

Inhibitory effects of high pressure processing on microbial growth and histamine formation in spotted mackerel (*Scomber australasicus*) during refrigerated storage

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ABSTRACT

Effects of high pressure processing (HPP) under 300, 400, 500 and 600 MPa for 5 min on microbiological growth and histamine content of spotted mackerel meats stored at 4 °C were evaluated. It was also found that the L^* (lightness), ΔE (color difference), and texture (hardness, cohesiveness and chewiness) of fish meat increased significantly with an increase in pressure, but a^* (redness) value decreased. With an increase of pressure, the loads of aerobic plate count (APC), psychrotrophic bacteria count (PBC), H_2S -producing bacteria count and coliform in mackerel meat significantly decreased. In addition, HPP significantly delayed the growth of APC and PBC during refrigerated storage for 15 days. Pressure up to 300 MPa significantly inhibited total volatile basic nitrogen (TVBN) and histamine formation, compared to control sample during storage. The results pointed to that the pressurization at least 300 MPa for 5 min on mackerel meat could extend shelf-life during refrigerated storage.

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Efectos inhibitorios del crecimiento microbiano y la formación de histamina en la caballa manchada (*Scomber australasicus*), durante el almacenamiento refrigerado. atribuibles al procesado a alta presión

RESUMEN

Este estudio se propuso evaluar los efectos del procesado a alta presión (HPP) —300, 400, 500 y 600 MPa durante 5 minutos— en el crecimiento microbiológico y el contenido de histamina de las carnes de caballa manchada almacenadas a 4°C. La evaluación permitió comprobar que el valor L^* (claridad), ΔE (diferencia de color) y la textura (dureza, cohesividad y masticabilidad) de la carne de pescado aumentaron significativamente al incrementar la presión, mientras que el valor a^* (enrojecimiento) disminuyó. Además, con el aumento de la presión en la carne de caballa se redujeron significativamente las cargas de recuento de placas aeróbicas (APC), así como el recuento de bacterias psicrótrofas (PBC), bacterias productoras de H_2S y coliformes. Por otra parte, el HPP retrasó de manera significativa el crecimiento de APC y PBC al realizarse el almacenamiento refrigerado durante 15 días. En este sentido, la comparación con la muestra de control mostró que la presión de hasta 300 MPa inhibió significativamente la formación de nitrógeno volátil básico total (TVBN) y de histamina durante el almacenamiento. Los resultados dan cuenta de que la presurización de la carne de caballa durante 5 minutos, aplicando una presión de, al menos, 300 MPa, podría prolongar su vida útil cuando se realiza su almacenamiento refrigerado.

1. Introduction

Spotted mackerel (*Scomber australasicus*) is a pelagic and widely distributed fish species in Indo-West Pacific region. Of the two main mackerel species caught (chub mackerel and spotted mackerel), the production of spotted mackerel accounts for 78% of total mackerel catch in Taiwan (Tzeng, 2004). In Taiwan, it is industrially manufactured as canning, salting and frozen for consumers (Tzeng, 2004). Histamine is a substance that can cause allergy-like poisoning; therefore, it commonly called scombrototoxicosis or histamine fish poisoning (Lehane & Olley, 2000). Histamine is produced by the decarboxylation of free histidine by histidine decarboxylase produced by histamine-producing bacteria in fish meat (Lehane & Olley, 2000). Scombroid fish, such as tuna, mackerel, bonito and saury, that 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 mackerel (Tsai et al., 2005), tuna (Chen et al., 2008), marlin (Chen et al., 2010), and milkfish (Tsai et al., 2007).

High pressure processing (HPP) is an emerging non-thermal processing technology, which aims to inactivate spoilage and pathogenic microorganisms with a pressure above 300 MPa to prolong the shelf-life of food and improve food safety (Lee et al., 2020; Wang et al., 2016). Compared with traditional thermal processing, this technology has the advantages of retaining more nutrients and aromatic substances in food; therefore, HPP is suitable for the processing of heat-sensitive food. For the purpose of food processing, preservation and sterilization, HPP can cause enzyme

inactivation due to the destruction of noncovalent bonds (such as hydrogen bonds and hydrophobic bonds) in food during the pressurization process, gelatinize starch, alter protein gel properties and destroy cell wall or membrane of microorganisms to reduce the number of microorganisms (Considine et al., 2008; Phuvasate & Su, 2015). At present, HPP has been widely used for meats, juice and beverages, vegetables, and aquatic products (Bonfim et al., 2019).

With regard to control 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 (Lee et al., 2016; Naila et al., 2010). The mechanism of those methods to control amines content is mainly by inhibiting growth of amines-producing bacteria and decarboxylase activities of amino acids. Kim et al. (2013) reported that hydrostatic pressures of 300 and 400 MPa could delay the growth and histamine production of histamine-producing bacteria (*Morganella morganii* and *Photobacterium phosphoreum*) inoculated in mackerel meat during low-temperature storage. In addition, previous studies examining the use of HPP in fish, such as albacore tuna (Ramirez-Suarez & Morrissey, 2006), salmon (Amanatidou et al., 2000), cod (Montiel et al., 2012), Atlantic mackerel (Matser et al., 2000), herring and haddock (Karim et al., 2011), and carp (Krzek et al., 2015), have been conducted. However, the formation of histamine in fish that was treated by high pressure in these studies were not performed.

There is little research regarding the pasteurization and preservation of spotted mackerel meats through high pressure processing. This aim of this study was to evaluate inhibitory effects of HPP on microbial growth and histamine formation in this fish during cold storage. Therefore, raw mackerel meats were treated under the pressure of 300–600 MPa for 5 min, immediately after which the changes in the color, texture, aerobic plate count (APC), psychrotrophic bacteria count (PBC), H₂S-producing bacteria count (HBC), coliform, and *Escherichia coli* of the fish meat were observed. In addition, the abovementioned pressurized fish meat was stored at 4 °C, and regularly sampled to analyze the changes in histamine-related quality to evaluate the effect of high-pressure processing on prolonging the storage life and freshness of spotted mackerel meats.

2. Materials and methods

2.1. Sample preparation

Fresh spotted mackerel (*Scomber australasicus*) was purchased from a fishery market in Kaohsiung, immediately placed in crushed ice and transported back to our Seafood Safety Laboratory of National Kaohsiung University of Science and Technology. Fish body was beheaded, eviscerated, and filleted. The skinless fillet was then cut into small pieces, soaked in sterile water to wash off the blood on the surface, drained the water off, and then packaged in vacuum bags (nylon/linear low-density polyethylene) on a clean bench. Each bag contained approximately 30 g of fish meat.

2.2. High pressure processing of the samples

The vacuum-packed mackerel samples were placed in a high-pressure device (BaoTou KeFa High Pressure

Technology Co. Ltd., 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, and 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. Under the high-pressure conditions of 300, 400, 500, and 600 MPa for 5 min, the color values (L^* , a^* , b^* and ΔE), texture, APC, PBC, HBC, coliform count and *E. coli* of fish meat were determined. The mackerel sample without high-pressure processing was used as the control group.

Control group and samples treated with different high pressures were subjected to storage at 4 °C for 15 days, and then sampled every 3 days. After sampling, the changes in the APC, PBC, TVBN and histamine content were analyzed. Three individual samples in each pressurized groups were taken for analysis at each sampling point.

2.3. Color analysis

A colorimeter (CR-300 Chroma meter, Konica Minolta, Inc., Tokyo, Japan) was used to analyze the color changes of fish meat samples after treated pressure (Ovissipour et al., 2013). The colorimeter was first calibrated with a white ceramic plate, and then the fish meat was placed in it. The values displayed on the screen were L^* (lightness), a^* (+a, red, -a, green) and b^* (+b, yellow; -b, blue). Each analysis was measured three times at different locations, while the ΔE (color difference) values were calculated according to the following equation, and the results were expressed as mean values for three individual samples.

The ΔE value was calculated as follows:

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

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

2.4. Texture profile analysis

A texture profile analysis (TPA) was performed on the mackerel meats using a TA-XT2 texture analyzer (Stable Micro System, Ltd., Surrey, UK) (Ovissipour et al., 2013). The TPA was used to measure the changes of texture, including hardness, cohesiveness, springiness, and chewiness in fish meat after high-pressure processing. A spherical probe (TA18 Sphere, 12.7 mm diameter) was used for detection; the fish meat 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 meat was measured three times at different locations, and the results were expressed as mean values for three individual samples.

2.5. Microbiological analysis

With regard to APC, a 10.0 g sample of finely minced fish meat was weighed, placed in a sterilized bottle (containing 90 mL of 0.85% sterile saline) for homogenizing at a 1,200 rpm speed for 2 min (Sae-leaw et al., 2018). Then,

1.0 mL of the stock solution was added into a sterilized test tube containing 9 mL of 0.85% sterile saline for serial dilution. Further, 0.1 mL taken from each dilution was spread on a trypticase soy agar (TSA, Difco, BD, Sparks, MD, USA) culture medium and incubated at 30 °C for 24–48 h. Finally, the number of colonies on the culture dish was counted and calculated as \log_{10} colony forming units (CFU) per gram.

To determine the PBC, 0.1 mL of the fish homogenate solution and a 10-fold serial dilution solution prepared during the abovementioned APC determination was spread on TSA culture medium and incubated at 7 °C for 10 days. Then, the number of colonies on the culture dish was calculated as the PBC (Cousin et al., 1992). H_2S -producing bacteria were enumerated from black colonies appearing on triple sugar iron agar (TSIA, Difco, BD, Sparks, MD, USA) after incubation at 25 °C for 3 days (Sae-leaw et al., 2018).

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 Lauryl Sulfate Tryptose (LST) broth. 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 Lactose Bile Broth (BGLB) tube and cultured at 35 °C for 48 h. Gas production in BGLB tube was considered 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) 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 a eosin methylene blue agar 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.

2.6. Total volatile basic nitrogen (TVBN) and histamine analysis

Mackerel meat samples (5 g) were placed in a 50 mL centrifuge tube contained 20 mL of 6% trichloroacetic acid (TCA) and the tube contents were homogenized at 1,200 rpm speed for 2 min. The homogenized solution was centrifuged at 3,000 $\times g$ for 10 min (4 °C) and filtered through Whatman No.1 filter paper. 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. The Conway's dish method proposed by Cobb et al. (1973) was used to determine the content of TVBN in fish meat. Boric acid absorption solution (1 mL) was

added into the inner chamber of Conway's dish, while saturated potassium carbonate (1 mL) and fish meat TCA extract (1 mL) was 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 meat.

In addition, histamine analysis was modified the method proposed by Chen et al. (2010). Histamine standard and TCA extract of fish meat were derivatized with dansyl chloride and injected into HPLC (Hitachi) for detection. The machine and analysis conditions are as follows:

Separating column: LiChrospher 100 (5 μ m) RP-18 reverse chromat-column (125 \times 4 mm i.e. E. Merck).

Mobile phase: Solution A was acetonitrile; 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 μ L

Column temperature: 40 °C

Setting of wavelength: 254 nm

2.7. Statistical analysis

The microbial counts, color, texture, TVBN, and histamine content of samples treated under different pressures were analyzed to determine the differences among pressure groups. The mean \pm 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.

3. Results and discussion

3.1. Effect of HPP on color of fish meates

Changes in color values (L^* , a^* , b^* and ΔE) were determined by a colorimeter as shown in Table 1. The L^* value (lightness) of the control increased from 57.06 to 66.10 (600 MPa) with an increase in pressure ($P < .05$). The increase in lightness is because of 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 (Kruk et al., 2011). Furthermore, after high-pressure processing, the a^* value (redness) of fish meat decreased significantly, from 6.43 in the control to 4.11 in the 600 MPa ($P < .05$). The b^* value (yellowness) showed a slightly downward trend with an

Table 1. The L^* , a^* , b^* and ΔE values of mackerel meat after HPP treatments at 300, 400, 500 and 600 MPa for 5 min.

Tabla 1. Valores L^* , a^* , b^* y ΔE de la carne de caballa después de tratamientos HPP a 300, 400, 500 y 600 MPa durante 5 minutos.

Color	Control (0.1 MPa)	Pressure (MPa)			
		300	400	500	600
L^*	57.06 \pm 0.48 ^d	63.32 \pm 0.17 ^c	64.69 \pm 0.13 ^b	65.45 \pm 0.26 ^a	66.10 \pm 0.28 ^a
a^*	6.43 \pm 0.29 ^a	5.53 \pm 0.36 ^b	5.25 \pm 0.13 ^{bc}	4.96 \pm 0.17 ^c	4.11 \pm 0.48 ^d
b^*	15.25 \pm 0.38 ^a	15.54 \pm 0.45 ^a	15.15 \pm 0.14 ^a	14.53 \pm 0.16 ^b	14.42 \pm 0.45 ^b
ΔE	-	6.68	8.00	8.47	9.15

Each value is the mean of three determinations \pm standard deviation; the different letters at the same row indicate significant difference ($P < .05$).

Cada valor es la media de tres determinaciones \pm desviación estándar; las distintas letras presentes en la misma fila indican una diferencia significativa ($P < .05$).

Table 2. Texture properties of mackerel meat after HPP treatments at 300, 400, 500 and 600 MPa for 5 min.**Tabla 2.** Propiedades de textura de la carne de caballa después de tratamientos HPP a 300, 400, 500 y 600 MPa durante 5 minutos.

Texture	Control (0.1 MPa)	Pressure (MPa)			
		300	400	500	600
Hardness (N)	1.16 ± 0.39 ^d	3.68 ± 0.11 ^c	4.29 ± 0.21 ^b	5.09 ± 0.08 ^a	5.25 ± 0.23 ^a
Cohesiveness	0.69 ± 0.03 ^b	0.70 ± 0.03 ^b	0.77 ± 0.01 ^a	0.80 ± 0.01 ^a	0.75 ± 0.03 ^a
Springiness (mm)	7.00 ± 0.25 ^a	6.90 ± 0.14 ^a	6.54 ± 0.36 ^a	6.55 ± 0.37 ^a	7.30 ± 0.42 ^a
Chewiness (mJ)	7.45 ± 0.15 ^c	24.52 ± 0.94 ^b	23.12 ± 1.65 ^b	25.62 ± 1.05 ^b	28.84 ± 0.97 ^a

*. Each value is the mean of three determinations ± standard deviation; the different letters at the same row indicate significant difference ($P < 0.05$).

*. Cada valor es la media de tres determinaciones ± desviación estándar; las distintas letras en la misma fila indican una diferencia significativa ($P < 0.05$).

increase in pressure. Similar results were reported by Montiel et al. (2012), who showed that smoked cod treated with high pressure had a brighter appearance and higher L^* value, but the a^* value decreased with the increase in pressure. Our study is also in accordance with De Alba et al. (2019), who found an increase of L^* and a decrease of a^* in Atlantic mackerel fillets at the higher pressure intensities. In addition, ΔE tended to increase significantly ($P < .05$) from 6.68 (300 MPa) to 9.15 (600 MPa) with an increase in pressure (Table 1). Ledward (1998) pointed out that HPP caused changes in the color parameters of fish meat, which may be because of the degeneration of myofibrillar and sarcoplasmic proteins. Additionally, the reduced fish redness caused by high pressure may be attributed to the degradation of pigments and myoglobin (Teixeira et al., 2014). In this study, the results are in agreement with a previous research reporting that the higher ΔE values of 5.80 and 12.11 were observed for hilsa fillets treated at 250 and 350 MPa for 10 min, respectively (Chouhan et al., 2015). A similar finding was also demonstrated by De Alba et al. (2019), who detected that the ΔE values of mackerel fillets increased significantly from 5.85 in the 100 MPa/5 min to 19.49 in the 500 MPa/5 min.

3.2. Effect of HPP on texture properties of fish meats

Changes in the texture of mackerel meats after high pressure processing are described in Table 2. The hardness of fish meat increased from 1.16 N in the control to 5.25 N with an increase in pressure, as seen in the 600 MPa ($P < .05$). The increase in the hardness of fish meat under high pressure may be related to the denaturation and aggregation of myofibrillar protein (Yagiz et al., 2007). The cohesiveness showed a slightly upward trend to increase from 0.69 in the control to 0.75 in the 600 MPa with increasing pressure ($P < .05$). The average springiness of the samples ranged from 6.54 to 7.30 (mm), and there was no significant difference between various pressures and control ($P > .05$). Chewiness showed similar results to hardness and increased from 7.45 (mJ) in the control to 28.84 (mJ) in the 600 MPa ($P < .05$). In summary, the average values of hardness, cohesiveness, and chewiness of fish meat increased with an increase in 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 (Yagiz et al., 2007). Christensen et al. (2017) found that after the cod flesh treated at 200 to 500 MPa for 2 min, the hardness increased with increasing pressure. Similar results were reported by Aubourg et al. (2013), who indicated that hardness, chewiness, and cohesiveness of Atlantic mackerel increased with higher levels of pressure

and longer pressure holding times, but springiness was affected less by pressure level and holding time.

3.3. Effect of HPP on microbial counts of fish meats

Microbial counts of mackerel samples after high pressure processing are presented in Table 3. The initial APC of control sample was 6.96 log CFU/g, which decreased to 6.56, 6.10, <2.0, and <2.0 log CFU/g with an increase in pressure at 300, 400, 500 and 600 MPa, respectively (i.e. reduced by 0.40, 0.86, 6.96 and 6.96 log CFU/g, respectively) ($P < .05$) (Table 1). This suggests that higher pressure is more effective in reducing APC in fish sample. APC is one of the important indexes for evaluating the quality of fresh and cold temperature stored aquatic products. A similar finding was also demonstrated by De Alba et al. (2019), who found that HPP at 300 and 500 MPa for 5 min significantly reduced the APC levels of Atlantic mackerel fillets, and reductions of 0.80 and 2.48 log CFU/g, respectively, were detected. Montiel et al. (2012) pressurized smoked cod at 400, 500, and 600 MPa for 5 and 10 min and observed that higher pressure significantly reduced the microbial count in smoked cod. Chéret et al. (2005) demonstrated similar results for sea bass fillets treated at higher pressure levels (300–500 MPa, 5 min) with higher reduction loads. When fresh salmon pressurized at 150 MPa for 15 min, the 3 log units reductions of APC were observed (Yagiz et al., 2009); but at a higher pressure level and holding time (200 MPa, 20 min) total plate counts did not apparently decrease in cold smoked salmon (Lakshmanan & Dalgaard, 2004). However, the APC load was significantly reduced by 2.21 and 2.40 log CFU/g after 250 and 350 MPa for 10 min, respectively (Chouhan et al., 2015). The different results on the microbial inactivation achieved between above studies are attributed to food composition

Table 3. The aerobic plate count (APC), psychrotrophic bacteria count (PBC), H_2S -producing bacteria count (HBC), coliform and *Escherichia coli* of mackerel meat after HPP treatments at 300, 400, 500 and 600 MPa for 5 min.**Tabla 3.** Recuento de placas aeróbicas (APC), de bacterias psicrótrofas (PBC), de bacterias productoras de H_2S (HBC), coliformes y *Escherichia coli* de la carne de caballa después de tratamientos HPP a 300, 400, 500 y 600 MPa durante 5 minutos.

Microorganisms	Control (0.1 MPa)	Pressure (MPa)			
		300	400	500	600
APC (log CFU/g)	6.96 ± 0.09 ^a	6.56 ± 0.04 ^b	6.10 ± 0.07 ^c	<2	<2
PBC (log CFU/g)	6.90 ± 0.08 ^a	6.51 ± 0.09 ^b	5.45 ± 0.08 ^c	<2	<2
HBC (log CFU/g)	4.19 ± 0.34 ^a	3.32 ± 0.44 ^b	<2	<2	<2
Coliform (MPN/g)	256 ± 20 ^a	70 ± 14 ^b	<3	<3	<3
<i>E. coli</i> (MPN/g)	<3	<3	<3	<3	<3

*. Each value is the mean of three determinations ± standard deviation; the different letters at the same row indicate significant difference ($P < 0.05$).

*. Cada valor es la media de tres determinaciones ± desviación estándar; las distintas letras en la misma fila indican una diferencia significativa ($P < 0.05$).

and characteristics, pressure levels and holding time, and bacterial growth phase and initial bacterial counts on food (De Alba et al., 2019).

The initial PBC of control sample was 6.90 log CFU/g, which decreased to 6.51, 5.45, <2.0 and <2.0 log CFU/g with an increase in pressure at 300, 400, 500, and 600 MPa, respectively (i.e. reduced by 0.39, 1.45, 6.90 and 6.90 log CFU/g, respectively) ($P < .05$) (Table 3). This suggests that higher pressure is more effective in reducing PBC in fish meat. Suemitsu and Cristianini (2019) demonstrated that HPP at 100 and 200 MPa for 3 min on tilapia fillets did not reduce PBC levels, but pressures at 300 and 400 MPa lead to significant reduction by 1.11 and 1.15 log CFU/g, respectively. Erkan et al. (2010) also reported that HPP at 330 MPa for 5 min on red mullet significantly reduced 1.0 log CFU/g of PBC.

With regard to HBC and coliform count, it was found that the initial HBC and coliform counts in control sample were 4.19 log CFU/g and 256 MPN/g, respectively, which decreased significantly with an increase in pressure. After the samples with pressures above 400 MPa, HBC and coliform were not detected. However, *E. coli* was not detected in any of the control and pressurized samples (Table 3). It is indicating that higher pressure is more effective in reducing HBC and coliform counts in mackerel meat.

3.4. The APC changes of pressurized mackerel meats during refrigerated storage

The changes in APC of fish meats after high pressure treatment during storage at 4 °C are shown in Figure 1. When stored at 4 °C, the APC of the control and 300 MPa increased gradually with an increase in storage time, and there was significant difference among both until day 9, but no difference after day 9 ($P > .05$). However, the APC of the 400 MPa increased slowly during the first 9 days of storage and subsequently increased significantly, but the count was lower than that of the control or 300 MPa ($P < .05$) during 15 days of

storage. In addition, the bacterial count of the 500 and 600 MPa was not detectable before day 3 and 9, respectively, and subsequently increased significantly, but the counts were lower than that of the control or other lower-pressure treatments ($P < .05$), indicating that higher pressure delays the increase in APC during refrigerated storage. According to Taiwan's microbial hygiene standards at 6.47 log CFU/g of APC for fresh aquatic products, the initial APC of the control and 300 MPa were 6.96 and 6.56 CFU/g, respectively, which exceeded the limit standard (6.47 log CFU/g). The APC of the 400 MPa did not exceed the limit standard until day 9 (6.53 log CFU/g). However, the APC in the 500 and 600 MPa did not exceed the limit standard until the 12th day (6.70 log CFU/g) and 15th day (7.10 log CFU/g), respectively. Therefore, when mackerel samples were stored at 4 °C after high pressure under 400, 500 and 600 MPa, the shelf life could be extended by 6, 9 and 12 days, respectively.

It can be concluded that high pressure (≥ 300 MPa) treatment could delay the increase in the APC of mackerel meats when stored at 4 °C, while pressure above 400 MPa for 5 min could prolonged storage life. Similarity, Montiel et al. (2012) pressurized smoked cod at 400, 500 and 600 MPa for 5 and 10 min, 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 minutes or 500 MPa for 5 min for prolonging storage life. De Alba et al. (2019) found that after treatment at 500 MPa for 2 min and 300 or 500 MPa for 5 min, the APC of Atlantic mackerel fillets could be effectively reduced, and the refrigerated storage life of mackerel slices could be prolonged.

3.5. The PBC changes of pressurized mackerel meats during refrigerated storage

The changes of PBC in fish samples after high pressure treatment during storage at 4 °C are shown in Figure 2.

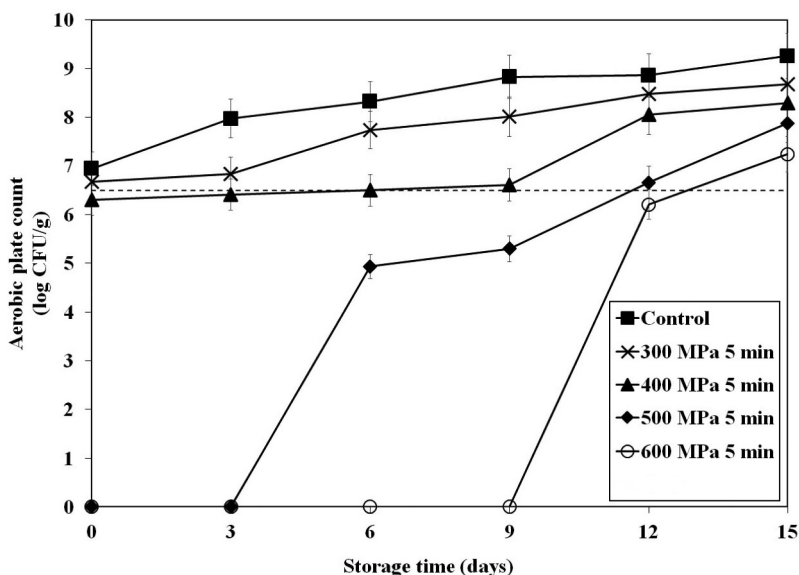


Figure 1. Change of aerobic plate count (APC) of mackerel meat after HPP treatments at 300, 400, 500 and 600 MPa for 5 min during storage at 4 °C. Dash-line represents 6.47 log CFU/g of APC as the regulatory standard for raw frozen fish.

Figura 1. Cambio del recuento de placas aeróbicas (APC) de la carne de caballa después de tratamientos HPP a 300, 400, 500 y 600 MPa durante 5 minutos cuando se realiza su almacenamiento a 4 °C. La línea punteada representa 6.47 log CFU/g de APC como norma reglamentaria para el pescado crudo congelado.

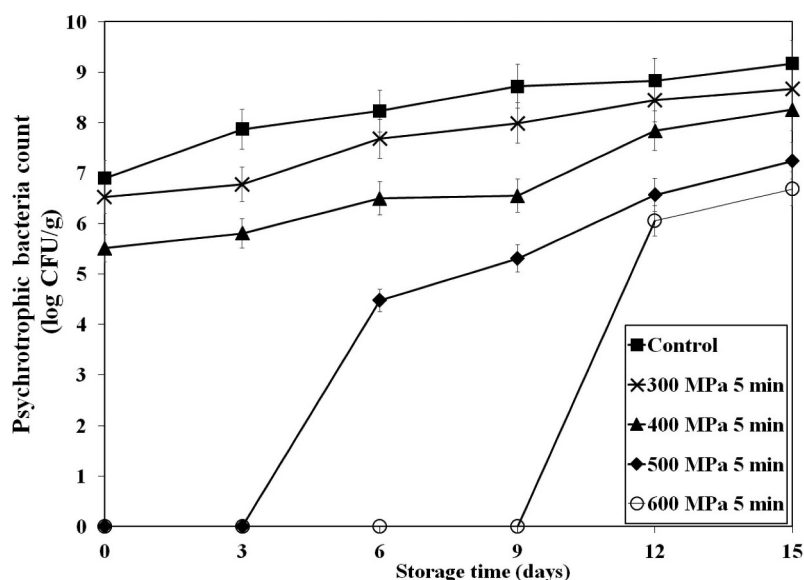


Figure 2. Change of psychrotrophic bacteria count (PBC) of mackerel meat after HPP treatments at 300, 400, 500 and 600 MPa for 5 min during storage at 4°C.

Figura 2. Cambio en el recuento de bacterias psicotróficas (PBC) de la carne de caballa después de los tratamientos HPP a 300, 400, 500 y 600 MPa durante 5 minutos cuando se realiza su almacenamiento a 4°C.

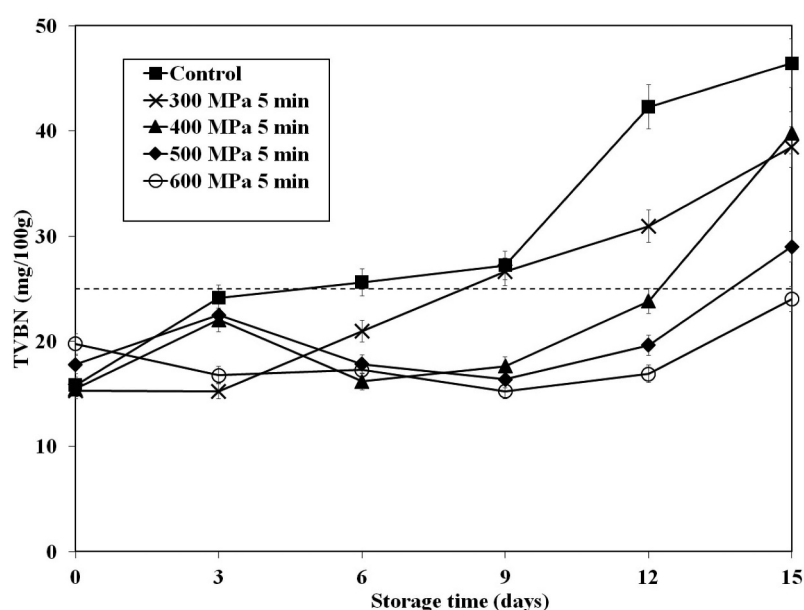


Figure 3. Change of total volatile basic nitrogen (TVBN) of mackerel meat after HPP treatments at 300, 400, 500 and 600 MPa for 5 min during storage at 4°C. Dash-line represents 25 mg/100 g of TVBN as a reference of fish decomposition.

Figura 3. Cambio del nitrógeno volátil básico total (TVBN) de la carne de caballa después de los tratamientos HPP a 300, 400, 500 y 600 MPa durante 5 minutos cuando se realiza su almacenamiento a 4°C. La línea punteada representa 25 mg/100 g de TVBN como referencia de la descomposición del pescado.

When stored at 4 °C, the growth trend was similar to that of APC, i.e. the PBC of the control and 300 MPa increased gradually with an increase in storage time, and there was significant difference among both until day 9, but no difference after day 9 ($P > .05$). However, the PBC of the 400 MPa increased slowly during the first 9 days of storage and subsequently increased significantly, but the count was lower than that of the control or 300 MPa ($P < .05$) during 15 days of storage. In addition, the PBC of the 500 and 600 MPa was not detectable before day 3 and 9, respectively, and subsequently increased significantly, but the counts were lower than that of the control or other lower-pressure treatments

($P < .05$). It indicates that higher pressure delays the increase of PBC in mackerel meat during cold storage.

3.6. The TVBN changes of pressurized mackerel meats during refrigerated storage

The changes in TVBN of mackerel meats after high pressure treatment during storage at 4 °C are shown in Figure 3. In control sample, the contents of TVBN increased gradually during storage, reaching 46.5 mg/100 g at the end of storage (day 15). TVBN content of 300 MPa increased slowly with an increase in storage time, reaching 38.0 mg/100 g at

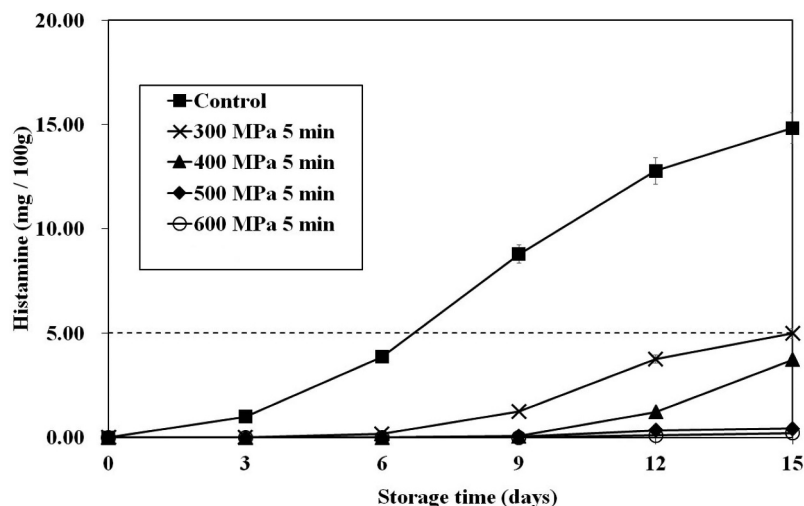


Figure 4. Change of histamine of mackerel meat after HPP treatments at 300, 400, 500 and 600 MPa for 5 min during storage at 4°C. Dash-line represents 5 mg/100 g of histamine as the allowable limit of USFDA.

Figura 4. Cambio de la histamina en la carne de caballa después de los tratamientos HPP a 300, 400, 500 y 600 MPa durante 5 minutos cuando se realiza su almacenamiento a 4°C. La línea punteada representa 5 mg/100 g de histamina como límite permitido por la USFDA.

the end of storage, but TVBN contents of the 400 and 500 MPa did not remarkably change during the first 12 days of storage, and increased progressively only after day 12. In addition, the TVBN content of 600 MPa did not significantly increase during storage and was lower than that in the control and other low-pressure treatments ($P < .05$). Therefore, it is indicated that high pressure significantly retarded the production of TVBN in mackerel samples during cold storage.

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 300 MPa when stored at 4 °C for 15 days already exceeded the limit standard on day 6 and 9, respectively (the TVBN values were 25.7 and 26.3 mg/100 g, respectively); the value in 400 and 500 MPa did not exceed the limit standard until day 15 (the TVBN values were 39.8 and 29.1 mg/100 g, respectively). In contrast, that of 600 MPa did not exceed the limit standard (25 mg/100 g) during the 15 days of storage. Therefore, when mackerel samples were stored at 4 °C, high pressure treatment under 300 MPa, 400 and 500 MPa, and 600 MPa could extend the shelf life from 3 days to 6, 12 and 15 days, respectively (Figure 3). Therefore, TVBN production was delayed and the storage life was prolonged in mackerel meats when stored at cold temperature (4°C) after treatment at high pressures above 300 MPa.

3.7. The histamine of pressurized mackerel meats during refrigerated storage

The changes in histamine content in mackerel meat after high pressure treatment during storage at 4 °C are shown in Figure 4. In control sample, the content of histamine increased gradually during storage, reaching 14.1 mg/ 100 g at the end of storage (day 15), while that of 300 and 400 MPa samples increased slowly with an increase in storage time, reaching 5.1 and 2.6 mg/100 g at the end of storage. Finally, the histamine contents in the 400, 500 and 600 MPa samples during storage were lower than 1.0 mg/100 g. In this study, the control and 300 MPa samples when stored at 4 °C for

9 days and 15 days (7.5 and 5.1 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). Therefore, when mackerel samples were stored at 4 °C, high pressure treatment under 300 MPa and >400 MPa could extend the shelf life from 6 days to 12 and 15 days, respectively (Figure 4). In summary, high pressure treatment of mackerel meats above 300 MPa could inhibit the formation of histamine and prolong the storage life during storage at 4 °C, as compared to control sample. The results of this study are similar to those of many published reports. For example, Křížek et al. (2014) reported that high pressures (300 and 500 MPa) could reduce the production of histamine in pike (*Esox lucius*) meat during storage at low temperatures (3.5 °C and 12 °C) and could prolong the storage life. In addition, high pressure (>200 MPa) could delay the growth of histamine-producing bacteria and histamine production in mackerel meat inoculated with histamine-producing bacteria (*M. organii* and *P. phosphoreum*) when stored at a low temperature (5 °C and 12 °C) (Kim et al., 2013). Matějková et al. (2013) treated rainbow trout meat at high pressures (300 and 500 MPa), which prolonged the storage life by 4–5 times and delayed the increase in histamine during refrigeration storage.

4. Conclusion

These results showed that HPP under 500 and 600 MPa significantly reduce APC, PBC, HBC and coliform loads to undetected level. However, the L^* and ΔE of the samples increased with the higher pressure, but the a^* (redness) decreased; HPP also caused a significant increase in hardness, cohesiveness, and chewiness of mackerel meat. Therefore, the high pressure may have some negative effects on mackerel color and texture. In addition, HPP above 300 MPa delayed the increase of APC and PBC, and retarded the production of TVBN and histamine in mackerel meats. In summary, HPP at least 300 MPa for 5 min was effective to preserve mackerel meats, as well as to extend the shelf life during refrigerated storage.

Authorship contributions

Chung-Saint Lin: Data curation, Methodology, Writing-original draft. Yi-Chen Lee: Conceptualization, Methodology, Supervision. Jhih-Wei Chiu: Methodology, Analysis. Chiu-Chu Hwang: Software, Project administration, Resources. Hsien-Feng Kung: Supervision, Validation, Writing-review & editing. Yung-Hsiang Tsai: Conceptualization, Supervision, Validation, Writing-review & editing. All authors have read and agreed to the published manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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