Impacts of high-pressure processing on quality and shelf-life of yellowfin tuna (Thunnus albacares) stored at 4°C and 15°C

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ABSTRACT
The objective of this work was to study the application of high-pressure processing (HPP) on the quality change and extending the shelf-life of yellowfin tuna (Thunnus albacares) stored at low temperature. The effects of HPP treatments (200, 300, 400, 500, and 600 MPa for 5 min) on microbiological and chemical quality, and histamine content of tuna flesh stored at 4°C and 15°C, respectively, were evaluated. With the increased pressure, the levels of aerobic plate count (APC) and coliform in tuna flesh significantly decreased. It was also found that the $L^*$ (lightness), $b^*$ (yellowness), $W$ (whiteness), $\Delta E$ (color difference), and texture (hardness, cohesiveness and chewiness) of fish flesh increased significantly with the increased pressure; however, $a^*$ (redness) value decreased. Furthermore, HPP with a pressure of $\geq 300$ MPa on tuna samples significantly delayed the increase in APC during storage at 4°C or 15°C, respectively, while the samples pressurized more than 200 MPa had significantly lower levels of total volatile basic nitrogen (TVBN) and histamine compared to the control samples during storage. The results indicated that tuna flesh pressurized at least 300 MPa for 5 min could be a technique employed to extend the shelf-life to 1 d at 15°C and 6 d at 4°C, based on the APC limit standard (6.47 log CFU/g). In this study, HPP can be seen as a potential useful technique to preserve tuna flesh due to its ability to reduce bacterial load and retard TVBN and histamine production.

Introduction
Yellowfin tuna (Thunnus albacares) is a highly migratory and widely distributed fish species in tropical and subtropical oceans. Of the four main tuna species caught (albacore, bigeye, skipjack, and yellowfin), the production of yellowfin tuna accounts for 42% of total tuna catch in the Indian Ocean by Taiwan’s fishing boat.[1] In Taiwan, this tuna is mainly frozen (whole fish, loins) and fresh (chilled whole fish, loins) types for sale in fishery markets; besides canning, it is served as sashimi, sushi, and deep or lightly-fried for consumers.[1] Histamine is a substance that can cause allergy-like poisoning; therefore, it commonly called scombrototoxicosis or histamine fish poisoning.[2] Histamine is produced by the decarboxylation of free histidine by histidine decarboxylase produced by histamine-producing bacteria.

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in fish meat.\textsuperscript{2} Scombroid fish, such as tuna, mackerel, bonito, and saury, which contain high levels of free histidine in their muscle tissue are often implicated in histamine poisoning incidents. In Taiwan, histamine fish poisoning most often occurs after eating tuna,\textsuperscript{3} marlin,\textsuperscript{4} mackerel,\textsuperscript{5} and milkfish.\textsuperscript{6}

With regard to controlling the accumulation of histamine or other biogenic amines in food products, some methods such as modified atmosphere packaging, irradiation, food additives and preservatives, and amine-degrading starter cultures have been studied.\textsuperscript{7,8} The mechanism employed to control amines content in those methods is mainly by inhibiting the growth of amines-producing bacteria and decarboxylase activities of amino acids. Information on the formation of histamine content in fish or fish products that were treated by high pressure is scarce. High-pressure processing (HPP) is an emerging non-thermal processing technology, which aims to kill spoilage and pathogenic microorganisms with pressure above 300 MPa to prolong the shelf-life of food and improve food safety.\textsuperscript{9,10} Compared with traditional thermal processing, this technology has the advantages of retaining more nutrients and aromatic substances in the food; therefore, HPP is especially suitable for the processing of heat-sensitive food. For the purpose of food processing, preservation, and sterilization, HPP can cause enzymes inactivation in food due to the destruction of noncovalent bonds (such as hydrogen bonds and hydrophobic bonds) during the pressurization process, gelatinize starch, alter protein gel properties, and destroy cell walls or membranes of microorganisms to reduce the number of microorganisms.\textsuperscript{11,12} At present, HPP is been widely used for meats, juice and beverages, vegetables, and aquatic products.\textsuperscript{13} Kim et al.\textsuperscript{14} reported that hydrostatic pressures of 300 and 400 MPa can delay the growth and histamine production of histamine-producing bacteria (Morganella morgani and Photobacterium phosphoreum) when inoculated in mackerel meat during low-temperature storage. In addition, previous studies have examined the use of HPP in fish, such as salmon,\textsuperscript{15} cod,\textsuperscript{16} mackerel,\textsuperscript{17} herring and haddock,\textsuperscript{18} and carp.\textsuperscript{19}

Ramirez-Suarez and Morrissey\textsuperscript{20} found that after albacore tuna (Thunnus alalunga) minced muscle was treated with HPP at 275 and 310 MPa for 2, 4, and 6 min, respectively, the pressure increased pH, offered protection from lipid oxidation, maintained low microbial counts, changed the color of muscle, and increased the hardness. Cartagena et al.\textsuperscript{21} reported that high pressures under 200 to 250 MPa for 2 min pressurized on albacore tuna steaks were suitable for decreasing weight loss in fish steaks without exerting a great impact on color and texture. Recently, after 12 months of frozen storage, HPP pretreatment at 200 MPa for 4 min on albacore tuna steaks enabled them to maintain similar texture values and reducing thawing losses, compared to the fresh steak control.\textsuperscript{22} Although research using HPP on tuna has been carried out on the above-mentioned albacore tuna steak, there is little research regarding the pasteurization and preservation of yellowfin tuna flesh through HPP treatments. In addition, the extant literature on the use of HPP treatments for reducing histamine and total volatile basic nitrogen (TVBN) formation, as well as maintaining freshness quality, in tuna flesh is scarce.

Therefore, the objective of this work was to study the effect of HPP under various pressures on the retarding quality change and extending the shelf-life of yellowfin tuna (Thunnus albacares) stored at low temperature, especially in regard to reducing histamine production in fish and maintaining freshness. To achieve this objective, fresh tuna flesh was treated at a pressure of 200–600 MPa for 5 min; immediately afterward the changes in the aerobic plate count (APC), coliform, Escherichia coli, appearance, texture, and color of the fish flesh were observed. In addition, the abovementioned pressurized fish flesh was stored at 4°C and 15°C, respectively, and regularly sampled to analyze the changes in histamine-related quality in order to evaluate the effect of high-pressure processing on prolonging the storage life and freshness of tuna flesh.

**Materials and methods**

**Sample preparation**

The yellowfin tuna (Thunnus albacares) loin was purchased from a fishery market in Kaohsiung, immediately placed in crushed ice and transported back to the laboratory. The fish flesh was then cut into cubes of $3 \times 3 \times 3 \text{ cm}$ (30 g), soaked in sterile water to wash off the blood on the surface,
the water off, and then vacuum-packed in vacuum bags (nylon/linear low-density polyethylene) at 0.02 MPa of vacuum degree. Each bag contained approximately 30 g of fish flesh. Because the small and experimental high-pressure equipment used in this study has limited capacity, the fish flesh had to be cut into small cubes for HPP treatment.

**High-pressure processing**

The vacuum-packed tuna flesh samples were placed in a high-pressure device (BaoTou KeFa High Pressure Technology Co. Ltd., Baotou, China) with a diameter × depth of 200 mm × 200 mm, a volume of 6.2 L and a working pressure range of 0.1–600 MPa; water was used as the pressure transmission medium at 20°C. The average heating rate of pressurized fluid was 2 ± 0.5°C per 100 MPa pressure. The maximum pressure was reached within 1.5 min, and the pressure reduction time was approximately 10–15 s. De Albu et al. found that after treatment at 300 or 500 MPa for 5 min, the APC of mackerel fillets could be effectively reduced, so we appropriately increased or decreased the pressure used and pressurized for 5 min; therefore, the high pressure conditions were set at 200, 300, 400, 500, and 600 MPa for 5 min. Under the high-pressure conditions of 200, 300, 400, 500, and 600 MPa for 5 min, the APC, coliform count, *E. coli*, color values (L*, a*, b*, W, and ΔE), and texture of fish flesh were determined. The tuna sample without high-pressure processing was used as the control group. The control group and samples treated with different high pressures were subjected to storage at 4°C and 15°C, respectively. The fish samples were stored at 4°C to simulate the temperature of a refrigerator, while other samples were stored at 15°C to simulate the temperature at which fish are placed on crushed ice while being sold by fishmongers. The samples stored at 4°C for 15 d were sampled every 3 d, while those stored at 15°C for 5 d were sampled once a day. After sampling, the changes in the APC, TVBN, and histamine content were analyzed. Three individual samples (n = 3) in each pressurized groups were taken for analysis at each sampling point.

**Analysis of microbiological quality**

With regard to APC, a 10.0 g sample of finely minced fish flesh was weighed, placed in a sterilized bottle (containing 90 mL of 0.85% sterile saline) for homogenizing at a 1200 rpm speed for 2 min. Then, 1 mL of the stock solution was added into a sterilized test tube containing 9 mL of 0.85% sterile saline for serial dilution. Subsequently, 0.1 mL taken from each dilution was spread on a tryptose soy agar (TSA, Difco, BD, Sparks, MD, USA) culture medium in duplicate and incubated at 30°C for 24–48 h. Finally, the colony numbers on the culture dish were counted and calculated as log₁₀ colony forming units (CFU) per gram. The results were expressed as mean values for three individual fish flesh samples (n = 3).

For the detection of coliform and *E. coli*, 1.0 mL of the fish homogenate solution and a 10-fold serial dilution solution prepared during the abovementioned APC determination was transferred to Lauril Sulfate Tryptose (LST) broth (Difco, BD, Sparks, MD, USA). After culturing at 35°C for 24–48 h, one loop of the bacterial solution taken from the gas-producing LST broth tube was added into Brilliant Green Bile Broth (BGLB) (Difco, BD, Sparks, MD, USA) tube and cultured at 35°C for 48 h. Gas production in BGLB tube was considered as a positive reaction, and the most probable number (MPN) of coliform was calculated immediately. In addition, one loop of the bacterial solution taken from the gas-producing BGLB tube was added into *E. coli* broth (EC) (Difco, BD, Sparks, MD, USA) and cultured at 45°C for 24 h. No gas production indicated a negative reaction of *E. coli*, and if there was gas production, a positive *E. coli* reaction was suspected. Then, one loop of the bacterial solution taken from the gas-producing tube (EC) was streaked onto an eosin methylene blue agar (EMB agar, Difco, BD, Sparks, MD, USA) and cultured for 18–24 h at 35°C. Colonies with a metallic luster were confirmed as *E. coli*, and the MPN of *E. coli* was calculated. The results were expressed as mean values for three individual fish flesh samples (n = 3).
**Appearance and color measurement**

The appearance of the fish flesh was recorded on a white background by using a SLR camera (EOS 60D, Canon Inc., Japan). A colorimeter (CR-300 Chroma meter, Konica Minolta, Inc., Tokyo, Japan) was used to analyze the color changes of fish flesh samples in the pressure-treated groups and control group.\(^{[25]}\) The colorimeter was first calibrated with a white ceramic plate, and the fish flesh was then placed in it. The values displayed on the screen were \(L^*\) (lightness), \(a^*\) (+a, red; -a, green) and \(b^*\) (+b, yellow; -b, blue). For each fish flesh sample, 3 measurements were performed at different locations, while the \(W\) (whiteness) and \(\Delta E\) (color difference) values were calculated according to the following equation. The results were expressed as mean values for three individual samples (\(n = 3\)). The \(W\) value was calculated as follows:

\[
W = 100 - \left\lfloor (100 - L^*)^2 + a^{*2} + b^{*2} \right\rfloor^{1/2}
\]

(1)

The \(\Delta E\) value was calculated as follows:

\[
\Delta E = \left[ (L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2 \right]^{1/2}
\]

(2)

where \(L_1^*, a_1^*,\) and \(b_1^*\) were original fish flesh colorimetric values and \(L_2^*, a_2^*,\) and \(b_2^*\) were the colorimetric values of fish flesh samples after high-pressure processing.

**Instrumental texture measurement**

A texture profile analysis (TPA) was performed on tuna flesh using a TA-XT2 texture analyzer (Stable Micro System, Ltd., Surrey, UK).\(^{[25]}\) The TPA was used to measure the changes of texture, including hardness, cohesiveness, springiness, and chewiness in fish flesh after high-pressure processing. A spherical probe (TA18 Sphere, 12.7 mm diameter) was used for detection; the fish flesh was placed on the stage at a distance of 0.5 cm from the probe; the probe pressing speed was set at 0.2 cm/s; the pressing depth was 1.0 cm; the holding time was 3 s, and the trigger force was 5 g. Each fish flesh sample was measured three times at different locations, and the results were expressed as mean values for three individual fish flesh samples (\(n = 3\)) from each pressurized treatment.

**Total volatile basic nitrogen (TVBN) and histamine**

Tuna flesh samples (5 g) were placed in a 50 mL centrifuge tube containing 20 mL of 6% trichloroacetic acid (TCA, Sigma-Aldrich, Burlington, MA, USA) and the tube contents were homogenized at 1,200 rpm speed for 2 min. The homogenized solution was centrifuged at 3,000 × g for 10 min (4°C) and filtered through Whatman No. 1 filter paper (Whatman, Maidstone, UK). The above steps were repeated twice. The filtrate was collected and constituted to 50 mL with 6% TCA (i.e., TCA extract) for analysis of TVBN and histamine content. Conway’s dish method proposed by Cobb et al.\(^{[26]}\) was used to determine the content of TVBN in the fish meat. Boric acid (Sigma-Aldrich, Burlington, MA, USA) absorption solution (1 mL) was added into the inner chamber of the Conway’s dish, while saturated potassium carbonate (1 mL) and fish flesh TCA extract (1 mL) were added into the outer chamber. The Conway’s dish was incubated at 37°C for 90 min and then titrated with 0.02 N HCl. The TVBN content was expressed as mg/100 g fish flesh. The results were expressed as mean values for three individual fish flesh samples (\(n = 3\)).

In addition, histamine analysis was modified via the method proposed by Chen et al.\(^{[4]}\) Histamine standard (Sigma-Aldrich, Burlington, MA, USA) and the TCA extract of fish flesh were derivatized with dansyl chloride (Sigma-Aldrich, Burlington, MA, USA) and injected into the HPLC (Hitachi, Ibaraki Prefecture, Japan) for detection. The machine and analysis conditions are as follows:

Separating column: LiChrospher 100 (5 μm) RP-18 reverse chromato-column (125 × 4 mm i.e., E. Merck, Darmstadt, Germany).
Mobile phase: Solution A was acetonitrile (E. Merck, Darmstadt, Germany); Solution B was water. Gradient conditions: Initially, A:B = 50%:50%; at 19 min, Solution A was increased to 90%; at the 20th min, Solution A was reduced to 50% and maintained for 10 min; the total analysis time was 30 min.

Flow rate: 1.0 mL/min
Injection volume: 20 μL
Column temperature: 40°C
Setting of wavelength: 254 nm

The results were expressed as mean values for three individual fish flesh samples (n = 3).

Statistical analysis

The microbial counts, color, texture, TVBN, and histamine content of the samples treated under different pressures were analyzed to determine the differences among the pressure groups. The mean ± standard deviation of the triplicate measurements was calculated, and statistical analysis was performed using SPSS 12.0 software (St. Armonk, New York, USA) using analysis of variance and Tukey's test, with p < .05 indicating a statistically significant difference.

Results and discussion

Microbial counts of pressurized fish samples

The microbial counts of tuna samples after high-pressure processing are presented in Table 1. The initial APC of fish flesh (control group) was 5.97 log CFU/g, which decreased to 5.51, 5.44, 4.75, 3.66, and 2.15 log CFU/g with the increased pressure at 200, 300, 400, 500, and 600 MPa, respectively (i.e., reduced by 0.46, 0.53, 1.22, 2.31, and 3.82 log CFU/g, respectively) (p < .05) (Table 1). The above result suggests that higher pressure is more effective in reducing the APC in fish samples. APC is one of the important indices for evaluating the quality of fresh and low-temperature-stored aquatic products. A similar finding was also demonstrated by de Alba et al. [23] who found that HPP at 300 and 500 MPa for 5 min significantly reduced the APC levels of mackerel fillets; reductions of 0.80 and 2.48 log CFU/g, respectively, were detected. Montiel et al. [16] pressurized smoked cod at 400, 500, and 600 MPa for 5 and 10 min, respectively, and observed that higher pressure significantly reduced the microbial count in smoked cod. A high hydrostatic pressure can induce irreversible denaturation of enzymes and proteins, causing rupturing of the cell membrane and the excretion of internal substances, resulting in bacterial death. [9] In addition, HPP has been reported to reduce cellular protein content by disrupting protein structures, lowering protein biosynthesis, and inhibiting protein repairs, ultimately resulting in bacterial damage or death. [10]

| Treatments                        | APC (log CFU/g) | Coliform (MPN/g) | E. coli (MPN/g) |
|-----------------------------------|-----------------|------------------|----------------|
| Control (0.1 MPa)                 | 5.97 ± 0.21a    | 120 ± 42b        | <3             |
| 200 MPa                           | 5.51 ± 0.32b    | 95 ± 10b         | <3             |
| 300 MPa                           | 5.44 ± 0.25b    | 15 ± 3.0b        | <3             |
| 400 MPa                           | 4.75 ± 0.30c    | <3               | <3             |
| 500 MPa                           | 3.66 ± 0.15d    | <3               | <3             |
| 600 MPa                           | 2.15 ± 0.15e    | <3               | <3             |

*Each value is the mean ± standard deviation (n = 3); the different letters at the same column indicate significant difference (p < 0.05).
With regard to coliform and *E. coli* count, it was found that the initial coliform count in the fish sample was 120 MPN/g, which decreased significantly with the increased pressure; at high pressure above 400 MPa, coliforms were not detected. Notably, *E. coli* was not detected in any of the groups (Table 1), indicating that higher pressure is more effective in reducing coliform count in fish flesh.

**Appearance and color values of pressurized fish samples**

The appearance of tuna flesh after high-pressure processing in this study is shown in Figure 1. The fresh tuna flesh was translucent and crimson, while after the 400 MPa treatment, the flesh became opaquer and light red. With the increased pressure (>400 MPa), it showed whiter with a more fading color. Therefore, the 300 MPa for 5 min is a critical pressure for acceptable visual properties after pressurizing tuna flesh.

In addition, the changes in color-related values (*L*,* a*, *b*, *W*, and Δ*E*) were measured by a colorimeter as shown in Table 2. The *L* value (lightness) of the control group increased from 28.90 to 56.85 (600 MPa group) with the increased pressure (*p* < .05). The increased lightness resulted from the decrease in pigment activity and protein coagulation (denaturation), as protein coagulation changes the characteristics of sample surface, increases light reflection, and produces a white appearance. [27] Furthermore, after high-pressure processing, the *a* value (redness) of fish flesh decreased significantly from 10.19 in the control group to 2.80 in the 600 MPa group (*p* < .05). On the contrast, the *b* value (yellowness) showed a slight upward trend with the increased pressure (*p* < .05). Similar results were reported by Cartagena et al. [21] who showed that albacore tuna steaks treated with high pressure had a brighter appearance and higher *L* and *b* values, but that the *a* value

![Figure 1. Changes in appearance of tuna fleshes after HPP treatment at 200, 300, 400, 500 and 600 MPa for 5 min.](image)

| Treatments | *L*  | *a*  | *b*  | *W*  | Δ*E* |
|------------|------|------|------|------|------|
| Control (0.1 MPa) | 28.90 ± 0.19 | 10.19 ± 0.04 | -4.29 ± 0.08 | 28.05 ± 0.18 | - |
| 200 MPa | 32.23 ± 0.09 | 9.68 ± 0.05 | -4.01 ± 0.04 | 31.42 ± 0.11 | 3.38 ± 0.04 |
| 300 MPa | 36.92 ± 0.13 | 9.57 ± 0.03 | -3.86 ± 0.03 | 36.08 ± 0.06 | 8.06 ± 0.03 |
| 400 MPa | 48.77 ± 0.23 | 9.00 ± 0.06 | -3.02 ± 0.01 | 47.90 ± 0.18 | 19.95 ± 0.06 |
| 500 MPa | 52.07 ± 0.23 | 3.78 ± 0.02 | -1.80 ± 0.07 | 51.89 ± 0.13 | 24.17 ± 0.04 |
| 600 MPa | 56.85 ± 0.07 | 2.80 ± 0.04 | -1.50 ± 0.08 | 56.73 ± 0.09 | 29.04 ± 0.07 |

*Each value is the mean ± standard deviation (n = 3); the different letters at the same column indicate significant difference (p < 0.05).*
decreased with the increased pressure. Our study results are also in accordance with de Alba et al.\textsuperscript{[23]} who found an increase of $L^*$ and a decrease of $a^*$ in mackerel fillets at higher-pressure intensities. In addition, the $W$ value of the control group increased from 28.05 to 56.73 (600 MPa group), and $\Delta E$ tended to increase significantly ($p < .05$) to 29.04 (600 MPa group) with the increased pressure (Table 2). Ledward\textsuperscript{[26]} pointed out that HPP caused changes in the color parameters of fish flesh, perhaps due to the degeneration of myofibrillar and sacroplasmic proteins. Additionally, the reduced fish redness caused by high pressure may be attributed to the degradation of pigments and myoglobin.\textsuperscript{[29]} In this study, the results are in agreement with a previous research reporting that the higher $\Delta E$ values of 5.80 and 12.11 were observed for hilsa fillets treated at 250 and 350 MPa for 10 min, respectively.\textsuperscript{[30]}

**Texture of pressurized fish samples**

The changes in the texture of tuna flesh after high-pressure processing are described in Table 3. The hardness of fish flesh increased from 2.79 N in the control group to 9.23 N with the increased pressure, as seen in the 600 MPa group ($p < .05$). The increased hardness of fish meat under high pressure may be related to the denaturation and aggregation of myofibrillar protein.\textsuperscript{[31]} The cohesiveness increased from 0.49 in the control group to 1.64 in the 600 MPa group with increasing pressure ($p < .05$). Conversely, the average springiness of the samples decreased slightly from 7.41 to 4.53 (mm) with the increased pressure ($p < .05$). Chewiness showed similar results to hardness and increased from 9.64 (ml) in the control group to 30.72 (ml) in the 600 MPa group ($p < .05$). Collectively, the average values of hardness, cohesiveness, and chewiness of the texture of fish meat increased with the increased pressure. The reason for the changes in tissue texture is the denaturation and aggregation of the muscle protein at a high pressure, which causes tissue structure shrinkage.\textsuperscript{[31]} The results of this study are in agreement with a previous study reporting that the increases in hardness and chewiness with increasing pressure in albacore tuna steaks at 50–500 MPa for 2 min.\textsuperscript{[21]} Similar results were reported by Aubourg et al.\textsuperscript{[32]} who indicated that hardness, chewiness, and cohesiveness of Atlantic mackerel increased with higher levels of pressure and longer pressure holding times, but that springiness was affected less by pressure level and holding time.

**APC changes of pressurized tuna samples during low-temperature storage**

The changes in APC of fresh fish flesh after high-pressure processing during storage at 15°C and 4°C are shown in Figure 2. When stored at 15°C, the APC of the control and 200 MPa groups increased with the increased storage time (Figure 2a); there was no significant difference among them during storage ($p > .05$). However, the APC of the 300 and 400 MPa groups increased gradually during the first 3 d of storage and was significantly lower than that of the control and 200 MPa groups ($p < .05$), but there was no difference after day 4 of storage. In addition, the bacterial count of the 500 and 600 MPa groups increased slowly during the first 2 d of storage and subsequently increased significantly; however, the count was lower than that of the control group or other pressure groups ($p < .05$).

| Treatments   | Hardness (N) | Cohesiveness | Springiness (mm) | Chewiness (ml) |
|--------------|--------------|--------------|-----------------|----------------|
| Control (0.1 MPa) | 2.79 ± 0.94<sup>a</sup> | 0.49 ± 0.03<sup>d</sup> | 7.41 ± 0.59<sup>a</sup> | 9.64 ± 2.08<sup>d</sup> |
| 200 MPa      | 4.19 ± 0.71<sup>de</sup> | 0.48 ± 0.04<sup>d</sup> | 7.09 ± 0.64<sup>ab</sup> | 14.95 ± 3.45<sup>cd</sup> |
| 300 MPa      | 4.77 ± 0.77<sup>c</sup> | 0.54 ± 0.05<sup>c</sup> | 6.91 ± 0.87<sup>ab</sup> | 16.75 ± 3.99<sup>c</sup> |
| 400 MPa      | 6.64 ± 0.54<sup>b</sup> | 0.95 ± 0.06<sup>b</sup> | 6.66 ± 0.59<sup>ab</sup> | 25.13 ± 2.53<sup>b</sup> |
| 500 MPa      | 8.52 ± 0.50<sup>a</sup> | 1.00 ± 0.08<sup>b</sup> | 6.09 ± 0.65<sup>b</sup> | 25.49 ± 3.22<sup>ab</sup> |
| 600 MPa      | 9.23 ± 0.96<sup>a</sup> | 1.64 ± 0.10<sup>a</sup> | 4.53 ± 0.41<sup>c</sup> | 30.72 ± 3.79<sup>ab</sup> |

* Each value is the mean ± standard deviation ($n = 3$); the different letters at the same column indicate significant difference ($p < .05$).
indicating that higher pressure delays the increase in APC. According to Taiwan’s microbial hygiene standards at 6.47 log CFU/g of APC for fresh aquatic products, the APC of the control and 200 MPa groups was between 6.50 and 6.48 log CFU/g on day 1 of storage at 15°C, which exceeded this hygienic limit standard. The APC of the 300 and 400 MPa groups (7.10 and 6.78 log CFU/g, respectively)

![Figure 2](image-url). Changes of aerobic plate count of tuna fleshes after HPP treatment at 200, 300, 400, 500 and 600 MPa for 5 min during storage at 15°C (a) and 4°C (b). Each value represents mean ± SD of three replications; Dash-line represents 6.47 log CFU/g of APC as the limit standard for fresh frozen fish.
exceeded the limit standard at day 2, while the APC of the 500 MPa group (6.50 log CFU/g) also exceeded the limit standard at day 3. However, the APC in 600 MPa group did not exceed the limit standard during storage of 5 d. Therefore, when the tuna samples were stored at 15°C, the shelf life could be extended by 1 d, 2 d and 5 d after HPP treatments at 300 and 400 MPa, 500 MPa, and 600 MPa, respectively.

In addition, when stored at 4°C, the APC of the control, 200 and 300 MPa groups increased gradually with the increased storage time (Figure 2b), and there was significant difference among them at day 3, but no difference after day 6 (p > .05). However, the APC of the 400 MPa group increased slowly during the first 9 d of storage and was significantly lower than that in the control, 200 MPa, and 300 MPa group (p < .05), but there was no difference after 12 d of storage. In addition, the bacterial count of the 500 and 600 MPa groups increased slowly and was lower than that in the control group and other low-pressure groups (p < .05), indicating that higher pressure delays the increase in APC. The APC of the control was 6.55 CFU/g on day 3 of storage at 4°C, which exceeded the limit standard (6.47 log CFU/g). In addition, the APC of the 200 and 300 MPa groups did not exceed the limit standard until day 6 (6.58 log CFU/g) and day 9 (6.59 log CFU/g), respectively. However, the APC in the 400 MPa group did not exceed the limit standard until the 12th d (6.80 log CFU/g), whereas that of the 500 and 600 MPa groups did not exceed the limit standard during storage. Therefore, when tuna samples were stored at 4°C after high-pressure processing at 200, 300, 400 MPa, and 500 and 600 MPa, the shelf life could be extended by 3, 6, 9 and 15 d, respectively.

Based on the above results, it can be concluded that high-pressure (≥300 MPa) processing can delay the increase in the APC of tuna flesh when stored at low temperatures (4°C and 15°C); the effect of delaying the growth of APC in fish flesh was more significant when combined with storage at a lower temperature (4°C). Similarity, Montiel et al. [16] pressurized smoked cod at 400, 500, and 600 MPa for 5 and 10 min, respectively, and observed that higher pressure significantly reduced the microbial count in smoked cod and delayed microbial growth in fish meat during cold storage (5°C). Therefore, it is suggested that smoked cod should be pressurized at 400 MPa for 10 min or 500 MPa for 5 min for prolonging storage life. De Albu et al. [23] found that after treatment at 500 MPa for 2 min and 300 or 500 MPa for 5 min, the APC of mackerel fillets could be effectively reduced, and the refrigerated storage life of mackerel slices could be prolonged.

**TVBN changes of pressurized tuna samples during low-temperature storage**

The changes in TVBN of tuna flesh after high-pressure processing during storage at 15°C and 4°C are shown in Figure 3. In the control group, the contents of TVBN increased rapidly during storage, reaching 46.1 mg/100 g at the end of storage (day 5). TVBN contents of the 200 MPa, and 300 MPa groups increased gradually with the increased storage time at 15°C for 5 d, but TVBN contents of the 400 MPa groups did not remarkably change during the first 3 d of storage, increased slowly only after day 4, and was significantly lower than that of the control, 200 MPa, and 300 MPa groups (p < .05) (Figure 3a). In addition, the TVBN contents of the 500 and 600 MPa groups did not significantly increase during storage and were lower than that in the control group and other low-pressure groups (p < .05). Similarity, when stored at 4°C for 15 d, TVBN content of the control group increased rapidly, reaching 51.8 mg/100 g at the end of storage (day 15) (Figure 3b). TVBN contents of the 200 MPa group increased gradually with the increased storage time, reaching 45.3 mg/100 g by the end, but TVBN contents of the 300 and 400 MPa groups did not remarkably change during the first 9 d of storage, and increased progressively only after day 12. In addition, the TVBN contents of the 500 and 600 MPa groups did not significantly increase during storage and were lower than that in the control group and other low-pressure groups (p < .05), indicating that high pressure significantly delayed the increase in TVBN production of tuna samples.

In summary, according to Taiwan’s hygienic standard for fresh aquatic products at 25 mg/100 g of TVBN, the values in the control and 200 MPa groups when stored at 15°C for 5 d already exceeded the limit standard on days 1 and 2, respectively (the TVBN values were 30.2 and 26.1 mg/100 g,
Figure 3. Changes of TVBN of tuna fleshes after HPP treatment at 200, 300, 400, 500 and 600 MPa for 5 min during storage at 15°C (a) and 4°C (b). Each value represents mean ± SD of three replications; Dash-line represents 25 mg/100 g of TVBN as the freshness standard for fresh aquatic products.
respectively); the values in the 300 and 400 MPa groups did not exceed the limit standard until day 3 and 4 (the TVBN values were 27.2 and 25.4 mg/100 g, respectively). In contrast, the 500 and 600 MPa groups did not exceed the limit standard (25 mg/100 g) during the 5 d of storage. Therefore, when tuna samples were stored at 15°C, high-pressure processing at 200, 300, 400, and >500 MPa could extend the shelf life to 1, 2, 3, and 5 days, respectively (Figure 3a). In addition, the TVBN in the control group on day 3 exceeded the limit standard and reached 33.2 mg/100 g when stored at 4°C for 15 d. The 200 MPa (31.9 mg/100 g) and 300 MPa (32.6 mg/100 g) groups exceeded the limit standard on days 6 and 12, respectively, and the 400 MPa (31.7 mg/100 g) group exceeded the limit standard on day 15. In contrast, the 500 and 600 MPa groups did not exceed the limit standard (25 mg/100 g) during the 15 d of storage. Therefore, when tuna samples were stored at 4°C, high-pressure processing at 200, 300, 400, and >500 MPa could extend the shelf life to 3, 9, 12 and 15 d, respectively (Figure 3b). Collectively, TVBN production was delayed and the storage life was prolonged in tuna when stored at low temperatures (<15°C) after treatment at high pressures above 200 MPa.

Histamine changes of pressurized tuna samples during low-temperature storage

The changes in histamine content in tuna flesh after high-pressure processing during storage at 15°C and 4°C are shown in Figure 4. The control and 200 MPa groups when stored at 15°C for 4 d and 5 d (16.0 and 10.2 mg/100 g, respectively) had histamine contents which exceeded the limit standard of 5 mg/100 g specified by the US Food and Drug Administration (USFDA) (Figure 4a). Furthermore, the histamine content of fish sample in the 300 MPa group stored till day 5 were 5.1 mg/100 g, which also exceeded the limit standard (5 mg/100 g) specified by the USFDA. Finally, the histamine contents in the 400, 500 and 600 MPa groups during storage were lower than 1.0 mg/100 g. Therefore, it can be seen that the >200 MPa high-pressure treatment groups showed a higher delaying effect of histamine production compared with the control group (Figure 4a). When stored at 4°C for 15 d, the detected histamine content in the control and 200 MPa groups was 2.5 and 0.5 mg/100 g, respectively, on day 15 of storage (Figure 4b). However, in the >300 MPa groups stored at 4°C for 15 d, the histamine content was lower than 1.0 mg/100 g. The >200 MPa pressure treatment groups had a greater effect of delaying histamine production than that in the control group (Figure 4b). In summary, high-pressure processing of tuna fleshes above 200 MPa could inhibit the production of histamine, i.e., high-pressure processing could delay the increased histamine in tuna flesh stored at low temperatures (4°C and 15°C).

The results of this study are similar to those of many published reports. For example, Křížek et al. reported that high pressures (300 and 500 MPa) could reduce the production of histamine and other biogenic amines in pike (Esox lucius) meat during storage at low temperatures (3.5°C and 12°C) and prolong the storage life. Ucak et al. applied high pressures (300 and 500 MPa) to vinegar-pickled herring inoculated with M. psychrotolerans, which could reduce the production of histamine and other biogenic amines in fish meat during cold storage and prolong the storage life. To the best of our knowledge, however, most published works normally involve two or three pressures on tuna, studying their impact on the weight loss, thawing loss, color, texture or lipid oxidation, so this is the first study to evaluate the use of HPP on retarding histamine and TVBN formation, as well as maintaining freshness quality, in tuna flesh during storage at low temperature.

Conclusion

The above results showed that HPP significantly reduced the APC and coliform loads. With the higher pressure, the whiter and more opaque appearance of fish flesh was observed because the L*, W, and ΔE of the samples increased, but the a* (redness) decreased. Regarding texture, HPP caused significantly increased hardness, cohesiveness, and chewiness of tuna flesh. Therefore, the higher pressure (>400 MPa) may have some negative effects on tuna color and texture. In addition, HPP above 200 MPa retarded the production in TVBN and histamine content in tuna flesh stored at low temperatures,
Figure 4. Changes of histamine content of tuna fleshes after HPP treatment at 200, 300, 400, 500 and 600 MPa for 5 min during storage at 15°C (a) and 4°C (b). Each value represents mean ± SD of three replications; Dash-line represent 5.0 mg/100 g of histamine as the limit standard of USFDA.
while the delaying effect on the increase of APC was significant at higher pressures (>300 MPa). In summary, HPP at least 300 MPa for 5 min was effective in preserving tuna flesh, as well as extending the shelf life during storage at low temperatures (4°C and 15°C). This result also suggests that HPP can provide a new alternative to prevent deterioration and quality loss of fish flesh, and prolong the storage life for seafood industry.

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Disclosure statement

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