

Gelation of Pangasius (*Pangasianodon hypophthalmus*) minced muscle by high pressure processing: The effects on selected qualities

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ARTICLE INFO	ABSTRACT
Research Paper Received: March 27, 2025 Revised: May 05, 2025 Accepted: May 09, 2025 Keywords FTIR Gelation High pressure processing (HPP) Pangasius Scanning electron microscopy (SEM) *Corresponding author Truong Quang Binh Email: tqbinh@hcmuaf.edu.vn	This study evaluated the changes of <i>Pangasius hypophthalmus</i> protein under high-pressure processing (HPP), focusing on key parameters such as color, gel structure, water-holding capacity (WHC), protein solubility, and mechanical properties to determine optimal conditions for improving product quality. Pangasius muscle were minced and subjected to pressures ranging from 100 to 600 MPa, at temperatures between 0 - 30°C, and for 0 - 15 min. The processed samples were then analyzed for color, gel texture, gel strength, WHC, protein solubility, scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results showed that treatment at 400 - 450 MPa enhanced the whiteness of Pangasius gels, improved microstructural texture, and increased water-holding capacity (WHC). In contrast, processing at 500 MPa at 30°C for 12.5 min reduced structural stability, led to the formation of porous networks, and decreased protein solubility. The gel's mechanical properties were optimal at 400 - 450 MPa but reduced the hardness at 500 MPa. Overall, the conditions of 400 - 450 MPa, 20 - 30°C, and 10 - 15 min were identified as optimal for enhancing Pangasius gel quality.

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1. Introduction

Pangasius (*Pangasianodon hypophthalmus*) is a species of catfish belonging to the family Pangasiidae, primarily found in the Mekong River basin, including countries such as Laos, Vietnam, Cambodia, and Thailand. Pangasius is widely farmed in Vietnam, particularly in the Mekong Delta region, and in recent years has become the country's leading freshwater fish for export, playing a significant role in the Vietnamese aquaculture industry.

However, the value added products from Pangasius, especially Pangasius gel, still encounter various challenges in maintaining high quality, extending shelf life, and improving sensory attributes. Common issues associated with Pangasius gel products include water loss, reduced gel strength, protein structure alterations, and color changes during storage. These factors not only affect the sensory value but also reduce the market competitiveness of the products. In this context, high pressure processing (HPP) has emerged as a promising solution to enhance the quality of Pangasius gel without compromising its nutritional and sensory properties (Truong et al., 2020). The HPP is a non-thermal food preservation technique that applies high pressure (typically between 100 - 600 MPa) to alter protein structures, thereby improving gel strength, water-holding capacity, and retaining the natural color of the product.

Nevertheless, the determination of the optimal processing conditions-such as pressure level, temperature, and holding time-remains a major challenge. Previous studies have shown that excessive pressurization may lead to undesirable protein denaturation, adversely affecting gel structure and product quality. Therefore, it is essential for researchers to identify suitable HPP

conditions to improve the quality of Pangasius gel.

This study aimed to evaluate the potential of applying HPP to Pangasius gel and to investigate the effects of different HPP conditions on its physicochemical properties. Treatments were conducted at pressure levels ranging from 400 to 500 MPa, temperatures between 20 - 30°C, and holding times of 10 - 15 min, in order to determine the most effective treatment combination for producing high quality Pangasius gel using HPP.

2. Materials and Methods

2.1. Materials

The study was conducted from November 2023 to December 2024 at the laboratories of the Faculty of Fisheries and the Faculty of Food Technology, Nong Lam University (Linh Trung Ward, Thu Duc City, Ho Chi Minh city), as well as the laboratory of the Faculty of Agro-Industry, Chiang Mai University, Thailand.

Pangasius fish (*Pangasianodon hypophthalmus*) weighing between 2 - 3 kg were purchased from local farms in Chiangmai, Thailand and filleted at the laboratory of the Faculty of Agro-Industry, Chiang Mai University, Thailand.

2.2. Experimental Design

Pangasius fillets were cut into small pieces, frozen, and subsequently minced with 2% salt for 2 min. The minced fish was stuffed into collagen sausage casings with a diameter of 24 mm and vacuum-packed. Gelation was carried out using a HPP system (Baotou Kefa High Pressure Technology Co., Ltd., China) at pressure levels ranging from 400 to 500 MPa, with treatment durations of 10 - 15 min at temperatures between 20 & 30°C.

Following HPP, the *Pangasius* gels were heated at 90°C for 30 min then cooling in ice water (4°C). The quality of the final product was assessed immediately after cooling. The treatment conditions were as follows:

NT1: HPP at 400 MPa/20°C in 12.5 min

NT2: HPP at 400 MPa/25°C in 10 min

NT3: HPP at 400 MPa/25°C in 15 min

NT4: HPP at 400 MPa/30°C in 12.5 min

NT5: HPP at 450 MPa/20°C in 10 min

NT6: HPP at 450 MPa/20°C in 15 min

NT7: HPP at 450 MPa/25°C in 12.5 min

NT8: HPP at 450 MPa/30°C in 10 min

NT9: HPP at 450 MPa/30°C in 15 min

NT10: HPP at 500 MPa/20°C in 12.5 min

NT11: HPP at 500 MPa/25°C in 10 min

NT12: HPP at 500 MPa/25°C in 15 min

NT13: HPP at 500 MPa/30°C in 12.5 min

The quality of *Pangasius* gels processed using high-pressure processing (HPP) was compared to that of control products, which were produced using conventional heat treatment (90°C for 30 min).

The following parameters were evaluated: color, mechanical properties (gel strength, hardness and springiness), water holding capacity (WHC), protein solubility, Fourier-transform infrared (FTIR) spectroscopy, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and scanning electron microscopy (SEM).

2.3. Analytical Methods

2.3.1. Color measurement

The color of *Pangasius* gels were measured by a Konica Minolta chromometer (CR-400, Tokyo, Japan). Color values including L^* (lightness), a^* (red-green), and b^* (yellow-blue) were used to calculate whiteness by the following formulas:

$$\text{Whiteness} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

2.3.2. Mechanical Properties

Pangasius gel samples with the size of 2.4 cm × 3 cm were removed from casings and tempered at 25°C for 2 h prior to gel strength analysis. A texture analyzer (TA-Xt plus, Stable Micro Systems, UK) with cylindrical plunger (40 mm in diameter) and 200 N load cell was used to investigate gel strength at a speed of 2 mm/min. Gel strength was calculated as breaking force × breaking deformation. Similarly, hardness and springiness of *Pangasius* gel samples (2.4 cm × 3 cm) were measured using the same texture analyzer with cylindrical plunger (40 mm in diameter) and 200 N load cell at a compression rate of 2 mm/min and compressing samples to 50% of fish gels height.

2.3.3. Protein solubility

Protein solubility was measured following the method of Truong et al. (2020) with minor modification. *Pangasius* gel samples (4 g) were homogenized with 40 mL of a 30 g/L NaCl solution for 1 min, followed by centrifugation at 1500 × g for 15 min at 5°C. A 1 mL aliquot of the supernatant was mixed with 10 mL of 30 g/L NaCl solution, and protein concentration was determined spectrophotometrically using the Lowry method at 550 nm with bovine serum albumin as the standard for quantifying protein.

2.3.4. Water-holding capacity

Pangasius gels (about 2 g) was wrapped around by filter papers then centrifuged at $9000 \times g$ at 20°C for 20 min (Universal 320R centrifuge, Hettich, USA). The WHC was calculated as the percentage of water retained relative to the initial water content (Uresti et al., 2005).

2.3.5. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR was conducted with an FT-IR 4700 spectrometer (JASCO, Tokyo, Japan) equipped with an ATR prism crystal accessory. Pangasius gels (approximately 1 mg) was placed on the ATR crystal surface at room temperature and applying pressure with a flat tip plunger to obtain clear spectral peaks. Infrared spectra were measured within the range of 4000 to 650 cm^{-1} and all measurements were performed in triplicate. The spectral resolution was maintained at 4 cm^{-1} . To improve spectral resolution, background subtraction and second derivative analysis was applied using OriginPro 2024 software (Ojagh et al., 2011).

2.3.6. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

+ Protein extraction

Total SDS-soluble and sarcoplasmic protein fractions were extracted following the method of Pazos et al. (2015). For the total SDS-soluble proteins, 0.5 g of Pangasius gels was mixed with eight volumes of Tris buffer (10 mM Tris-HCl, pH 7.2) containing 2% SDS as a denaturing agent and 5 mM PMSF as a protease inhibitor and homogenized thoroughly. The homogenate was boiled, then homogenized again using an Ultra-Turrax device, and centrifuged at $40,000 \times g$ for 12 min at 4°C . The collected supernatant was designated as the total SDS-soluble protein fraction.

+ SDS-Polyacrylamide gel electrophoresis

SDS-PAGE was done with 1-D laboratory-prepared 10% (v/v) polyacrylamide gels with an acrylamide:N,N'-ethylene bis-acrylamide ratio of 200:1. The stacking gel (4% polyacrylamide) was loaded with $30\text{ }\mu\text{g}$ of protein for each well, and run at 60V for 30 min, followed by the separating gel at 100V for 90 min in a Mini-PROTEAN 3 cell system (Bio-Rad, Hercules, CA, Singapore) (Laemmli, 1970). The running buffer consisted of 1.44% (w/v) glycine, 0.67% Tris-base, and 0.1% SDS. The gels were stained overnight with Coomassie PhastGel Blue R-350 dye (GE Healthcare, Uppsala, Sweden).

2.3.7. Scanning Electron Microscopy (SEM)

The microstructure of Pangasius gels was observed following the method of Iwasaki et al. (2005) with minor modifications. The centre of gel samples was cut into square blocks (1 mm in thickness $\times$ 2 cm^2 in area) then fixed in 2.5% glutaraldehyde and dehydrated by ethanol with increased concentration (50%, 70%, 90%, and 100%). The dehydrated samples were immersed in 2-methyl-2-propanol to replace ethanol and freeze-dried. Samples were sputter coated in vacuum with platinum-palladium. The microstructure of Pangasius gels was observed by a Hitachi TM4000 plus SEM (Tokyo, Japan) at 10 kV.

2.3.8. Statistical Analysis

All analytical measurements were performed in four replications, and results were expressed as mean $\pm$ standard deviation. Data were analyzed by one-way ANOVA followed by LSD post-hoc tests to assess differences among treatments. Statistical significance was set at $P < 0.05$ (SPSS version 21.0, IBM, USA).

3. Results and Discussion

3.1. Color characteristics of Pangasius gel under different high-pressure processing conditions

Figure 1 shows the color characteristics of Pangasius gel under different treatment conditions. The gels subjected to high-pressure processing (HPP) exhibited whiteness values ranging from 62.72 to 64.12, which were significantly higher than those of the heat treated gel (60.52 ± 0.10) ($P < 0.05$) (data not shown). These results are consistent with the findings of Cando et al. (2015), who observed an increase in lightness in Alaska Pollock gels as pressure levels increased. HPP may induce

greater protein denaturation, contributing to a whiter appearance of the gel. Additionally, high pressure may displace or disrupt the heme and cause globin denaturation, leading to increased whiteness in the gel (de Oliveira et al., 2017). Changes in color are also associated with protein aggregation and cross linking, which result in more compact structures capable of reflecting more light (Uresti et al., 2004). Other studies also have similar results indicated that HPP induced protein aggregation enhances gel whiteness. This observation aligns with previous reports showing that pressures above 200 MPa significantly alter the color of white fish muscle (Buckow & Bull, 2013).



Figure 1. Visual color of Pangasius gel under different high pressure processing conditions.

3.2. Mechanical properties

The results of the mechanical properties of Pangasius gel are presented in Table 1. The hardness of the gel samples ranged from 6.53 N to 10.74 N. High-pressure processing (HPP) significantly increased the hardness of all Pangasius gel treatments compared to

the control group that did not undergo pressure treatment ($P < 0.05$). These results are consistent with previous studies on barramundi (Truong et al., 2017), tilapia (Hwang et al., 2007), and *Nemipterus virgatus* (Ma et al., 2015), which also reported enhanced gel hardness with HPP treatment.

Among the HPP treated samples, the treatments at 400 MPa/20°C in 12.5 min and 450 MPa/20°C in 10 min exhibited the highest hardness values (10.73 N and 10.74 N, respectively), which were significantly higher ($P < 0.05$) than those of treatments at 450 MPa/25°C in 12.5 min, 450 MPa/30°C in 10 min, 450 MPa/30°C in 15 min, 500 MPa/20°C in 12.5 min, 500 MPa/25°C in 10 min, 500 MPa/25°C in 15 min, and 500 MPa/30°C in 12.5 min.

The difference in hardness values among these HPP treatments could be attributed to the interaction between pressure and temperature

conditions. Treatments with moderate pressure and temperature likely promoted optimal protein aggregation and gel network formation, whereas excessively high pressure or temperature may disrupt gel structure, resulting in reduced gel quality and lower hardness values.

In terms of springiness, there were no statistically significant differences ($P \geq 0.05$) between HPP-treated and non-HPP-treated gels. This finding is consistent with the report by Truong et al. (2017), indicating that HPP may have limited influence on the elasticity of Pangasius gels

Table 1. Mechanical properties of Pangasius gel treated under different high pressure processing conditions

Treatment	Hardness (N)	Springiness (mm)	Gel strength (N.mm)
Control	7.21 ^a ± 0.18	0.89 ^a ± 0.01	360.62 ^a ± 18.95
400 MPa/20° in 12.5 min	10.73 ^b ± 1.27	0.86 ^a ± 0.02	514.88 ^{bc} ± 117.71
400 MPa /25° in 10 min	10.62 ^{bc} ± 1.23	0.89 ^a ± 0.08	495.08 ^{bcd} ± 106.14
400 MPa /25° in 15 min	10.29 ^{bcdh} ± 0.62	0.89 ^a ± 0.01	479.37 ^{bcd} ± 55.38
400 MPa /30° in 12.5 min	9.62 ^{cdh} ± 0.33	0.86 ^a ± 0.02	461.32 ^{bcd} ± 5.89
450 MPa /20° in 10 min	10.74 ^b ± 0.18	0.88 ^a ± 0.01	512.99 ^{bc} ± 77.98
450 MPa /20° in 15 min	10.37 ^{bch} ± 0.67	0.87 ^a ± 0.02	504.88 ^{bc} ± 24.19
450 MPa /25° in 12.5 min	9.38 ^{deh} ± 0.24	0.88 ^a ± 0.02	484.11 ^{bcd} ± 53.24
450 MPa /30° in 10 min	8.99 ^{efi} ± 0.36	0.88 ^a ± 0.02	477.47 ^{bcd} ± 35.82
450 MPa /30° in 15 min	8.51 ^{eg} ± 0.36	0.87 ^a ± 0.02	491.62 ^{bcd} ± 77.86
500 MPa /20° in 12.5 min	9.31 ^{ce} ± 1.40	0.86 ^a ± 0.02	494.66 ^{bcd} ± 107.62
500 MPa /25° in 10 min	8.61 ^{efg} ± 0.29	0.87 ^a ± 0.02	427.35 ^{ac} ± 77.76
500 MPa /25° in 15 min	8.24 ^{gi} ± 0.57	0.88 ^a ± 0.03	397.85 ^{ad} ± 19.67
500 MPa /30° in 12.5 min	8.56 ^{efg} ± 0.31	0.88 ^a ± 0.02	359.20 ^a ± 66.27

Different letters (a, b, c, d) indicate significant differences ($P < 0.05$).

The gel strength of Pangasius gels ranged from 359.20 to 514.88 N.mm. High pressure processing (HPP) significantly increased the gel strength of most treatments compared to the control (heat only) samples ($P < 0.05$), except for the treatments at 500 MPa/25°C in 10 min, 500 MPa/25°C in 15 min, and 500 MPa/30°C in

12.5 min, where no significant differences were observed ($P \geq 0.05$). These results suggest that HPP can increase the gel strength of Pangasius muscle under appropriate conditions. However, the enhancement effect of HPP was diminished in treatments with excessive pressurization, temperature, and prolonged holding time.

Among the HPP treatments, gel strength values were closely aligned with hardness trends. Specifically, the treatments at 400 MPa/20°C in 12.5 min and 450 MPa/20°C in 10 min achieved the highest gel strength (514.88 N·mm and 512.99 N·mm, respectively), which were significantly greater ($P < 0.05$) than those observed in 500 MPa/30°C in 12.5 min and 500 MPa/25°C in 15 min. This indicates that higher pressure (500 MPa) combined with elevated temperatures (25 - 30°C) may reduce the gel strength of *Pangasius* gels.

Overall, the application of high pressure improved the mechanical properties of *Pangasius* gels, resulting in higher gel hardness and strength compared to conventional heat treatment. This improvement may be attributed to the differing gelation mechanisms between pressure and heat. During thermal processing, proteins denature and unfold, forming an ordered three-dimensional protein network stabilized by hydrophobic interactions, hydrogen bonds, and disulfide linkages (Colmenero, 2002). In contrast, high-pressure gelation begins with the disruption of hydrophobic interactions in the native protein structure, promoting solubilization and unfolding (Hwang et al., 2007). Disulfide bonds are also formed under pressure, and upon decompression, heat sensitive hydrogen bonds and hydrophobic interactions are established. Consequently, HPP induced gelation is stabilized by three types of intramolecular forces: disulfide bonds, thermally sensitive hydrogen bonds, and hydrophobic interactions (Hwang et al., 2007).

Moreover, in HPP induced gels, myosin filaments are primarily linked via their head regions, as opposed to the tail to tail associations observed in heat-induced gels. This head to head interaction contributes to the specific rheological characteristics of pressure induced gels (Iwasaki et al., 2005).

When pressure exceeds optimal levels (e.g., 500 MPa) and temperatures over 25°C, gel strength declines significantly. This weakening may be attributed to two factors: first, excessive pressure may break down the gel matrix; second, the intense protein denaturation can promote non specific aggregation rather than controlled cross linking, leading to gel instability. These mechanisms likely account for the weakened gel texture observed under extreme conditions (Ma et al., 2015).

3.3. Protein solubility

The protein solubility of *Pangasius* gel is presented in Figure 2, ranging from 7.63 mg to 8.69 mg, with no significant differences observed among the treatments ($P \geq 0.05$). A decrease in protein solubility is indicative of protein denaturation and the precipitation of myofibrillar proteins, particularly myosin, following denaturation (Ramírez et al., 2000). Both high-pressure and thermal treatments induced substantial protein denaturation and precipitation, which contributed to the proper formation of the *Pangasius* gel structure.

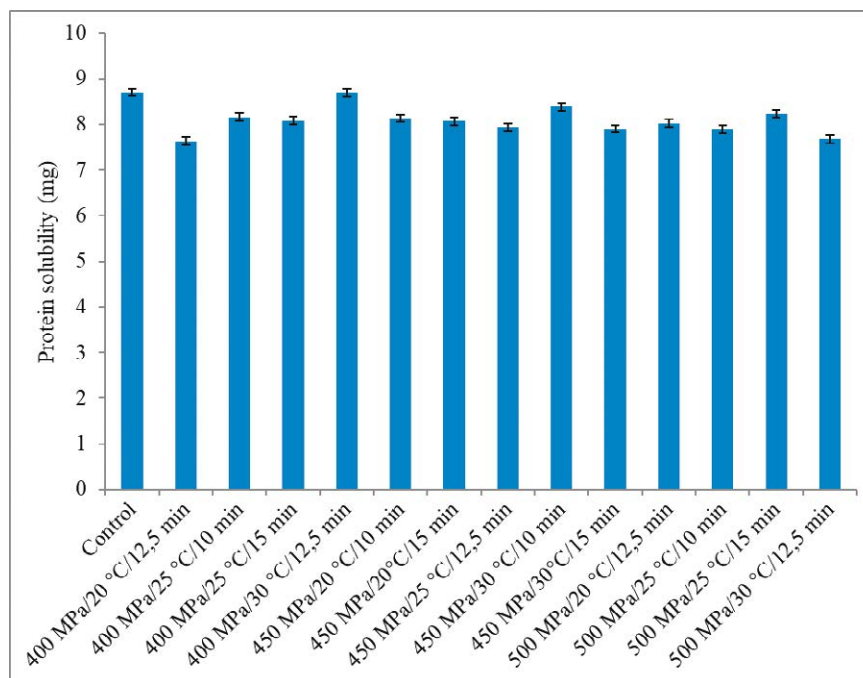


Figure 2. Protein solubility (mg) of Pangasius gel under different high pressure processing conditions. Different letters (a, b, c, d) indicate significant differences ($P < 0.05$).

3.4. Water-Holding Capacity (WHC)

The WHC of Pangasius gel is presented in Figure 3, with the highest value observed at 90.95% for the treatment at 450 MPa/20°C in 10 min, and the lowest at 75.8% in the control (heat-only) group. HPP significantly increased the WHC of all Pangasius gel treatments compared to the control ($P < 0.05$).

Treatments at 400 MPa/20°C in 12.5 min, 400 MPa/25°C in 10 min, 400 MPa/25°C in 15 min, 400 MPa/30°C in 12.5 min, 450 MPa/20°C in 10 min, and 450 MPa/20°C in 15 min showed significantly higher WHC than those at 450 MPa/30°C in 15 min, 500 MPa/20°C in 15 min, 500 MPa/25°C in 10 min, 500 MPa/25°C in 15 min, and 500 MPa/30°C in 12.5 min ($P < 0.05$).

The increase in WHC may be attributed to the ability of high pressure to promote the formation of a stable gel network, which enables the

retention of more immobilized water within the matrix and reduces water loss during cooking. Moreover, HPP enhances protein unfolding and solubility, allowing more free water to interact with protein molecules, thereby improving protein hydration (Tabilo-Munizaga & Barbosa-Cánovas, 2004).

However, when pressure exceeds 450 MPa and temperatures above 25°C, the combined effects of high pressure and elevated temperature appear to reduce WHC. This may be due to the disruption of the three-dimensional gel matrix structure. Excessive pressure can damage the protein network, resulting in a weaker gel matrix that cannot effectively retain water, allowing more free water to be released. Additionally, elevated temperature may denature proteins before the gel network is fully formed, further decreasing the gel's ability to retain moisture.

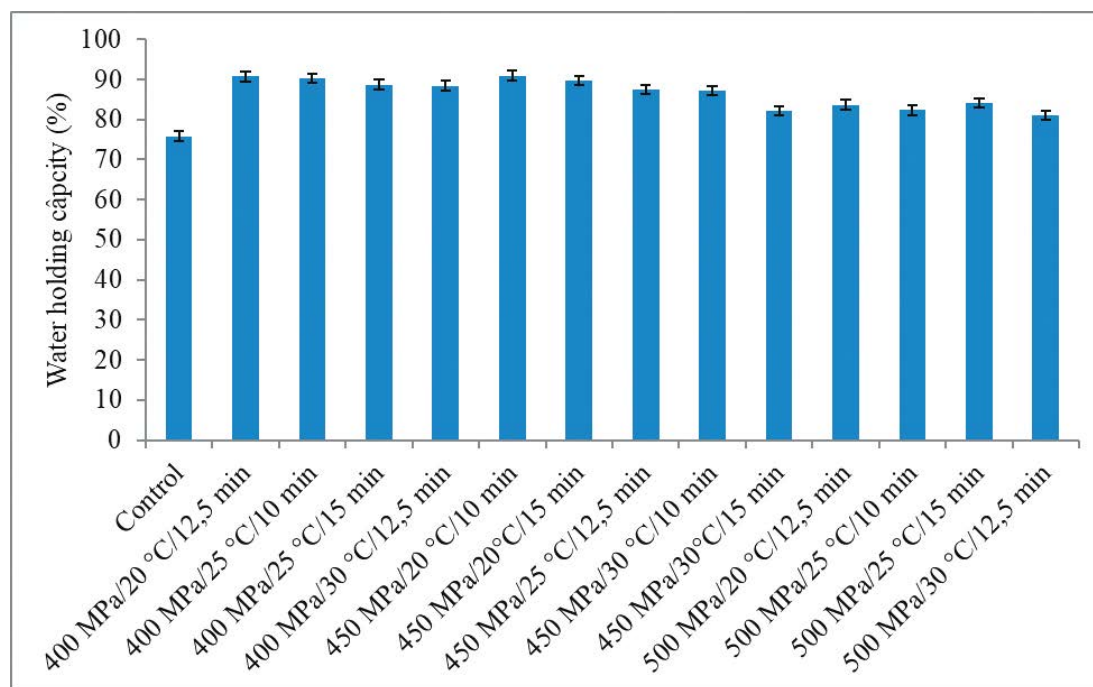


Figure 3. Water holding capacity of Pangasius gel under different high pressure processing conditions. Different letters (a, b, c, d) indicate significant differences ($P < 0.05$).

3.5. Fourier-transform infrared (FTIR) spectroscopic analysis

FTIR spectroscopy was used to obtain the vibrational spectra of each sample, providing information on the secondary structure of proteins after high-pressure treatment under different conditions. Figure 4 shows the spectra of all samples in the wavenumber range of 4000–650 cm^{-1} . In general, all spectra exhibited similar patterns across treatments, with characteristic peaks commonly observed at the following positions: 1038–1061 cm^{-1} , 1131–1146 cm^{-1} , 1207–1218 cm^{-1} , 1397–1399 cm^{-1} , 1452–1455 cm^{-1} , 1538 cm^{-1} , 1626 cm^{-1} , 1703 cm^{-1} , 2809–2836 cm^{-1} , 2901–2907 cm^{-1} , 2930–2936 cm^{-1} , and 3291 cm^{-1} . The peaks between 1038–1061 cm^{-1} are associated with C–O and C–C stretching vibrations, while the peak at 1397–1399 cm^{-1} corresponds to C–H stretching (Ye et al., 2019). The FTIR band at 1452–1455 cm^{-1} is attributed

to the bending vibration of CH_2 or CH_3 groups, originating from the side chains of proteins and/or lipids. Most notably, the amide I peak at 1626 cm^{-1} and the amide II peak at 1538 cm^{-1} are of key importance.

For myofibrillar proteins, the amide I band (1700 – 1600 cm^{-1}) is particularly significant, as it is highly sensitive to hydrogen bonding, dipole–dipole interactions, and the conformation of the polypeptide backbone (Krimm & Bandekar, 1986). Thus, the amide I band is commonly used to assess secondary structural changes in proteins. This band corresponds to the C=O stretching vibration/hydrogen bonding interactions, typically associated with carboxyl groups ($-\text{COO}^-$), while the amide II band reflects N–H bending and C–N stretching. The amide I peak near 1626 cm^{-1} is assigned to β -sheet structures, whereas the amide II peak at approximately 1538 cm^{-1} corresponds to α -helix

structures (Kobayashi et al., 2017). Additionally, the peak at $2930 - 2936 \text{ cm}^{-1}$ is related to C–H stretching, and the broad band at 3291 cm^{-1} , located between 3000 and 3500 cm^{-1} , is referred to as the amide A band, which is commonly used to assess the interaction between protein molecules and water (Ye et al., 2019).

The application of high-pressure processing caused no substantial shifts in the FTIR peak patterns compared to the control group, suggesting that no new secondary protein structures were formed under pressure. However, the variations in peak intensities indicate possible differences in the formation or disruption of specific secondary structures, which need quantitative analysis in future studies

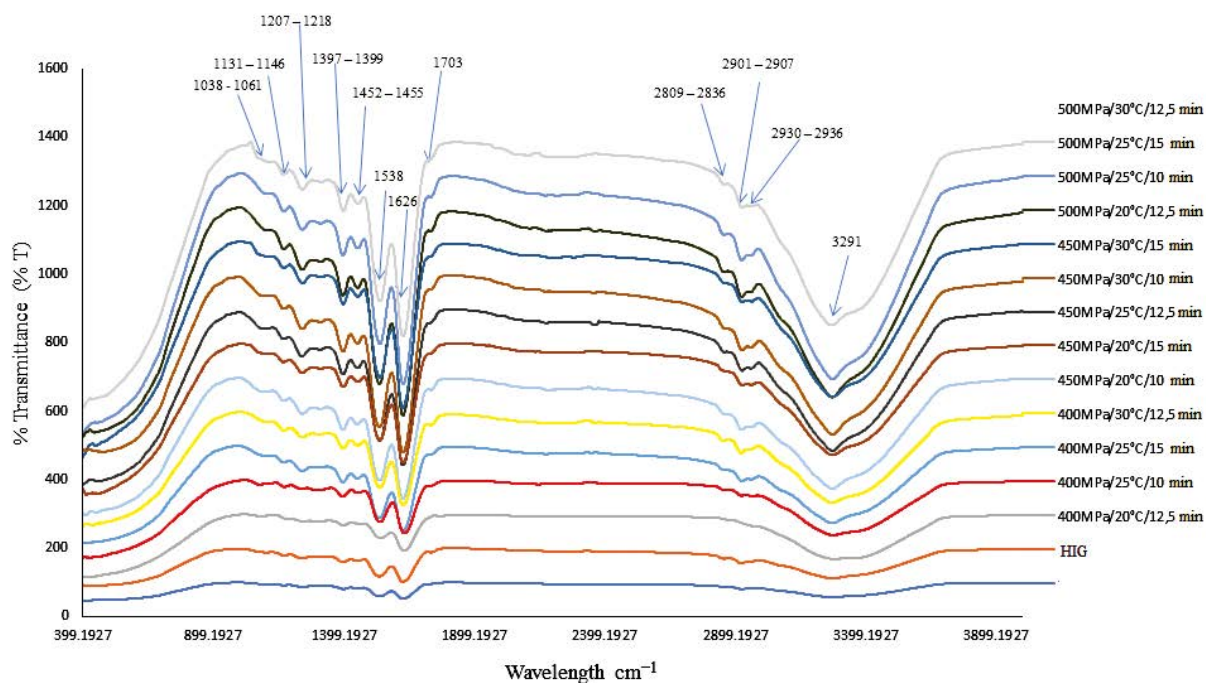


Figure 4. FTIR spectra of Pangasius gel under different high pressure processing conditions at the wavenumber range of $4000 - 650 \text{ cm}^{-1}$.

3.6. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE results of total SDS-soluble proteins extracted from Pangasius gel are shown in Figure 5. Compared to the heat-treated gel, Pangasius gels subjected to high-pressure processing (HPP) exhibited a similar protein molecular weight profile. Similarly, no major differences were observed in protein composition

among the different HPP treatments. In fish muscle, two key proteins are myosin, a high molecular weight protein ($\sim 200 - 240 \text{ kDa}$), and actin, a lower molecular weight protein ($\sim 45 \text{ kDa}$). Other bands ranging from 21.9 to 94.9 kDa are associated with various myofibrillar proteins such as tropomyosin, troponins, and sarcoplasmic proteins. Myosin is considered the principal protein responsible for gel-forming ability (Uresti et al., 2004).

Several studies have shown that high pressure often affects the intensity of the myosin band in fish gels. In particular, gels subjected to HPP followed by thermal treatment (90°C for 15 min) exhibited a stronger myosin band than those subjected to heat treatment alone, suggesting that less myosin aggregation occurred during heat-induced gelation in HPP-treated samples. Other research has indicated that surimi treated with HPP alone tends to show a gradual decrease in myosin band intensity as pressure increases, due to the formation of non-disulfide bonds under high pressure. Myosin, especially

its head region, is known to be sensitive to high pressure and may aggregate under saline conditions (Uresti et al., 2004).

These observations suggest that the mechanisms of protein unfolding and myosin aggregation differ between HPP and thermal gelation. In the present study, myosin bands in HPP-treated gels appeared at lower molecular weights compared to those in heat-treated gels, indicating that high pressure caused partial denaturation and aggregation of myosin, thereby contributing to a denser gel structure than that formed by thermal treatment alone.

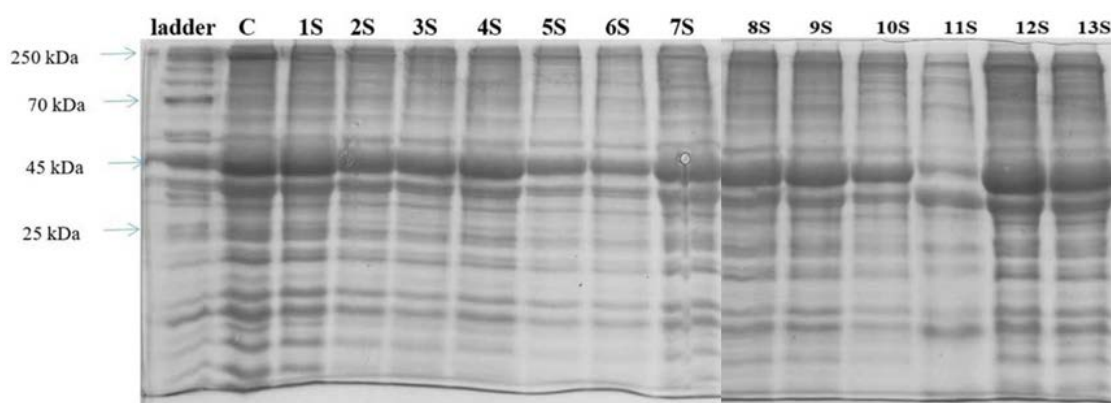


Figure 5. SDS-PAGE profile of total SDS-soluble proteins from *Pangasius* gels induced by different high pressure processing conditions. Control (C); 400 MPa/20°C in 12.5 min (1S); 400 MPa/25°C in 15 min (2S); 400 MPa/25°C in 10 min (3S); 400 MPa/30°C in 12.5 min (4S); 500 MPa/20°C in 12.5 min (5S); 500 MPa/25°C in 10 min (6S); 500 MPa/25°C in 15 min (7S); 450 MPa/20°C in 10 min (8S); 450 MPa/20°C in 15 min (9S); 450 MPa/25°C in 12.5 min (10S); 450 MPa/30°C in 10 min (11S); 450 MPa/30°C in 15 min (12S); 500 MPa/30°C in 12.5 min (13S).

3.7. Scanning electron microscopy (SEM) analysis

As shown in Figure 6, *Pangasius* gels subjected to heat treatment alone exhibited a rough, porous surface with an uneven distribution of the protein network and numerous voids. In contrast, gels treated with high pressure prior to thermal processing showed smoother surfaces and more compact, cohesive structures,

particularly in treatments with moderate HPP conditions such as 400 MPa/20°C in 12.5 min, 400 MPa/25°C in 10 min, and 450 MPa/20°C in 10 min. However, high-pressure treatments at elevated temperature and pressure combinations such as 500 MPa/25°C in 15 min and 500 MPa/30°C in 12.5 min appeared to reduce the effects of HPP, resulting in less uniform, less compact, and rougher gel structures.

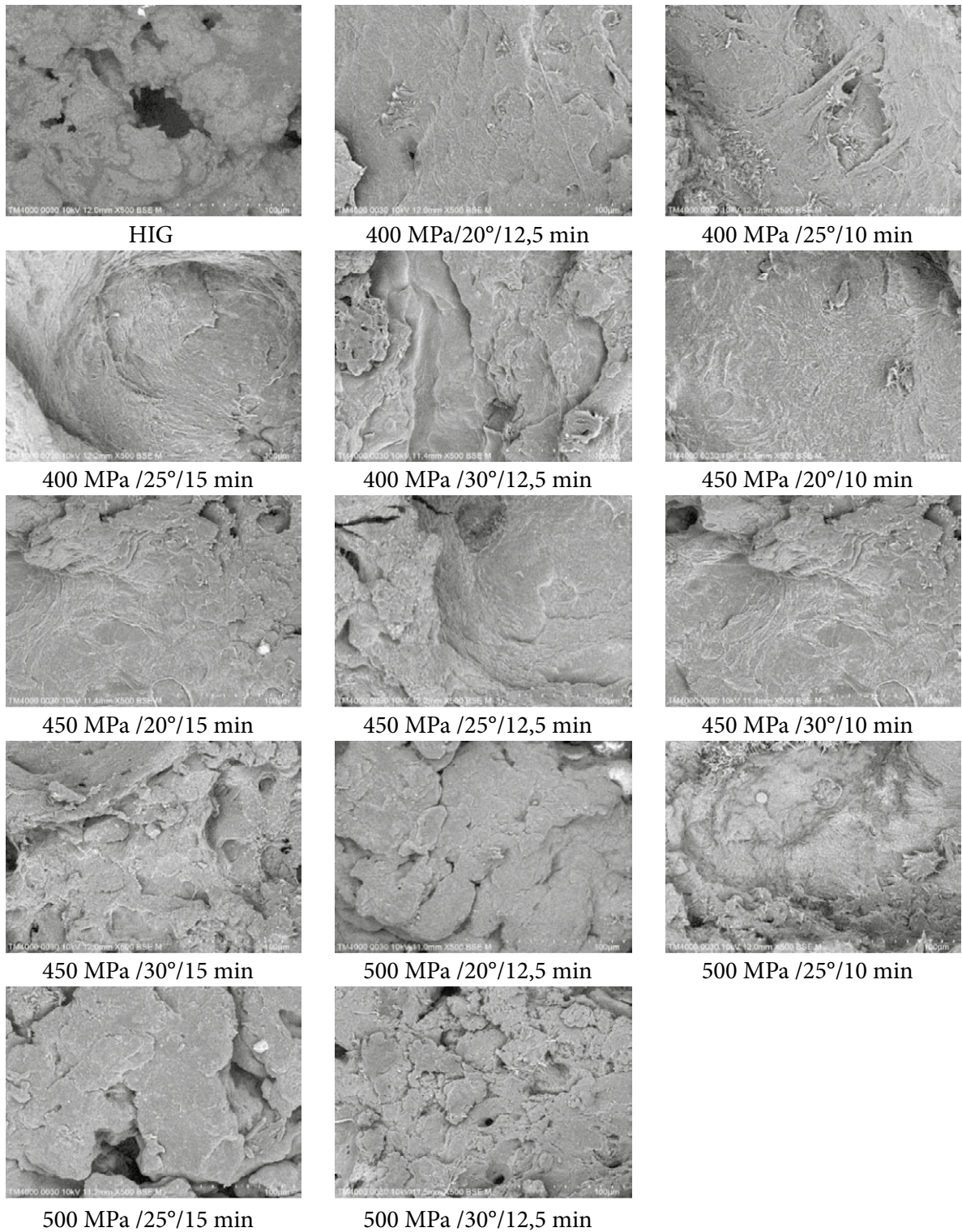


Figure 6. Scanning electron microscopy (SEM) images of Pangasius gels under different high-pressure processing conditions.

The HPP prior to heating promoted the formation of typical pressure induced gel structures, similar to those reported for other fish species such as barramundi gels (Truong et al., 2017), sardine surimi (Perez-Mateos & Montero, 1997), and threadfin bream gels (Ma et al., 2015). The mechanism of pressure-induced gel formation has been explained by greater protein denaturation and/or aggregation under the combined effects of high pressure and subsequent heat, as opposed to heat alone. Under pressure, fibrous proteins such as filamentous actin or globular proteins like sarcoplasmic actin are disrupted, leading to solubilization of myosin (Davis, 1985), actin, and other proteins (Iwasaki et al., 2006) for gel formation.

Protein structure is primarily maintained through ionic bonds, hydrogen bonding, hydrophobic interactions, disulfide bonds, and non-disulfide covalent linkages (Ma et al., 2015). Under high pressure, disruption of hydrophobic and electrostatic interactions, along with formation of hydrogen and disulfide bonds, leads to conformational changes in soluble proteins and promotes protein aggregation (Sikes et al., 2009). Ikeuchi et al. (1992) also proposed that during the heating of pressure treated proteins, more hydrophobic groups is exposed in aqueous solution, reducing solubility and increasing protein aggregation. Furthermore, protein unfolding under pressure may reorganize the protein network, resulting in increased aggregation during subsequent thermal processing (Uresti et al., 2004).

4. Conclusions

The results showed that HPP at 400 - 450 MPa improved the color of Pangasius gel, resulting in a brighter appearance. Gels treated at 400 - 450 MPa exhibited a more uniform, smoother,

and more stable structure compared to control samples. However, at 500 MPa, the gel structure was weakened, with the appearance of voids caused by excessive protein denaturation. The highest water-holding capacity was observed at 400 - 450 MPa; however, when the pressure exceeded this range, WHC decreased due to disruption of the gel network. Regarding mechanical properties, gels treated at 400 - 450 MPa showed the best gel strength, springiness, and hardness. In contrast, gels treated at 500 MPa became fragile and less stable. In conclusion, HPP at 400 - 450 MPa, combined with mild thermal conditions (20 - 30°C for 10 - 15 min), was identified as the optimal range for improving the overall quality of Pangasius gels.

Although this study provides insights into the influence of HPP on Pangasius gel quality, certain limitations remain. Future research should focus on optimizing processing parameters of HPP conditions and investigation of bond formation. In addition, comprehensive evaluations of sensory attributes, nutritional content, and storage stability are necessary to confirm the feasibility and consumer acceptance of Pangasius gels processed using high-pressure techniques

Conflict of interest

The authors declare that they have no competing interests.

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