Combined effect of brine salting and high-hydrostatic-pressure processing to improve the microbial quality and physicochemical properties of milkfish fillet

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ABSTRACT
The effects of soaking milkfish fillet in 3% or 9% NaCl brines for 90 min at 4°C, and followed by processing with varying hydrostatic pressures (300, 400, 500, and 600 MPa) for 5 min on microbial quality and physicochemical properties were evaluated. After brine salting, the color of the fillet, the L* (lightness), W (whiteness), and ΔE (color difference) values increased with the increases in pressure, whereas the a* (redness) and b* (yellowness) values decreased, indicating that the fillet became brighter and whiter. Among them, 3% brine salting combined with pressures of 300 and 400 MPa groups had higher redness (a* value) and lower ΔE value compared to the higher pressure groups. Regarding the texture of the fillet, the hardness and chewiness of unsalted fish were significantly higher than that of brine salted fish under the same pressure, whereas the cohesiveness of the unsalted fillet was significantly lower than that of brine salted fillet. The results indicates that brine salting could make the instrumental texture of fish softer, which can compensate for the disadvantages of increased hardness and chewiness of the fillet caused by high-pressure processing. Brine salting combined with high pressure produced a significantly reducing effect on aerobic plate count (APC), psychrotrophic bacteria count (PBC), and total volatile basic nitrogen (TVBN) values of the fish as compared with that by brine salting or high pressure alone. Therefore, brine salting at a proper brine concentration (3%) and followed by high-pressure processing at 300 or 400 MPa for 5 min can improve or maintain a relatively good color and texture, as well as result in a synergistic bactericidal effect.

Introduction
High hydrostatic-pressure processing or high-pressure processing (HPP) is a non-thermal processing technique that devotes to inactivate enzymes and pathogens under a pressure above 300 MPa to extend the storage life of food and enhance food safety. [1,2] When HPP compares with conventional thermal processing, it has the merits of maintaining more nutritional contents and flavors in food; and therefore, HPP is proper for the processing of thermo-labile food. [3,4] Currently, HPP has been generally applied for meat products, juice and vegetable products, beverages, and shellfish...
products.\(^5\) However, HPP technology has some disadvantages on the quality of fish, including color changes with higher \(L^*\) value and lower \(a^*\) value associated with higher opacity, and harder textures.\(^5\)

Salting is a traditional method that uses osmotic pressure to cause cell rupture of bacteria to inhibit or kill pathogenic bacteria and spoilage bacteria, and it is used to maintain food quality and prolong food storage life, especially for fish that are difficult to preserve and easily rot.\(^6\) In addition, salting in food processing plays an important role to improve the texture properties, such as tenderness and juiciness.\(^7\) Nevertheless, lightly salted aquatic products have become more popular among consumers with the needs to make changes in dietary habits. At present, brine salting is often used by the seafood industry to manufacture lightly salted aquatic products.\(^8\) In recent years, combining several physical and chemical treatments to replace the use of chemical preservatives and bactericides in food, that is, adopting the hurdle technology, has become a trend in food processing.\(^9\) If the heights of hurdles (i.e., the strengths of preservation factors) are moderately increased in food, the influence of microorganisms on food can be effectively limited to meet the requirements of food hygiene and safety.\(^9\) Common methods in the hurdle technology include sugaring, salting, low-temperature storage, addition of bactericides/preservatives, reduction of water activity, adjustment of \(pH\) value, and high-pressure processing. Recently, Balamurugan et al.\(^10\) inoculated \textit{Listeria monocytogenes} to minced chicken, which had been added with \(1.5–2.5\%\) CaCl\(_2\) and processed with HPP at 600 MPa for 60s. The combined effect of salting and HPP lowered the log values of bacterial (Listeria) count in the minced chicken by 1.12–1.21, when compared to HPP alone.

Milkfish (\textit{Chanos chanos}), widely distributed in Indo-Pacific waters, is one of the important inland cultured fish species in Taiwan. It has been cultivated in Taiwan for more than 350 years, and the annual output is approximately 50,000–60,000 metric tons.\(^11\) In Taiwan, milkfish is mainly the fresh types, that is, chilled whole fish or fish belly fillet for sale in the fish market. Milkfish also has been processed into fried floss, fish ball, and salted fish for consumers. Recently, brine- and light-salted milkfish has gained popularity in Taiwan.\(^8\) Previous studies examining the use of HPP in combination with sodium chloride have been done on meat products. For instance, Ros-Polski et al.\(^7\) reported that salting at low concentration of NaCl in combination with HPP treatment can improve the texture and color in white chicken meat. Balamurugan et al.\(^10\) demonstrated that HPP treatment could be used to improve the microbial safety of ground chicken with reduced NaCl concentration. The functionality of frankfurters with lower salt contents can be improved using HPP treatments (150 and 300 MPa).\(^12\) However, there are no reports on the application of HPP combined with brine salting as a hurdle technology for aquatic products.

This study had three objectives: 1) to investigate how the brine-salting treatment on milkfish flesh prior to HPP improve the texture and color caused by high pressure; 2) to evaluate the effects of high-hydrostatic-pressure processing, in combination with brine salting at high or low NaCl concentrations, on the microbial counts and physicochemical properties of milkfish flesh; and 3) to understand whether the hurdle technology of combining HPP with brine salting has an synergistic bactericidal effect. Therefore, milkfish fillet was soaked in 3\% or 9\% brine at 4°C for 90 min and then treated with different high pressures (300, 400, 500, and 600 MPa) for 5 min, after which the changes in microorganisms and physicochemical properties were observed and compared with fish flesh treated at the same high pressures alone.

**Materials and methods**

**Preparation of milkfish samples**

Twelve raw milkfish (\textit{C. chanos}), with an average length and weight of 48 ± 4 cm and 800–900 g, were purchased from a fish market in Kaohsiung and immediately placed in crushed ice and transported to the laboratory within 1 h. The milkfish were deheaded, skinned, gutted, and filleted on a clean bench for the subsequent steps. Four fillet pieces (with total average weight of 200 g) were obtained from each milkfish. The fish fillet pieces were divided into three groups: the first group was
the control group (non-high pressure group), which included the raw fish flesh subgroup, 3% NaCl brine salting subgroup, and 9% NaCl brine salting subgroup (three subgroups in total); the second group was the solely high-pressure group, in which the raw fish flesh was pressurized at 300, 400, 500, and 600 MPa for 5 min (four subgroups in total); and the third group was the brine salting and high pressure combined treatment group, in which the fish flesh salted in 3% or 9% brine as described above was pressurized at 300, 400, 500, and 600 MPa for 5 min (eight subgroups in total). The experiment was conducted in triplicate for each subgroup. With regard to brine salting, the fish flesh was soaked in 3% or 9% brine at a fish:brine ratio of 1:2 (w/v) at 4°C for 90 min. The salted fish flesh was hung on a clean bench to allow brine water to drip off for subsequent experiments. The use of 9% high salt concentration was to simulate the traditional salted milkfish processing method in the past, while the 3% low salt concentration was a processing method that was design to meet to the current trend of low salt intake. The dimension and weight of each piece of fillet for vacuum packaged and high-pressure treatment was in the length × width × thick: 9 ± 2 × 3 ± 1 × 2 ± 0.5 cm and 47–52 g, respectively.

**High-pressure processing**

The unsalted or salted fish samples were packaged into vacuum bags (NY/LLDPE), vacuumed, heat sealed and set in a high-pressure apparatus (BaoTou KeFa High Pressure Technology Co. Ltd., China) with a diameter: 20 cm × height 20 cm, a volume of 6.2 L and a working pressure range of 0.1–600 MPa, and water was used as the medium for pressure transmission at 20°C. The pressure was increased at a rate of 300 MPa/min, and the pressure decrease time was approximately 15–20 s. The high-pressure treatment conditions were set to 300, 400, 500, and 600 MPa for 5 min.

**Appearance and color analysis**

The appearance of the fish samples was measured on a white background by using a SLR camera (EOS 60D, Canon Inc., Japan). In terms of color changes, fish samples in the control group, pressure-treated group, and combination group were analyzed by colorimeter (CR-300 Chroma meter, Konica Minolta, Inc., Tokyo, Japan). The colorimeter was calibrated before determining the fish samples using a standard white plate. The values displayed on the screen were $L^*$ (lightness), $a^*$ (+ a, red, -a, green) and $b^*$ (+ b, yellow; -b, blue). Each piece of fish fillet was measured three times, the $W$ (whiteness) and $\Delta E$ (color difference) values were determined in accordance with the following equation, and the results were presented as average values. The $W$ value was calculated as follows:

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

The $\Delta E$ value was calculated as follows:

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

where $L_1^*$, $a_1^*$, and $b_1^*$ corresponded to the colorimetric values of fish fillets in control and $L_2^*$, $a_2^*$, and $b_2^*$ corresponded to the colorimetric values of fish fillets after brine salting, HPP, or combination.

**Instrumental texture determination**

A TA-XT2 texture analyzer (Stable Micro System, Ltd., Surrey, UK) was used to analyze texture profile analysis (TPA) on the milkfish fillet to characterize the changes in texture, including hardness, cohesiveness, springiness, and chewiness as affected by treatments. TPA was performed with a stainless steel spherical probe (TA18 Sphere, 12.7 mm diameter) and a loading distance from the probe to fish sample was set at 0.5 cm. The probe operating at a speed of 0.2 cm/s was performed and
the compression rate was 50% of the original height, the pressing depth was 1.0 cm, the holding time was 3 s, and the trigger force was 5 g. Each piece of fish flesh was measured three times, and the results were expressed as mean values.

**Measurement of salt content and moisture content in samples**

Finely minced fish flesh sample (5.0 g) was weighed and placed in a crucible, placed into an furnace, and ashed at 550°C for 12–14 h till the ashes turned white or gray-white, and then placed in a desiccator to allow it to reach room temperature. Each of the ashed sample was made up to 100 mL with deionized water in a graduated flask and mixed thoroughly. The salt content was measured by a precipitation titration (Mohr’s method). Moisture content was performed using the standard gravimetric method by oven-drying 1–3 g of a minced sample at 105.0 ± 2.0°C for 3 h. The weight was measured by extra drying for 1 h or longer until the variance in weight did not exceed 0.5 mg.

**Analysis of microbial counts**

To determine the APC, sample (10.0 g) of minced fillet was homogenized in a 90 mL of 0.85% sterile saline using a Oster blender with a 1300 rpm speed for 90s. After homogenization, decimal dilutions were performed by using 9 mL of 0.85% sterile saline. Then, 0.1 mL of serial dilutions of fish homogenates was spread on the surface of trypticase soy agar (TSA, Difco, BD, Sparks, MD, USA) in duplicate, and incubated at 35°C for 1–2 d. After incubation, the APC colonies formed on the medium were enumerated and presented as \( \log_{10} \) colony-forming units (CFU)/g of fish sample.

With regard to PBC, sample (0.1 mL) of serial decimal dilution prepared from the above APC preparation method was spread on TSA medium and then incubated at 7°C for 10 d. After incubation, the PBC colonies on the culture medium were enumerated and presented as \( \log_{10} \) colony-forming units (CFU)/g of fish sample.

**Analysis of total volatile basic nitrogen (TVBN)**

The Conway’s dish method proposed by Cobb et al. was used to determine the content of TVBN in the fish samples. The trichloroacetic acid (TCA; Sigma, St. Louis, MO, USA) extract of the finely minced fish samples in 6% TCA was absorbed by boric acid and then titrated with 0.02 N HCl. The TVBN content was expressed as mg/100 g fish flesh.

**Statistical analysis**

The color, texture, salt content, moisture content, microbial counts, and TVBN content of samples treated under different brine salt concentrations, pressures, and combinations were evaluated to confirm the differences among groups. The results were expressed as means ± standard deviations of the triplicate individual samples. The SPSS 12.0 software (St. Armonk, New York, USA) for statistical analysis was adopted to conduct the analysis of variance and Tukey’s test. The statistically significant difference was indicated at \( p < .05 \).

**Results and discussion**

**Effect of HPP combined with brine salting on the appearance and color values of milkfish fillet**

The changes in the appearance of milkfish fillet treated with high pressures of 300–600 MPa for 5 min combined with brine salting are shown in Figure 1. Fish fillet treated with 3% and 9% brine salting alone was slightly moist and bright, and the fillet treated with 9% brine salting was slightly whiter than raw fish fillet and that treated with 3% brine salting. After combining brine salting with HPP, the color
The value of fillets became whiter with higher pressures, regardless of the brine concentration. Under the same pressure, the fillet in the 9% brine salting group became whiter than that in the 3% brine salting group (Figure 1).

The degrees of changes in color-related values (\(L^*\), \(a^*\), \(b^*\), \(W\), and \(\Delta E\)) as measured by the colorimeter are shown in Table 1. The \(L^*\) values (lightness) in the 3% and 9% brine salting groups increased from the original 59.89 and 57.83 to 83.04 and 80.97, respectively, with the increase in pressure to 600 MPa \((p < .05)\). Furthermore, under the same pressure conditions, the \(L^*\) values of the

Table 1. The \(L^*, a^*, b^*, W, \Delta E\) value of milkfish fillet soaked in 3% and 9% NaCl brine for 90 min at 4°C, and then pressurized with 300, 400, 500, and 600 MPa for 5 min.

| Brine concentration (%) | Control (0.1 MPa) | HPP treatment (MPa) |
|-------------------------|-------------------|---------------------|
|                         | 300               | 400                 | 500                 | 600                 |
| \(L^*\)                 | Raw meat          | 52.74 ± 0.08<sup>T</sup> | 68.92 ± 0.49<sup>Bd</sup> | 72.47 ± 2.51<sup>Ca</sup> | 74.13 ± 0.20<sup>Ca</sup> | 75.46 ± 0.54<sup>Ca</sup> |
| 3                       | 59.89 ± 0.10<sup>Ab</sup> | 69.90 ± 0.46<sup>Ad</sup> | 73.55 ± 0.18<sup>Bc</sup> | 79.28 ± 0.12<sup>Ab</sup> | 83.04 ± 0.06<sup>Ab</sup> |
| 9                       | 57.83 ± 0.04<sup>Bd</sup> | 70.70 ± 0.35<sup>Ac</sup> | 76.90 ± 0.37<sup>Ab</sup> | 76.13 ± 0.49<sup>Br</sup> | 80.97 ± 0.11<sup>Ba</sup> |
| \(a^*\)                 | Raw meat          | 5.66 ± 0.22<sup>Ba</sup> | 3.32 ± 0.34<sup>Ba</sup> | 1.87 ± 0.07<sup>Ab</sup> | 1.43 ± 1.71<sup>Ab</sup> | 1.06 ± 0.21<sup>Ab</sup> |
| 3                       | 7.94 ± 0.13<sup>Ab</sup> | 4.17 ± 0.35<sup>Ab</sup> | 2.70 ± 0.13<sup>Ac</sup> | 1.16 ± 0.35<sup>Ad</sup> | 0.84 ± 0.31<sup>Ad</sup> |
| 9                       | 0.88 ± 0.05<sup>Ca</sup> | -0.13 ± 0.06<sup>Ab</sup> | -0.71 ± 0.10<sup>Cc</sup> | -0.73 ± 0.18<sup>BC</sup> | -0.87 ± 0.14<sup>BC</sup> |
| \(b^*\)                 | Raw meat          | 11.00 ± 0.10<sup>Ba</sup> | 9.54 ± 0.07<sup>Ab</sup> | 8.45 ± 1.21<sup>Ab</sup> | 8.35 ± 0.73<sup>Br</sup> | 7.79 ± 0.42<sup>Ab</sup> |
| 3                       | 11.83 ± 0.03<sup>Ab</sup> | 6.92 ± 0.06<sup>Bd</sup> | 7.35 ± 0.21<sup>Bc</sup> | 9.09 ± 0.07<sup>Ab</sup> | 6.88 ± 0.11<sup>Bd</sup> |
| 9                       | 6.42 ± 0.11<sup>Ca</sup> | 6.00 ± 0.13<sup>Cb</sup> | 5.19 ± 0.21<sup>Cc</sup> | 4.90 ± 0.05<sup>Cd</sup> | 2.17 ± 0.07<sup>Ce</sup> |
| \(W\)                   | Raw meat          | 51.15 ± 0.09<sup>Bc</sup> | 66.93 ± 0.33<sup>Cb</sup> | 71.16 ± 2.66<sup>Ca</sup> | 72.96 ± 0.06<sup>Ca</sup> | 73.97 ± 0.99<sup>Ca</sup> |
| 3                       | 57.43 ± 0.13<sup>Ab</sup> | 68.02 ± 0.08<sup>Br</sup> | 72.23 ± 0.17<sup>Bc</sup> | 77.34 ± 0.13<sup>Ab</sup> | 81.67 ± 0.04<sup>Ab</sup> |
| 9                       | 57.33 ± 0.05<sup>Ac</sup> | 70.61 ± 0.03<sup>Ad</sup> | 76.38 ± 0.06<sup>Ac</sup> | 75.55 ± 0.51<sup>Br</sup> | 80.02 ± 0.12<sup>Ba</sup> |
| \(\Delta E\)            | Raw meat          | -                | 16.26 ± 0.43<sup>Ab</sup> | 20.26 ± 2.26<sup>Ab</sup> | 22.11 ± 0.09<sup>Ab</sup> | 23.29 ± 0.97<sup>Ba</sup> |
| 3                       | -                | 11.52 ± 0.10<sup>Br</sup> | 14.87 ± 0.19<sup>Cc</sup> | 20.73 ± 0.23<sup>Br</sup> | 24.72 ± 0.11<sup>Ab</sup> |
| 9                       | -                | 16.35 ± 1.16<sup>Bc</sup> | 19.92 ± 0.30<sup>Bb</sup> | 19.93 ± 0.57<sup>Cb</sup> | 23.01 ± 0.17<sup>Ba</sup> |

<sup>1</sup>Each value is the mean ± standard deviation (n = 3); Different lower case letters indicates significant differences at the same salt concentration (p < 0.05); Different upper case letters indicates significant differences at the same pressure (p < 0.05).
brine salting group were higher than that of the unsalted group. With respect to a* value (redness), the
redness of fish fillet in the 9% brine salting group (0.88) was significantly lower than that of the raw fish
fillet (5.66) and the sample in the 3% brine salting groups (7.94), among the control groups. The b*
values ( yellowness) showed the same trend as the a* values. The yellowness of the fillet in the 9% brine
salted group (6.42) was significantly lower than that of the raw fish fillet (11.00) and that fillet in the 3%
brine salting group (11.83), among the control groups. After HPP treatments, the a* and b* values of
fish samples decreased significantly with increases in pressures. Under the same pressure conditions,
the a* and b* values of the 9% brine salting group were significantly lower than those of the unsalted
group and 3% brine salted group (p < .05). Especially, the a* values of the 300 and 400 MPa groups
combined with 3% brine salting were 4.17 and 2.70, respectively, which were significantly (p < .05)
higher than those of the other groups (Table 1). In contrast, the W value of the unsalted group was
significantly lower than that of the 3% and 9% brine salted groups (p < .05) under the same pressure,
while the W value of the fillet increased with the increases in pressures. In addition, the ΔE value
increased significantly with the increase in pressure (p < .05). The ΔE values of the 300 and 400 MPa
groups combined with 3% brine salting were 11.52 and 14.87, respectively, which were significantly
(p < .05) lower than that of the other groups (Table 1).

Under the same pressure, the color of fish flesh after brine salting was lighter and whiter than that of
fillets without brine salting (with the lowest values of L* and W). Lauritzen et al. [16] reported that the
presence of calcium and magnesium ions in salt could change the appearance of cod muscle to white.
Yang et al. [17] found that salting led to an increase in the L* value (lightness) of grass carp flesh, and
salting at a low-concentration for a short period of time enhanced the lightness of grass carp flesh.
However, a previous research showed that the higher a* values for white chicken meats treated at 1.5–
2.5% of NaCl, compared to the non-salted control. [7] The addition NaCl at low concentrations in
Turkey meat had resulted in a* value increase, and was probably due to the solubilization of
myofibrillar proteins, which could react with heme pigments. [18] However, our milkfish fillet in the
high-concentration brine salting (9%) group (with the lowest values of a* and b*) was significantly
discolored (reduced redness) compared with that in the unsalted and 3% brine salted groups.
Therefore, it was possible that the high brine concentration could lead to the dehydration of fish
flesh, resulting in discoloration because of the loss (denaturation) of the water-soluble proteins,
containing heme pigments. [17] Ledward [19] had attributed the HPP-induced changes in the color
parameters of fish flesh to the degeneration of myofibrillar and sarcoplasmic proteins. Teixeira et al. [20]
also attributed the reduced fish flesh redness caused by high pressure to the degradation of pigments
and myoglobin.

Overall, this study found that the 3% brine salting combined with pressures of 300 and 400 MPa
groups had lower ΔE value and higher a* value compared to other groups of HPP combined with 9%
NaCl or HPP alone. Therefore, treatments of milkfish flesh with low-concentration (3%) brine salting
combined with appropriate pressures (300 and 400 MPa) could maintain more of the original red
color of the milkfish flesh.

**Effect of HPP combined with brine salting on the texture of milkfish fillet**

The texture characteristics of milkfish fillet treated with high pressure (300–600 MPa for 5 min)
combined with 3% and 9% brine salting are shown in Table 2. The hardness and chewiness of fish
flesh after 3% and 9% brine salting in the control group were significantly lower than that of the raw
fish fillet (p < .05). The hardness and chewiness of fish flesh increased significantly with the increases
in pressures, regardless of brine salting. The hardness in the 3% and 9% brine salted groups
increased from 1.01 and 0.88 N to 4.75 and 3.76 N (p < .05), respectively, in the 600 MPa group
(p < .05), and the chewiness in the 3% and 9% brine salted groups increased from 0.55 and 0.83 mJ
to 7.09 and 7.07 mJ, respectively, in the 600 MPa group (p < .05). In contrast, the cohesiveness of the
brine salted groups increased significantly. Cohesiveness of a food is the degree of force-deformation
before the food ruptures or crumbles. Therefore, a high cohesiveness indicates the components of
the fish were able to stick together better. An increase in cohesiveness by salt indicated that the molecular bonding between fish muscle fibers became stronger and that was due to the solubilization of fish muscles by salt since it is well known that myofibrillar proteins are salt-soluble. Therefore, it made sense that an increase in cohesiveness accompanied with a decrease in firmness and chewiness. Increasing had an effect on cohesiveness since the cohesiveness in the unsalted fillet and 3% brine salted group increased with the increase in pressures. However, fish flesh in the 9% brine salted group had no significant differences under different pressures (p > .05). Therefore, pressure effect was dependent upon salt concentration. The average springiness of fish tended to increase slowly, with slight changes and without any statistically significant differences with the increases in pressure, regardless of brine salting.

Collectively, the hardness and chewiness of fish flesh without brine salting were significantly higher than those of fish fillet with brine salting under the same pressure, whereas the cohesiveness of fish fillet without brine salting was significantly lower than that of fish flesh with brine salting. This indicates that brine salting could compensate for the disadvantages of increased hardness and chewiness of fish flesh caused by HPP and resulted in a softer texture. Therefore, proper brine salting combined with HPP could improve or maintain a relatively good texture of milkfish flesh.

Similar results had been reported by Ros-Polski et al.\(^{[7]}\) who found that salting at low concentration of NaCl in combination with HPP treatment can improve the texture in white chicken meat. Pszczola\(^{[21]}\) also had reported that NaCl improved tenderness and juiciness of processed meat products because NaCl activated proteins resulting in an increase of hydration and water-binding capacity. In addition, after high-pressure treatment, an increase in the hardness of fish flesh samples may be due to the denaturation and aggregation of myofibrillar protein.\(^{[22]}\) On the other hand, the denaturation and aggregation of the muscle proteins under a high pressure had been reported to cause tissue structure shrinkage.\(^{[22]}\) Christensen et al.\(^{[23]}\) had demonstrated that the hardness of cod flesh increased with pressure increased under a pressure ranged from 200 to 500 MPa for 2 min. Same research also had been revealed by Aubourg et al.,\(^{[24]}\) who showed that a increase in the hardness, chewiness, and cohesiveness of Atlantic mackerel meat under higher intensity of pressure and longer holding times; however, springiness was influenced less by pressure intensity and holding time. Our research showed that the potential muscle protein solubilization caused by 9% salting for 90 min might have helped reduce the firming effect induced by high-pressure protein aggregation in fish fillet. It is quite well known, myofibrillar proteins are soluble in 0.6 M NaCl or higher concentrations. A 9% brine soaking could produce much higher than 0.6 M NaCl in fish fillet tissues.

| Table 2. Texture properties of milkfish fillet soaked in 3% and 9% NaCl brine for 90 min at 4°C, and then pressurized with 300, 400, 500, and 600 MPa for 5 min. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Hardness (N)                   | Control (0.1 MPa) | HPP treatment (MPa) |
| Raw meat                       | 3.38 ± 0.43\(^{Ta}\) | 3.40 ± 0.22\(^{Ab}\) | 4.21 ± 0.15\(^{Ac}\) | 7.19 ± 0.06\(^{AB}\) | 10.84 ± 0.25\(^{Aa}\) |
| 3                              | 1.01 ± 0.15\(^{Bc}\) | 2.43 ± 0.26\(^{Ab}\) | 2.77 ± 0.48\(^{Ac}\) | 3.56 ± 0.20\(^{Bb}\) | 4.75 ± 0.71\(^{Ba}\) |
| 9                              | 0.88 ± 0.22\(^{Bc}\) | 1.46 ± 0.71\(^{Cc}\) | 2.36 ± 0.70\(^{Bb}\) | 2.39 ± 0.48\(^{Cc}\) | 3.76 ± 0.53\(^{Ca}\) |
| Cohesiveness                   | Raw meat         | 0.25 ± 0.03\(^{Bc}\) | 0.34 ± 0.02\(^{Ab}\) | 0.40 ± 0.01\(^{Ab}\) | 0.43 ± 0.01\(^{Ba}\) |
| 3                              | 0.35 ± 0.06\(^{Bc}\) | 0.48 ± 0.07\(^{Ab}\) | 0.53 ± 0.08\(^{Ab}\) | 0.56 ± 0.05\(^{Ab}\) | 0.69 ± 0.11\(^{Aa}\) |
| 9                              | 0.55 ± 0.07\(^{Aa}\) | 0.57 ± 0.12\(^{Aa}\) | 0.56 ± 0.07\(^{Aa}\) | 0.55 ± 0.05\(^{Aa}\) | 0.69 ± 0.15\(^{Aa}\) |
| Springiness (mm)               | Raw meat         | 1.77 ± 0.17\(^{Aa}\) | 2.07 ± 0.54\(^{Aa}\) | 2.30 ± 0.85\(^{Aa}\) | 2.32 ± 0.57\(^{Aa}\) | 2.69 ± 0.14\(^{Aa}\) |
| 3                              | 1.59 ± 0.22\(^{Bb}\) | 2.05 ± 0.63\(^{Ab}\) | 1.78 ± 0.41\(^{Ab}\) | 1.62 ± 0.14\(^{Ab}\) | 2.29 ± 0.28\(^{Ca}\) |
| 9                              | 1.88 ± 0.22\(^{Bb}\) | 1.99 ± 0.64\(^{Ab}\) | 2.39 ± 0.66\(^{Ab}\) | 2.20 ± 0.30\(^{Ab}\) | 2.46 ± 0.64\(^{Aa}\) |
| Chewiness (mJ)                 | Raw meat         | 1.28 ± 0.94\(^{Ad}\) | 2.72 ± 0.70\(^{Ac}\) | 3.78 ± 1.11\(^{Ac}\) | 7.23 ± 0.10\(^{Ab}\) | 12.48 ± 2.01\(^{Aa}\) |
| 3                              | 0.55 ± 0.10\(^{Bb}\) | 1.64 ± 0.21\(^{Bc}\) | 2.88 ± 0.71\(^{Bb}\) | 3.48 ± 0.62\(^{Bb}\) | 7.09 ± 2.17\(^{Bb}\) |
| 9                              | 0.83 ± 0.23\(^{Aa}\) | 1.80 ± 0.50\(^{Bc}\) | 2.77 ± 0.48\(^{Bb}\) | 3.05 ± 0.43\(^{Bb}\) | 7.07 ± 2.26\(^{Bb}\) |

\(^{1}\)Each value is the mean ± standard deviation (n = 3); Different lower case letters indicates significant differences at the same salt concentration (p < 0.05); Different upper case letters indicates significant differences at the same pressure (p < 0.05).
Table 3. The salt and moisture content (%) of milkfish fillet soaked in 3% and 9% NaCl brine for 90 min at 4°C, and then pressurized with 300, 400, 500, and 600 MPa for 5 min.

| Brine concentration (%) | Control (0.1 MPa) | HPP treatments (MPa) |
|-------------------------|-------------------|----------------------|
|                         |                    | 300                  | 400 | 500 | 600 |
| Salt content            |                    |                      |     |     |     |
| Raw meat                | 0.02 ± 0.01a       | 0.04 ± 0.02a         | 0.02 ± 0.01a | 0.04 ± 0.03a | 0.02 ± 0.01a |
| 3                       | 1.39 ± 0.03a       | 1.35 ± 0.02ab        | 1.32 ± 0.03abc | 1.28 ± 0.02bc | 1.25 ± 0.02c |
| 9                       | 5.11 ± 0.01a       | 4.88 ± 0.02b         | 4.77 ± 0.03c  | 4.80 ± 0.01c  | 4.78 ± 0.01c  |
| 300°C                   | 72.08 ± 0.44a      | 71.54 ± 0.59ab       | 70.88 ± 0.46bc | 70.40 ± 0.33bc | 70.02 ± 0.21c |
| Moisture content        |                    |                      |     |     |     |
| Raw meat                | 74.35 ± 0.53a      | 73.26 ± 0.46ab       | 72.50 ± 0.35bc | 72.07 ± 0.62b  | 70.67 ± 0.74c  |
| 3                       | 74.29 ± 0.40a      | 73.52 ± 0.46ab       | 73.16 ± 0.52bc | 72.10 ± 0.64b  | 69.30 ± 0.35c  |
| 9                       |                    |                      |     |     |     |

1 All data were the means ± standard deviation of three replicates. Different letters in the same row indicate significant differences (p < 0.05).

Effect of HPP combined with brine salting on the salt and moisture content of milkfish fillet

The changes in salt and moisture content of milkfish flesh treated with high pressures (300–600 MPa for 5 min) combined with 3% and 9% brine salting are shown in Table 3. The salt content of raw fish flesh treated with high pressures was between 0.02% and 0.04%, showing that high pressure alone had no effect on salt content of fish. In addition, the salt content of fish fillet after 3% and 9% brine salting was 1.39% and 5.11%, respectively; for the respective combinations of 3% and 9% brine salting with HPP, the average salt contents of fish fillets decreased slightly with the increases in pressures to 1.25% and 4.78% in the 600 MPa group, respectively (p < .05) (Table 3). With regard to moisture content, the moisture content of fish fillet after 3% and 9% brine salting was 74.35% and 74.29%, respectively; for the respective combinations of 3% and 9% brine salting with HPP, the average moisture contents of fish fillets decreased slightly with the increases in pressures to 70.67% and 69.30% in the 600 MPa group, respectively (p < .05) (Table 3). Therefore, HPP treatment reduced the salt and moisture content of fish fillet with brine salting, with higher pressure further lowering the salt and moisture content. This effect might be attributed to high pressures that could destroy non-covalent bonds (such as hydrogen bonds and hydrophobic bonds) between molecules, thus denaturing proteins in fish muscle, reducing the water-binding capacity, and leading to the loss of drip, moisture, and salt.[7]

Effect of HPP combined with brine salting on the microbial count of milkfish fillet

The APC of milkfish fillet treated with high pressures (300–600 MPa for 5 min) combined with brine salting (3% and 9%) is shown in Figure 2. The initial APC of raw milkfish fillet (control) was 6.38 log CFU/g. After 3% and 9% brine salting, APC decreased to 5.09 and 4.59 log CFU/g, respectively (i.e., reduction by 1.29 and 1.79 log CFU/g, respectively), indicating that treating fillet with brine salting alone could reduce the APC value. Moreover, the higher brine concentration (9%) significantly increased this effect, probably because brine could help release bacteria attached to the surface of fish flesh or inhibit the growth of bacteria in the fillet, particularly by the high salt concentration. The same trend was observed in the high-pressure groups. The APC of fish flesh treated by 3% and 9% brine salting combined with 300 MPa high pressure decreased from 5.99 log CFU/g in the group treated at 300 MPa alone to 4.68 and 4.32 log CFU/g, respectively (i.e., reduction by 1.31 and 1.67 log CFU/g, respectively). The APC of fish fillet treated by 3% and 9% brine salting combined with 400 MPa high pressure decreased from 5.13 log CFU/g in the no-salt treated group at 400 MPa to 3.48 and 3.44 log CFU/g, respectively (i.e., reduction by 1.65 and 1.69 log CFU/g, respectively). Specifically, when 3% and 9% brine salting was combined with 500 and 600 MPa high pressure, respectively, no APC was detected in the fillet after processing. In addition, under the same brine concentrations (3% or 9%), the APC of the fillet decreased significantly with the increase in pressure (p < .05). In summary, treatment of milkfish fillet with brine salting combined with high pressure had an additive bactericidal effect on APC, indicating that high pressure combined with brine salting can be regarded as an effective hurdle technology.
The PBC of milkfish flesh treated with high pressures (300–600 MPa for 5 min) combined with brine salting (3% and 9%) is shown in Figure 3. The initial PBC of the raw milkfish fillet was 6.23 log CFU/g, and it decreased to 5.17 and 4.66 log CFU/g (i.e., reduction by 1.06 and 1.57 log CFU/g, respectively) after 3% and 9% brine salting, respectively, indicating that brine salting alone could reduce PBC. PBC showed similar results to APC, that is, under the same pressure, higher brine concentration reduced the PBC in fillet products, and under the same brine concentration, the PBC of the fillet decreased significantly with the increase in pressure (p < .05). In summary, treatment of milkfish flesh by brine salting combined with high pressure was more effective than brine salting or high pressure alone, with a synergistic effect on eliminating PBC.

The mechanism of inhibiting microorganisms by NaCl is mainly due to the reduced water activity in foods that caused an inhibition on the delivery of nutrients to cells, imbalance of osmotic pressure, and changes in turgor pressure, etc. A high hydrostatic pressure can induce irreversible denaturation of enzymes and proteins and causes rupturing of the cell membrane and the excretion of internal substances, resulting in bacterial death. In addition, HPP had 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.

Effect of HPP combined with brine salting on the TVBN of milkfish fillet

The TVBN of milkfish fillet treated by high pressures (300–600 MPa for 5 min) combined with brine salting (3% and 9%) is shown in Figure 4. Generally, TVBN includes trimethylamine (TMA), dimethylamine (DMA), and ammonia (NH3), which are widely used as indicators for freshness and spoilage of fish. The initial TVBN of raw milkfish fillet was 25.9 mg/100 g and it decreased to 13.58 mg/100 g and 13.44 mg/100 g after 3% and 9% brine salting, respectively. These results showed that the TVBN values could be reduced by brine salting alone, which might be due to the dehydrating effect of salt resulting in the loss of volatile amines in the brine, and/or the volatile amines on the surface of fish flesh being washed off by brine. In addition, under the same pressure conditions, higher brine
Figure 3. The psychrotrophic bacteria count of milkfish fillet soaked in 3% and 9% NaCl brine for 90 min at 4°C, and then hydrostatically pressurized with 300, 400, 500 and 600 MPa for 5 min. Different lower case letters indicates significant differences at the same salt concentration ($p < .05$); Different upper case letters indicates significant differences at the same pressure ($p < .05$).

Figure 4. The TVBN of milkfish fillet soaked in 3% and 9% NaCl brine for 90 min at 4°C, and then hydrostatically pressurized with 300, 400, 500 and 600 MPa for 5 min. Different lower case letters indicates significant differences at the same salt concentration ($p < .05$); Different upper case letters indicates significant differences at the same pressure ($p < .05$).
concentrations lowered the TVBN value in fish flesh \( p < .05 \), and under the same brine concentration, the TVBN value of fish fillet decreased significantly with the increase in pressure \( p < .05 \), corresponding to the trend of bacterial inactivation. In summary, treating milkfish flesh by brine salting combined with HPP produced synergistic effect on TVBN reduction than brine salting alone or HPP treatment alone. A similar finding had been demonstrated by Chouhan et al.,\textsuperscript{13} who found that HPP at 250 and 350 MPa for 10 min significantly reduced the TVBN levels of hilsa fillets, and decreased from 7.16 mg/100 g in the control to 3.52 and 2.16 mg/100 g, respectively. The authors attributed this reduction of TVBN to the degeneration of muscle protein, shrinkage of tissue structure, and release of water, which led to the loss of volatile amines in water.\textsuperscript{13} Similar to the results of this study, Chaijan\textsuperscript{26} salted tilapia and found that the TVBN content during storage was reduced, and attributed the effect to the leaching of salt-soluble media or the diffusion and loss of volatile amines from tilapia.

Most of the previous studies on HPP combined with NaCl mainly used high pressure for the meat and its products as an alternative processing method to reduce the amount of salt used, which can reduce the number of microorganisms while maintaining the physical characteristics and functional quality of the meat products.\textsuperscript{7,27,28} This study is the first to examine the effect of brine salting in combination with HPP on fish fillet for improving the physicochemical properties and ensure microbial safety. As shown in the results described above, the 3\% brine salting combined with the 300 or 400 MPa pressure groups had significantly higher \( a^* \) value and lower \( \Delta E \) value than those of the other treatment groups. Additionally, brine salting alone reduced APC and PBC as well as the contents of TVBN, and this reduction increased with increased pressure and/or brine concentrations. However, in the 9\% brine group, the original red color of fish flesh significantly faded (the \( a^* \) and \( b^* \) values were the lowest), while the salt content after 9\% brine salting was excessively high (>4.77\%) (Table 3). Therefore, 9\% brine salting does not meet the health requirements of modern diets. Considering all factors, the optimum processing conditions that caused minimum negative effects to milkfish fillet are 3\% brine salting combined with a moderate pressures of 300 or 400 MPa for 5 min.

In a study, after cooking, HPP pretreatment at 200 MPa for 6 min on albacore meat maintained a similar color and texture as compared to the control (non-HPP treated sample).\textsuperscript{29} In addition, the sensorial acceptability of oven cooked HPP-treated Atlantic mackerel samples was similar to that of cooked control samples.\textsuperscript{24} Thus, HPP treatment on fish could be a suitable technology for ensuring seafood safety, especially when it is intended to its subsequent consumption once cooked or canning process.\textsuperscript{29} Further research is necessary to evaluate the impact of HPP combined with brine salting on texture and sensory quality of milkfish fillets after cooking.

**Conclusion**

Our results showed that under the same pressure conditions, the color of milkfish fillet treated by high pressure combined with brine salting (3\% and 9\%) became brighter and whiter than that of milkfish fillet without brine salting, with almost a total loss of redness at the combinations of 9\% salt and high-pressure ranges (500–600 MPa). Brine salting could compensate for the disadvantages of increased hardness and chewiness of fish flesh caused by HPP to result in a softer texture. A combination of brine salting and HPP treatment had synergistically bactericidal effects on APC and PBC, and had a reducing effect on the TVBN value. Overall, these results from this study demonstrate that proper selection of high pressures in combination with brine salting can be regarded as an effective hurdle technology for improving the quality of fish fillet in the aquatic food industry.

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Disclosure statement

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

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