets were very pale indicated by the L* values which were far higher than untreated samples. Only a*
component which represent greenness/redness of fillet remained unchanged. The b* which represent blueness/yellowness also significantly different than that of untreated samples.

This phenomenon was in agreement with the work of Ji et al. (2012), which reported much paler shrimp after scCO2 exposure. The color changes could be due to the loss of heme proteins, either because of leaching and/or denaturation. Richards et al. (2007) reported the heme protein of fish could withstand against 50°C heating but prone to oxidation at pH 7.4. This scCO2 pasteurization might result heme protein damage at temperature lower than 50°C. A study from Nuchuchua et al. (2016) confirmed that CO2 exposure cause a change in the heme binding and aggregation of myoglobin, one of the heme proteins.

**Barrel and fillet temperature differences.**

During pasteurization, the temperature differences between the barrel and fillet were recorded. It was found that only at subcritical CO2 pasteurization (70 bar), fillet temperature could reach the same level of barrel temperature; although the increase of fillet temperature was lagged behind barrel temperature during preheating stage. The differences between barrel and fillet temperature under subcritical phase were shown in Fig. 3A for pasteurization at 40°C and Fig. 3B for pasteurization at 50°C.

During scCO2 pasteurization, fillet temperature was lower than barrel temperature. The temperature difference between the barrel and fillet was found to be wider for higher barrel temperature. Fig. 3C shows the fillet temperature under pasteurization at 40°C and Fig. 3D shows pasteurization at 50°C, both were at 80 bar.

The temperature of fillet and barrel which achieved the same level during subcritical CO2 pasteurization (70 bar), might be attributed by continuous conversion between liquid and gaseous CO2 in the pressure vessel. The triple point of CO2 is 5.18 bar (Span and Wagner, 1996), in which both liquid and gaseous CO2 coexist. At 40°C and 50°C, liquid CO2 gained heat and evaporated. Since the concentration of CO2 vapor already at saturated level (13.7614 kg.m⁻³), the vapor was condensed and liquified again, back and forth. The evaporation and condensation cycle, resulted gas convection within the system. This convection stream distributed heat evenly into the object of pasteurization. Meanwhile, in the supercritical system, there is no phase difference. Therefore, there was no driving force for the gas and the liquid to be circulated in and out of
pasteurization object. Thus, it is plausible that in subcritical CO\textsubscript{2} system, heat distribution between pasteurizing agent and pasteurization object was better than that of scCO\textsubscript{2} system. However, since heat carry out minor role for deactivating enzyme in this temperature range (< 50°C), the direct impact of experimental temperature on enzyme inactivation was not significant.

**CONCLUSION**

Supercritical CO\textsubscript{2} had been found was capable to reduce enzyme activity, particularly protease, at significant level. It was equally capable to reduce the microbial loads of Nile tilapia fillet significantly. These findings promise the potential of scCO\textsubscript{2} as pasteurization medium at mild temperature. In order to achieve significant pasteurization effect, fish must be pasteurized at 80 bar scCO\textsubscript{2} or higher. Holding time 30 min and experimental pressure 50°C were found to have adverse effect on fillet texture. The color of fillet was whitish, regardless the level of pressure, holding time, and temperature.

![Figure 3 Fillet temperature during CO2 pasteurization](image)

A. 70 bar, 40°C B. 70 bar, 50°C

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**REFERENCES**


ICMSF. (1986). Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific


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