Effects of marination and high-pressure processing on the physicochemical and sensory quality of ready-to-cook yellowfin (*Thunnus albacares*) tuna steak

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Academic Editor: Jelena Popović-Djordjević, PhD, Department for Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

Received: 13 November 2025; Accepted: 8 April 2026; Published: 15 May 2026

ORIGINAL ARTICLE

Abstract

To promote the development of convenient, safe and healthy ready-to-cook (RTC) tuna products, this study investigated the combined effect of marination and high-pressure processing (HPP) on the quality and safety of yellowfin tuna steaks. Treatments included marination alone and marination together with HPP at 300 MPa or 600 MPa, compared with untreated control samples. The findings revealed that combining marination with moderate-pressure processing (300 MPa) significantly improved texture by reducing hardness, chewiness and shear force with sustenance of consumer-preferred aroma and taste. Although colour differences increased with pressure, sensory colour acceptability remained unaffected. Marination and HPP also effectively reduced histamine and total volatile basic nitrogen levels, indicating enhanced freshness and microbial stability. All samples maintained microbial counts below detectable limits (<10 CFU/g). Scanning electron microscopy further supported the acceptable structural modifications at 300 MPa. This study demonstrates that HPP-assisted marination is a promising preservation strategy for improving the safety, texture, and acceptability of RTC tuna without compromising quality. The approach offers valuable insight for sustainable seafood processing industries seeking cleaner and safer alternatives to thermal treatment.

Keywords: hardness; histamine; texture analysis; TVBN; SEM

ISSN 1757-837X online, DOI 10.15586/qas.v18i2.1628

Introduction

In recent years, the growing demand for nutritious, convenient, and safe seafood products has driven innovation in food preservation technologies. Among all marine resources, tuna stands out as a globally recognised and widely consumed fish, valued for its exceptional nutritional profile and culinary versatility. Tuna is notably rich in high-quality protein (Zhou *et al*., 2023), omega-3 fatty acids and essential micronutrients (Ramona *et al*., 2023), which have various health benefits, including cardiovascular health, brain function, and muscle development (Von Schacky, 2021). Beyond its nutritional value, tuna also holds considerable economic importance, contributing substantially to the global seafood market through a wide range of products, such as sashimi, canned tuna, frozen steaks, and ready-to-cook (RTC) meals (Yi-Li *et al*., 2025).

Despite its advantages, tuna is highly perishable, primarily because of its high moisture content, protein-rich matrix, and susceptibility to microbial spoilage and enzymatic degradation (Yi-Li *et al*., 2025). Unlike thermal processing, high-pressure processing (HPP) maintains the fresh-like attributes of seafood by minimising heat-induced damage to proteins and lipids (Roobab *et al*., 2022). Studies have shown that HPP can significantly reduce microbial loads in tuna and other seafoods without compromising nutritional value (Boziaris *et al.*, 2021; Tsai *et al*., 2022). However, recent proteomic and lipidomic research indicates that pressures between 300 and 600 MPa can induce structural changes in muscle proteins and promote lipid oxidation because of membrane disruption (Gokul Nath *et al.*, 2023). These effects may be pronounced in tuna because of its relatively firm, low-collagen, and highly oxidative muscle, which behaves differently from white-fleshed fish under pressure.

To mitigate these challenges and improve product performance, marination is commonly used to enhance flavour, tenderness and stability. Ingredients such as salt, acid or sugars can modify muscle proteins, increase ionic strength, and influence water–protein interaction. When marination is combined with HPP, previous studies on pork, beef, and certain seafoods have reported deeper marinade penetration, altered textural properties, and improved flavour development (Joseph *et al*., 2021; Kim *et al*., 2018; O'Neill *et al*., 2019). HPP and marination have been previously applied to various seafood and meat systems. However, only limited studies have examined marinated yellowfin tuna, particularly using a marinade consisting of salt, pepper, oil, vinegar, water and sugar prior to HPP treatment. The behaviour of marinated tuna muscle under pressure may differ from other species because of its fibre structure, connective tissue content, and lipid composition, which may influence texture, seasoning distribution, and retention of flavour in RTC products.

Therefore, this study aims to investigate the combined effects of marination and HPP on the physicochemical quality, microbial stability, and consumer acceptability of yellowfin tuna steaks. This study aims to evaluate parameters such as texture, colour, pH, water-holding capacity (WHC), total volatile basic nitrogen (TVB-N), histamine levels, and microstructure to determine whether this combined treatment can effectively enhance product safety, extend shelf life, and maintain desirable sensory attributes for the modern seafood market.

Materials and Methods

Preparation of tuna samples

A whole yellowfin tuna was sourced from a local seafood supplier in Sabah, Malaysia. The fish was freshly caught and frozen immediately. It was transported to the laboratory under frozen conditions. Upon arrival, the frozen tuna was immediately processed into loins, manually skinned, deboned, and sliced into steaks of 2 cm thick and an average weight of 200 g each. The tuna steaks were then stored at -20°C until further use. Prior to treatment, the frozen tuna steaks were thawed in a refrigerator (4°C). The fish-to-marinade ratio was maintained at 1.5:1 (w/w). The marinade consisted of 3% sugar, 2.5% salt, and 1.5% pepper, with the remaining liquid phase comprising oil, water, and vinegar in a fixed ratio of 1:1:0.2. All ingredients were weighed and homogenised using a high-speed homogeniser (IKA T-25 ULTRA-TURRAX, Cole-Parmer, Illinois) to ensure uniform distribution.

All tuna steaks were first immersed in marinade and vacuum-sealed in embossed vacuum bags to initiate the marination process. For combined treatment groups, the vacuum-sealed samples were immediately subjected to HPP using a 55 L HPP unit (Hiperbaric, Burgos, Spain) with a 5-min holding time and rapid depressurisation (<5 s). After HPP treatment, all samples were stored at 4°C for 24 h to complete the marination period.

Four groups were prepared. The initial sample without treatment was labelled as the untreated control (C) and served as a baseline for comparison. Other groups included a marinated only group (M), a marinated and HPP-treated group at 300 MPa (M + HPP300), and a marinated and HPP-treated group at 600 MPa (M + HPP600).

Marinade uptake

The initial weight of each tuna sample was recorded. After treatment, marinated samples were removed from the marinade liquid, and excess marinade was gently scraped off before reweighing. The marinade uptake was calculated using the following formula:

$$Marinade\ uptake = \frac{W_t - W_i}{W_i} \times 100$$

where W_i represents the initial untreated weight; and W_t represents the weight after 24 h marination. Negative values indicate net weight loss, which occurs if the water is lost during marination.

Cooking yield and dimensional shrinkage

The weight, thickness, and diameter of each sample were measured before and after cooking. The thickness and diameter of each marinated tuna steak were measured using a digital vernier calliper (±0.01-mm accuracy). The samples were then baked in an oven at 170°C, and the internal temperature was monitored using a digital kitchen thermometer (Thermopro TP02S; ThermoPro Co., USA) inserted into the centre of each steak. Cooking continued until the internal temperature reached 70°C, which exceeds the US Department of Agriculture (USDA, 2024)-recommended minimum of 63°C for tuna. The cooking yield was calculated using the following equation:

$$Cooking\ yield\ (\%) = \frac{W_c}{W_t} \times 100$$

where W_c represents the weight of the cooked sample; and W_t represents the weight after 24-h marination.

Calculation of dimensional shrinkage is as follows:

$$Dimensional\ shrinkage\ (\%) = \frac{(T_1 - T_2) + (D_1 - D_2)}{(T_1 + D_1)} \times 100$$

where T_1 represents marinated (uncooked) thickness; T_2 represents cooked thickness; D_1 represents marinated (uncooked) diameter; and D_2 represents cooked diameter.

Water-holding capacity

The WHC refers to the tuna steak's ability to retain water when subjected to external forces, such as centrifugation. The WHC was determined using the methodology outlined by Zhang *et al.* (2022). After thawing the tuna meat, the marinade liquid on the surface was wiped off with filter paper. The thawed tuna meat (2 g) was wrapped in two layers of filter paper (Whatman No. 1) and centrifuged for 10 min at 4°C and 5,500 RPM using a refrigerated microcentrifuge (Kubota 3740, Japan). After centrifugation, the meat was removed from the filter paper and weighed accurately. WHC was determined using the following formula:

$$WHC\ (\%) = \frac{W_a}{W_b} \times 100$$

where W_a represents the weight of the sample after centrifugation; and W_b represents the weight of the sample before it was centrifuged.

Colour analysis

The surface colour of tuna samples was measured using a hand-held chromameter (CR400, Minolta Camera Co., Osaka, Japan) with an 8-mm aperture. Observations were made at three different locations using three separate samples from each treatment, which were placed in Petri dishes at room temperature (25°C). The average values of CIE L^*, a^*, and b^* were recorded, and ΔE was calculated (Wei *et al.*, 2024). ΔE *(*d*E)* was determined using the following formula:

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2},$$

Where L_1^*, a_1^*, and b_1^* represent the colour values before treatment, and L_2^*, a_2^* and b_2^* represent the colour values after treatment.

Texture profile analysis and shear force test

The texture profile of cooked tuna samples was evaluated using a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, UK) in accordance with the methodology outlined by Ismail *et al.* (2021), with appropriate adjustments applied. The cooked samples were shaped into 20-mm × 20-mm cubes and subjected to compression twice with a P/75 probe at a trigger force of 5 g, reducing their height to 75% of the original. The texture analyser was configured with a pre-test speed of 3.0 mm/s, a test speed of 1.0 mm/s, and a post-test speed of 1.0 mm/s for measurements. The evaluated characteristics were hardness, gumminess, chewiness, and cohesiveness.

The Warner–Bratzler shear test was conducted according to the methodology outlined by Ismail *et al.* (2021), with

appropriate adjustments applied. The cooked tuna samples, measuring 30 mm in length and 10 mm in diameter, were positioned horizontally and sliced using a Warner–Bratzler V-slot blade with a triangular, slow-cutting edge (strain mode) at a test speed of 1.5 mm/s. The maximum shear force (kg) was determined.

Scanning electron microscopy (SEM)

The microstructure of tuna samples was examined using SEM (LEO 1455 VPSEM, Cambridge, UK) after freeze-drying. The specimens were mounted on stubs using double-sided carbon-conductive tape and coated with a thin layer of gold. Images were obtained at 100× magnification (Faridah *et al*., 2023).

pH analysis

Approximately 1 g of tuna samples was homogenised (1:10 w/v) with distilled water using a homogeniser (Diax 900; Heidolph, Germany). The pH was determined with a pH meter (PB-10, Sartorius, Germany) (Alemán *et al*., 2016).

Total volatile basic nitrogen

The TVB-N content of tuna samples was determined using the Kjeltec 8400 Analyser (FOSS, Denmark), following a modified Association of Official Analytical Chemists (AOAC) method (European Union, 1995). A 10-g portion of the homogenised sample was weighed in a centrifuge tube. Subsequently, perchloric acid solution was added to bring the volume to 25 mL, and the mixture was homogenised thoroughly. The volume was then adjusted to 40 mL with additional perchloric acid solution. The homogenate was filtered to obtain a clear extract.

Depending on the expected TVB-N content, an aliquot of 1–20 mL of filtrate was transferred into a digestion tube. If the volume was less than 20 mL, perchloric acid solution was added to bring the total volume to 20 mL. Then, 3 drops of phenolphthalein indicator were added to the solution.

The digestion tubes were loaded into the Kjeltec system, and the TVB-N analysis program was selected. The apparatus automatically added 100 mL of 40% sodium hydroxide solution to the digestion tube and 30 mL of 1% boric acid solution to the receiving flask. After a 5-s delay, steam distillation was performed at 60% power for 6 min to release ammonia, which was absorbed by the boric acid solution.

The released ammonia was titrated with 0.1-N hydrochloric acid standard solution until the endpoint, indicated by a colour change to grey with a slight blue tint. A reagent blank was prepared and titrated in triplicate to obtain a corrected blank value, which was used to adjust final TVB-N values. The following formula was used:

$$X = \frac{(V - V') \times 0.14}{m} \times F \times 100$$

where X represents the amount of TVB-N in samples (mg/100 g); V represents the volume of HCl standard solution consumed by the test portion (mL); V' represents the volume of HCl standard solution consumed by blank reagents (mL); m represents mass of test portion (g); F represents the dilution factor of the sample; 0.14 mg of TVB-N equals to 1 mL of HCl standard solution.

Histamine content

Histamine content in tuna samples was determined using a colourimetric assay kit MAK432 (Sigma-Aldrich, USA) following the manufacturer's protocol (Shahid *et al*., 2011).

Sample preparation

Approximately 200 mg of the homogenised tuna sample was mixed with 500 µL of histamine extraction buffer (prepared by diluting assay buffer with 100% methanol at a 1:1 v/v ratio). The mixture was then water-bathed at 90°C for 20 min, cooled on ice, and centrifuged at 10,000×g for 5 min. The resulting supernatant was collected and used for analysis.

Standard curve preparation

Histamine stock solution, 50 µg/mL (mg/kg), was prepared by diluting 4 µL of 5 mg/mL histamine standard with 396 µL of assay buffer. Serial dilution was performed in a 96-well plate to generate a standard curve. Wells A1 and A2 were filled with 100 µL of stock standard, and 50 µL of assay buffer was added to wells B1 and B2 through H1 and H2. A two-fold serial dilution was performed from wells A to G by transferring 50 µL from one well to the next, mixing thoroughly after each transfer. No solution was added to wells H1 and H2.

Reaction mix

A sufficient volume of reagent was prepared based on the total number of assay wells required. For each well, 50 µL of reaction mix was prepared by combining 46 µL of assay buffer, 2 µL of reaction enzyme, and 2 µL of detection solution. The prepared reaction mix was used immediately for the assay.

Assay reaction

In all, a 50-µL sample was added to each sample well, followed by 50 µL of reaction mix in each standard and sample well. The plate was then incubated at room temperature for 30 min, protected from light. Absorbance was read at 450 nm using a microplate reader. Measurements within 60 min were accepted for analysis.

Microbial analysis

Tuna samples were assessed for microbiological quality based on four key indicators: total plate count (TPC), yeast and mould count (YMC), coliform count, and *Enterobacteriaceae* count. A 25-g portion of each tuna sample was aseptically transferred into 225 mL of sterile peptone water in stomacher bags and homogenised for 3 min using a stomacher (BagMixer; Interscience, France). All microbiological analyses were conducted following the procedures outlined in the Bacteriological Analytical Manual (Merker, 1998).

From homogenised samples, 1-mL aliquots were subjected to 10-fold serial dilutions ranging from 10^{-1} to 10^{-6}. For microbial enumeration, 0.1 mL of each dilution was plated onto the appropriate agar medium using the spread plate technique. TPC was carried out using plate count agar (PCA), YMC on potato dextrose agar (PDA), and both coliform and *Enterobacteriaceae* counts were determined using MacConkey Agar. All media were obtained from Merck (Germany). The inoculated plates were incubated at 35°C for 24 h (Suhaili *et al*., 2021), after which colony-forming units (CFU) were recorded and expressed as $\log_{10}$ CFU/g. Incubation at 35°C was performed following established methodologies for assessing food microbiological quality indicators and was not designed for the isolation of marine-specific bacteria (Tsai *et al*., 2022; Üçok *et al*., 2025).

Sensory test

The sensory properties of tuna samples, including appearance, colour, aroma, texture, taste, and overall acceptability, were evaluated by 100 untrained panellists (50 panellists per session) using a 9-point hedonic scale. All tuna samples were cooked as described in Section 2.3. Each sample was labelled with a three-digit random code and presented to panellists in a randomised order, along with a cup of water and cucumber cubes for palate cleansing between samples. The hedonic scale ranged from 1 = 'dislike extremely' to 9 = 'like extremely', allowing panellists to rate their preference for each sensory attribute.

Ethical Statement

Ethical approval for this study was granted by the Universiti Putra Malaysia Ethics Committee for Research Involving Human Subjects (JKEUPM) (ethics approval number: UPM.TNCPI.800-2/1/7). All participants were informed about the study procedures, and written informed consent was obtained prior to participation.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 26. Significant differences in data were determined statistically using one-way analysis of variance (ANOVA) with Tukey's multiple-comparison tests at a 95% confidence level. The values were expressed as a mean ± standard deviation (SD).

Results

Marination uptake, cooking yield, dimensional shrinkage, and WHC of HPP marinated tuna steaks

Both marinade uptake and cooking yield of tuna samples were significantly affected ($P < 0.05$) by the combined effect of marination and HPP (Table 1). While marination alone (M) resulted in the highest marinade uptake, the incorporation of HPP (M + HPP300 and M + HPP600) led to a significant reduction in marinade absorption. This reduction could be attributed to pressure-induced protein denaturation and structural tightening of muscle fibres, which could reduce permeability and limit marinade penetration (Cartagena *et al*., 2020; Tsai *et al*., 2022). Additionally, HPP may cause partial expulsion of marinade because of compressive forces during pressurisation, further decreasing the net uptake (Muntean *et al*., 2016).

The cooking yield of the control sample was significantly higher than that of all other treatment groups. Cooking yield decreased after M + HPP300 and M + HPP600. This could be due to increased protein denaturation, which reduces water retention during cooking (Cartagena *et al.*, 2019). A similar result was reported by Wang *et al*. (2015). Control pork chops exhibited lower cooking loss than most treatment groups. However, samples treated at 400 MPa had values comparable to those of the control.

In contrast, dimensional shrinkage and WHC were not significantly affected ($P > 0.05$) by either marination or its combination with HPP. WHC refers to the ability of muscle tissue to retain moisture under external forces such as cutting and heating, and is a critical determinant

of product juiciness and texture (Drisya and Sukumar, 2026). In this study, WHC remained stable across treatments. This suggests that although marinade retention and cooking yield were altered, the muscle's internal moisture-binding properties were largely unaffected. This outcome differs from the findings of Ma *et al*. (2019), who reported that combining enzymatic tenderization (papain) with low-pressure HPP improved WHC in yak meat. The discrepancy may stem from differences in protein structure, treatment sequence, and pressure intensity. For example, pressures above 300 MPa, as used in this study, may exceed the optimal range for improving WHC, thereby promoting structural degradation or water loss.

Overall, marination combined with HPP altered the marinade uptake and cooking yield of tuna samples. However, it did not significantly influence WHC or dimensional shrinkage. These results highlight the importance of optimising pressure parameters and marination protocols to preserve desirable quality attributes in RTC tuna products.

Colour profile of tuna samples

Colour is an important quality attribute of an RTC product because it strongly influences consumer food choices (Uçak and Afreen, 2022). As shown in Table 2, L^* of the samples increased significantly ($P < 0.05$) in both HPP-treated groups, with the highest L^* observed in M + HPP600, followed by M + HPP300, while the control samples showed the lowest L^*. A similar trend was observed for b^* value, whereas a^* value decreased significantly in the treated groups, with the lowest redness in marinated-only samples.

The calculated ΔE values indicated significant differences among treatment groups ($P = 0.026$). The M + HPP600 group exhibited the highest total colour difference, followed by M + HPP300 and M groups. ΔE was not calculated for control, as it served as a reference point.

The M group sample showed a slight colour change ($\Delta E = 10.57$). This could be due to pigment diffusion and pH-related protein changes. However, the application of HPP, particularly at higher pressures, significantly increased the lightness and yellowness while reducing redness. An increase in L^* value in HPP-treated samples could be due to protein denaturation. In particular, sarcoplasmic protein precipitation can reflect more light and gives a paler appearance (Joo *et al*., 1999; Marcos *et al.*, 2010).

As reported by Marcos *et al*. (2010), ΔE values around 10 were perceptible but not drastic, while values exceeding 20 resulted in noticeable visual changes. Therefore, the M + HPP600 group substantially altered the appearance of tuna meat. This could influence consumer perception,

Table 1. Marinade uptake, cooking yield, dimensional shrinkage, and water-holding capacity (WHC) of tuna samples.

Analyses	C	M	M + HPP300	M + HPP600
Marinade uptake (%)	N/A	9.45 ± 0.69^{b}	4.86 ± 1.61^{a}	3.80 ± 2.23^{a}
Cooking yield (%)	82.07 ± 4.62^{b}	73.56 ± 1.18^{a}	69.30 ± 1.71^{a}	66.95 ± 1.00^{a}
Dimensional shrinkage (%)	11.49 ± 2.19	14.37 ± 0.83	10.36 ± 5.99	13.06 ± 5.43
WHC (%)	65.20 ± 1.72	61.67 ± 4.45	55.52 ± 8.32	53.48 ± 2.43

Notes: Values are mean ± standard deviation (SD) of three replicates (n = 3). Mean values in the same row that do not share the same lowercase superscript alphabet are significantly different ($P < 0.05$).

Table 2. Colour profile of tuna samples across different treatments and their total colour difference.

Colour	C	M	M + HPP300	M + HPP600
L^*	44.38 ± 3.36^{a}	47.93 ± 0.74^{a}	60.10 ± 1.08^{b}	62.07 ± 0.45^{b}
a^*	16.20 ± 1.31^{b}	6.49 ± 0.89^{a}	8.06 ± 0.39^{b}	8.35 ± 0.16^{b}
b^*	11.22 ± 1.65^{a}	11.47 ± 1.47^{a}	16.55 ± 0.33^{b}	20.20 ± 0.52^{c}
Δ*E* (d*E*)	N/A	10.57 ± 2.47^{a}	18.51 ± 4.44^{a,b}	21.35 ± 3.64^{b}

Note: Different superscript alphabets in the same row indicate significant differences ($P < 0.05$) among treatment groups.

especially for products marketed as raw or minimally processed forms. A significant increase in ΔE in the M + HPP600 group suggests that both marination and high-pressure levels may work together to influence surface pigmentation.

Texture profile and shear force test of tuna samples

Texture parameters and shear force values for tuna samples subjected to different treatments are presented in Table 3. Hardness is significantly affected by different treatments. The M + HPP300 group had the lowest, while the M + HPP600 group had the highest hardness values. Springiness did not differ significantly across treatments. Both gumminess and chewiness were significantly reduced in the M + HPP300 group, while the M + HPP600 group showed the highest value among all treated groups. Cohesiveness and resilience also differed significantly, with M + HPP300 samples being the least cohesive and resilient. Shear force values increased progressively with treatment intensity, with the control sample showing the lowest value and the M + HPP600 group having the highest value.

Results of both texture profile and shear force revealed that marination and HPP had distinct and sometimes opposing effects on tuna muscle structure. The M + HPP300 group showed significantly lower hardness, gumminess, and chewiness, compared with other groups, indicating enhanced tenderness. This can be attributed to moderate pressure-induced denaturation of myofibrillar proteins and weakening of muscle fibre integrity, consistent with the findings of Ma *et al*. (2019). Gokul Nath *et al*. (2023) also reported similar effects on meat marinated and treated with HPP (~300 MPa). Although shear force increased following HPP treatment, this result suggests that both shear force and TPA represent different aspects of muscle texture. Moderate pressure may soften the internal structure while increasing resistance to blade cutting because of protein restructuring.

In contrast, M + HPP600 significantly increased hardness and shear force. This suggests that excessive pressure may cause protein aggregation or gelation, making the meat tougher (O'Neill *et al*., 2019). Increase in gumminess and chewiness supports this interpretation. Similar trends were noted in studies on beef and poultry. Ultra-high level HPP resulted in firmer texture because of irreversible protein–protein interactions (Kaur *et al*., 2016; Muntean *et al*., 2016; Tsai *et al*., 2022).

Marination alone reduced hardness and shear force compared with the control. However, its effect was less pronounced than when combined with moderate-pressure HPP. Low cohesiveness and resilience observed in M + HPP300 may reflect structural loosening due to pressure-enhanced marinade diffusion and enzymatic softening.

Overall, the synergy of marination and moderate-pressure HPP (300 MPa) improved tenderness and reduced mechanical resistance, which are desirable for RTC tuna products. However, excessively high pressure (600 MPa) may adversely affect textural quality, underscoring the importance of optimising pressure parameters based on product goals.

Microstructure

The SEM micrographs revealed distinct microstructural differences among the four treatment groups (Figure 1). Control sample (C), which did not undergo marination or HPP, exhibited well-organised muscle fibre structure with compact alignment and small, uniform pores. This structural integrity reflects the native myofibrillar arrangement typically observed in fresh tuna muscle.

Table 3. Texture parameters and shear force test of tuna steaks.

Texture	C	M	M + HPP300	M + HPP600
Hardness (g)	14,168.64 ± 3,162.97^{b}	10,039.70 ± 1,320.48^{a,b}	5,694.83 ± 104.75^{a}	21,751.40 ± 3,998.46^{c}
Springiness	0.65 ± 0.05	0.57 ± 0.05	0.53 ± 0.09	0.71 ± 0.72
Gumminess (g)	7,048.20 ± 1,880.79^{b}	4,046.50 ± 1,161.18^{a,b}	1,580.64 ± 316.59^{a}	10,881.84 ± 1,734.52^{c}
Chewiness (J)	4,632.45 ± 1,415.03^{b}	2,341.53 ± 882.86^{a,b}	732.41 ± 120.54^{a}	8,654.67 ± 1,181.59^{c}
Cohesiveness	0.49 ± 0.26^{b}	0.40 ± 0.06^{a,b}	0.24 ± 0.00^{a}	0.44 ± 0.10^{b}
Resilience	0.15 ± 0.01^{b}	0.12 ± 0.01^{a,b}	0.08 ± 0.05 ^{a}	0.15 ± 0.03^{b}
Shear force (kg)	1.76 ± 0.09^{a}	2.95 ± 0.51^{a}	6.19 ± 0.81^{b}	7.88 ± 0.28^{c}

Note: Different superscript alphabets in the same row indicate a significant difference ($P < 0.05$) among treatment groups.

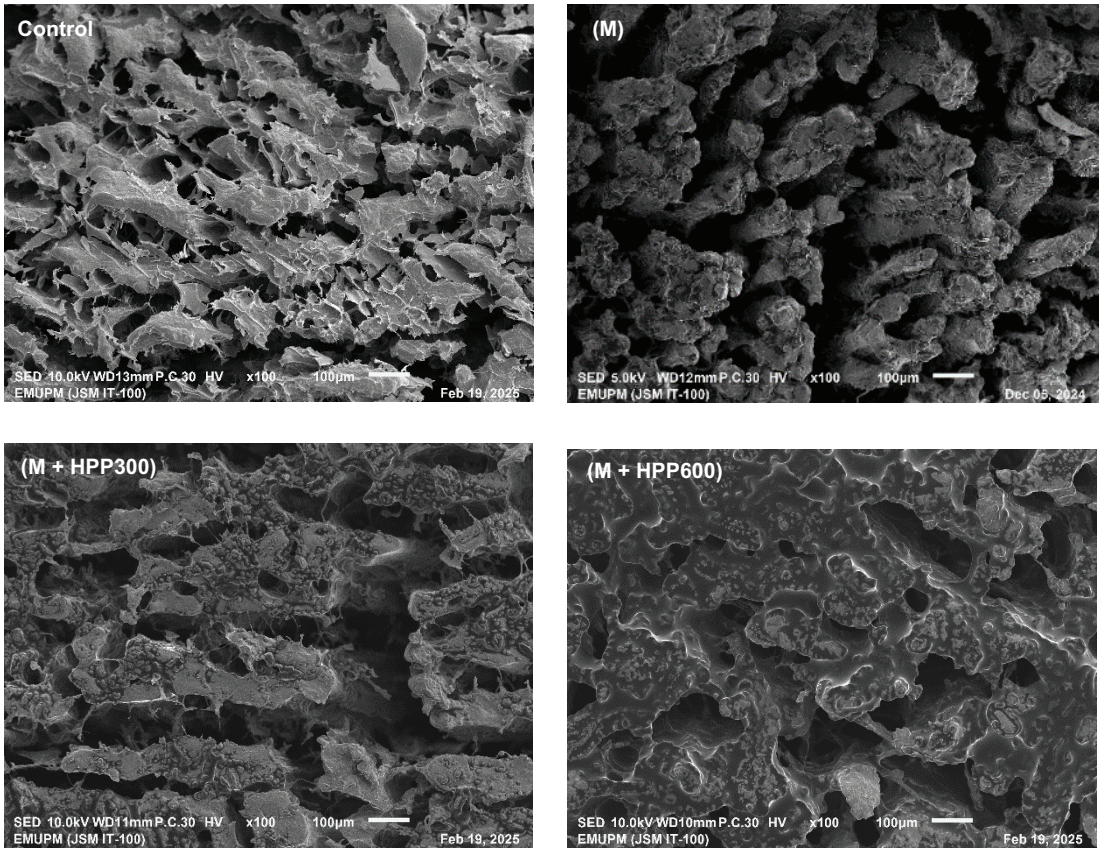

Figure 1. SEM photomicrograph of tuna samples with various treatment groups. All images were captured at 100× magnification. Raw untreated tuna (control); raw SP3-marinated tuna (M); raw SP3-marinated + HPP 300 MPa (M + HPP300); and raw SP3-marinated + HPP 600 MPa (M + HPP600).

In contrast, the marinated-only sample displayed the largest and the most elongated voids among all treatments, often appearing as straight channels between muscle fibres. This disruption potentially was due to osmotic activity during marination, where salt and acid infiltration weakened inter-fibre linkages, resulting in a looser and more open structure. Increased marinade uptake and reduced cohesiveness observed in the texture profile analysis further supported this interpretation.

The sample treated with marination and HPP at 300 MPa (M + HPP300) showed partial preservation of muscle fibre alignment, with visible structural damage and a moderate pore size. Some fibres remained intact and clustered, suggesting that this pressure level partially disrupted the muscle matrix while still maintaining some of the original fibre morphology. This correlates well with the favourable texture outcomes of this group, such as lower hardness and gumminess. This indicates an optimal balance between tenderisation and structural integrity.

In the M + HPP600 group, muscle fibres appeared densely aggregated. They formed a gel-like structure with only a few tiny pores remaining. This major structural change was probably due to protein denaturation and aggregation at high pressure. It could lead to the formation of a protein gel network. This compact microstructure aligned with significantly higher hardness, gumminess, and shear force values observed in this group. As a result, meat had a firmer and less juicy texture during consumption.

Overall, the SEM results provide strong microstructural evidence that supports physicochemical and textural outcomes reported in previous sections. Moderate-pressure HPP (300 MPa) in combination with marination appears to strike a balance between muscle fibre disruption and preservation. In contrast, excessive pressure (600 MPa) may cause over-aggregation. This can reduce eating quality because of gelation and densification of the muscle matrix.

pH and chemical analysis

The pH values of tuna samples were not significantly affected ($P > 0.05$) by the combined effect of marination and HPP (Table 4); these ranged from 5.5 to 5.67,

Table 4. pH values and TVB-N content of tuna samples.

Analyses	C	M	M + HPP300	M + HPP600
pH	5.67 ± 0.58	5.50 ± 0.20	5.50 ± 0.10	5.60 ± 0.10
TVB-N (mg/100 g)	14.51 ± 0.19^{b}	12.59 ± 0.70^{a}	11.28 ± 0.98^{a}	12.10 ± 0.35^{a}
Histamine (mg/kg)	31.14 ± 0.36^{d}	25.21 ± 0.66^{c}	11.89 ± 0.24^{b}	9.40 ± 0.62^{a}

Note: Different superscript alphabets in the same row indicate significant differences ($P < 0.05$) among treatment groups.

showing no notable difference between treatment groups. This finding aligned with those of Wei *et al*. (2023), who reported no significant difference in pH values between HPP-marinated clams and frozen-marinated clams. Similarly, Kim *et al*. (2018) found that although HPP increased the pH of beef samples, compared with non-HPP-treated controls, HPP-marinated with different sauce treatments had no significant effect.

All samples, including the control, maintained similar pH values. The pH remained low and stable. This was mainly due to the acidic components of marinade (vinegar, etc.), with naturally occurring lactic acid bacteria potentially contributing minor amounts of organic acids over time. These observations are relevant to RTC yellowfin tuna, where maintaining a low, stable pH immediately after treatment is essential to preserve freshness and delay microbial deterioration.

The TVB-N values of tuna steaks subjected to different treatments are presented in Table 4. TVB-N is commonly used as an indicator of protein breakdown and spoilage in fish and seafood products (Tsai *et al*., 2022). The C group showed the highest TVB-N content at 14.51 ± 0.19 mg/100 g, which was significantly higher ($P < 0.05$) than all treated groups. In contrast, the M sample, as well as M + HPP300 and M + HPP600, exhibited significantly lower TVB-N values, which were 11.28 and 12.1 mg/100 g, respectively. No significant differences were observed among these three treated groups. This suggests that marination, with or without HPP, reduced TVB-N levels. This reduction indicates improved freshness and suppression of spoilage-related biochemical reactions. It is especially important for RTC tuna products. These products must maintain freshness during distribution without thermal processing.

According to Kimura and Kuma Kura (1934), a TVB-N level of <10 mg/100 g is considered fresh. Levels between 20 mg/100 g and 30 mg/100 g indicate early spoilage. Values of >30 mg/100 g indicate spoilage (Kimura and Kuma Kura, 1934). Similarly, the rejection thresholds set by the European Commission Regulation No. 2074/2005 (now repealed) were <35 mg/100 g (Bekhit *et al*., 2021). These findings confirm that tuna samples retained acceptable freshness regardless of treatment, with the treated groups showing higher freshness quality.

The lower TVB-N values observed in the treated samples may be attributed to reduced microbial activity and enzymatic protein breakdown. These results are in line with the TVB-N findings of Tsai *et al*. (2022). Furthermore, this researcher reported that HPP treatment at ≥400 MPa significantly delayed the increase in TVB-N in tuna at 4°C and 5°C. In untreated tuna, TVB-N rapidly exceeded 25 mg/100 g within just a few days. In contrast, HPP-treated samples, especially at 500 MPa or 600 MPa, remained below the threshold throughout 5–15 days of storage. This highlights HPP's ability to maintain quality and extend shelf life by suppressing microbial growth and volatile nitrogen formation.

Moreover, previous work demonstrated that frozen samples are inclined to exhibit higher TVB-N levels than those stored in ice. This is due to protein denaturation and lipid oxidation, which can release substrates for microbial metabolism (Bita and Sharifian, 2024). Freezing may also disrupt cell membranes, making nutrients more accessible to microorganisms during frozen storage. Overall, the results suggest a synergistic preservation effect. Marination combined with HPP helps to reduce TVB-N accumulation and maintain tuna freshness.

As shown in Table 4, the control sample had the highest histamine level recorded at 31.14 mg/kg. Marination alone reduced histamine to 25.21 mg/kg. The combination of marination with HPP further suppressed histamine to 11.89 mg/kg at 300 MPa and 9.40 mg/kg at 600 MPa. These results indicate that HPP, particularly at pressures of >300 MPa, significantly suppresses histamine formation. Marination appears to enhance this effect.

All samples, including the control, exhibited low histamine levels. This could be due to the immediate effects of marination and HPP on histamine precursors or enzyme

activity. This result aligned with previous studies done by Tsai *et al*. (2022), who reported that pressure of >200 MPa could inhibit histamine formation during storage at 4°C. The observed decline in histamine content with increasing pressure further supports the role of HPP. It helps to control biogenic amine accumulation and improve tuna safety and shelf life.

Additionally, all samples, including both C and M samples, had histamine values below the safety threshold of 50 mg/kg reported by the US Food and Drug Administration (US FDA) (Tsai *et al*., 2022). The combined treated groups showed significantly lower levels. This highlights the effectiveness of HPP, especially when combined with marination, in inhibiting histamine formation.

Microbial growth

Table 5 shows TPC, YMC, coliform count, and *Enterobacteriaceae* count in all tuna samples. All tuna samples, including control and treated groups, had values below the detection limit (<10 CFU/g). This indicates minimal microbial contamination across all samples, and none exceeded acceptable safety limits.

A low microbial load is attributed to proper sample handling, immediate freezing after catching, hygienic processing, and effective pre-treatment steps. No significant differences were observed between treatment groups. However, the results are consistent with previous studies. HPP and marination are shown to inhibit microbial growth by inactivating spoilage and pathogenic microorganisms (Tsai *et al*., 2022; Uçak *et al*., 2019). This preventive effect is practically important for RTC yellowfin tuna, as maintaining low microbial levels after processing helps to ensure stable quality during storage.

According to Yi-Li *et al*. (2025), HPP disrupts microbial cell membrane integrity and inhibits enzymatic activities, thereby reducing microbial growth and proliferation. Although untreated samples already had low microbial counts, HPP and marination remain important. They help to prevent microbial proliferation during extended storage and distribution (Tsai *et al*., 2022).

Sensory test

Among the evaluated sensory attributes (Table 6), significant differences ($P < 0.05$) were observed in aroma,

Table 5. Microbial growth of tuna samples across different treatments.

Sample	Analyses	TPC (CFU/g)	YMC (CFU/g)	Coliform (CFU/g)	*Enterobacteriaceae* (CFU/g)
C	C1	<10	<10	<10	<10
	C2	<10	<10	<10	<10
M	M1	<10	<10	<10	<10
	M2	<10	<10	<10	<10
M + HPP300	A1	<10	<10	<10	<10
	A2	<10	<10	<10	<10
M + HPP600	B1	<10	<10	<10	<10
	B2	<10	<10	<10	<10

Note: C1 and C2 represent analysis 1 and analysis 2 for the control sample (C); M1 and M2 represent analysis 1 and analysis 2 for marinated samples (M); A1 and A2 represent analysis 1 and analysis 2 for samples treated with HPP300; B1 and B2 represent analysis 1 and analysis 2 for samples treated with HPP600. All analyses were carried out in triplicate.

Table 6. Sensory profiles of tuna samples.

Parameters	C	M	M + HPP300	M + HPP600
Appearance	4.89 ± 1.26	6.84 ± 0.0	7.21 ± 0.83	6.70 ± 0.14
Colour	4.89 ± 1.23	6.96 ± 0.11	7.34 ± 0.76	6.64 ± 0.11
Aroma	4.74 ± 1.07^{a}	6.92 ± 0.25^{a,b}	7.40 ± 0.65^{b}	6.62 ± 0.23^{a,b}
Texture	4.87 ± 1.34	7.22 ± 0.20	7.22 ± 0.60	5.74 ± 0.11
Taste	4.05 ± 0.72^{a}	7.36 ± 0.34^{b}	7.69 ± 0.55^{b}	6.44 ± 0.28^{b}
Overall acceptability	4.45 ± 1.06^{a}	7.40 ± 0.40^{b}	7.74 ± 0.45^{b}	6.62 ± 0.23^{a,b}

Note: Different superscript alphabets in the same row indicate significant differences ($P < 0.05$) among treatment groups.

taste, and the overall acceptability across different treatment groups. Chen *et al*. (2022) reported that laboratory instruments, such as spectrophotometers, texture analysers, and electronic noses, could detect pressure-induced changes. These include changes in appearance, texture, aroma, and flavour as well as early lipid oxidation. However, in the present study, the sensory panel did not perceive any unfavourable aroma or taste in the marinated and HPP-treated samples.

The M + HPP300 sample consistently received the highest scores. Aroma (7.4 ± 0.65), taste (7.69 ± 0.55), and the overall acceptability (7.74 ± 0.45) indicate superior sensory performance. The higher acceptability of this treatment is explained by its favourable textural properties, supported by TPA and shear force results. The M + HPP300 samples exhibited significantly lower hardness, gumminess, and chewiness, compared with other treatment groups. This indicates improved tenderness and reduced mechanical resistance. Such textural characteristics are commonly associated with improved eating quality and consumer preference.

In contrast, the untreated control (C) group received the lowest ratings for aroma (4.74 ± 1.07), taste (4.05 ± 0.72), and overall acceptability (4.45 ± 1.06), reflecting a significantly lower preference. This may be related to its comparatively higher hardness and firmer texture, as indicated by instrumental measurements.

Marination alone improved their aroma, taste, and overall acceptability. However, the improvement was less than when combined with HPP. The M + HPP600 group showed slightly lower ratings than the M + HPP300 group. This could be due to pressure-induced textural changes at higher intensities. Excessive pressure can promote protein aggregation and muscle fibre compaction. This leads to increased hardness and reduced tenderness, which can negatively affect sensory perception.

These results have practical implications for the seafood industry. RTC yellowfin tuna marinated with a formula consisting of salt, pepper, oil, vinegar, water, and sugar can undergo HPP without compromising sensory quality. This approach can enhance both shelf-life and safety while maintaining desirable flavour and aroma. It offers a practical solution for minimally processed, consumer-ready tuna products.

Conclusion

The combined application of marination and HPP has shown promising effects in enhancing the physico chemical, microbial, and sensory qualities of RTC tuna steaks. The M + HPP300 group (300 MPa) exhibited the most desirable texture attributes, with significant reduction in hardness, gumminess, chewiness, and shear force, indicating improved tenderness. Colour analysis revealed the highest total colour difference in M + HPP600. However, both HPP-treated groups demonstrated noticeable visual changes; the colour acceptance during the sensory test showed no significant difference between samples. While pH values showed no significant differences among treatments, all marinated samples and HPP effectively reduced TVB-N and histamine levels, with both M + HPP300 and M + HPP600 showing significant reductions, compared with the control. Microbial analysis indicated that all samples, including the untreated control, remained below the detectable microbial limit (<10 CFU/g), suggesting minimal contamination. Sensory evaluation identified M + HPP300 as the most preferred group in terms of aroma, taste, and overall acceptability. These results affirm the potential of marination combined with moderate-pressure HPP (300 MPa) to enhance safety, freshness, texture, and consumer acceptance of tuna products, making it a viable method for quality preservation in seafood processing.

Acknowledgements

Authors gratefully acknowledged the Department of Fisheries, Malaysia, Ministry of Agriculture and Food Security, Malaysia, for funding this project. Special thanks to Arthur Besther Sujang, Nor Azlin Mokhtar, and Muhammad Shakirin Suhaimi from the *Bahagian Sumber Perikanan Tangkapan Cawangan Pembangunan Tuna* for their assistance.

Mandatory Disclosure on Use of Artificial Intelligence

The authors used Grammarly to assist with grammar checking and language refinement in the preparation of this manuscript. No artificial intelligence tools were used for data analysis, interpretation, or generation of scientific content. The authors take full responsibility for the integrity and accuracy of the work.

Author Contributions

T.Y.-L. Original draft; Methodology; Investigation; Formal analysis; Data curation. M.R.I.-F. Supervision; Project administration; Funding acquisition; Conceptualisation. M.R.I.-F., N.H.J., N.N.J., A.R., M.A.R.N.-K., R.N., N.H., T.Y.-L. Writing – review & editing; Resources; Methodology; Validation.

Conflict of Interest

The authors declared that they had no known competing financial interests or personal relationships that could have influenced the research reported in this paper.

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