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Effects of Fish Bone Utilization Patterns on Protein Conformation and Gelation Behavior of Anchovy (*Stolephorus commersonii*) Mince

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ABSTRACT

Anchovy (*Stolephorus commersonii*) is an important marine resource with high nutritional value. Efficient utilization of fish bones is important for improving the value of low-value anchovy resources, yet the effects of different bone utilization strategies on protein functionality and gel formation remain poorly understood. This study systematically compared anchovy mince (AM), anchovy mince supplemented with finely processed fish bone meal (AMBM), and anchovy mince produced by direct whole-fish comminution with bones (AMWB), with particular emphasis on gel properties, protein structural changes, water distribution, and product quality. AMBM exhibited markedly improved gel-forming performance, showing the highest hardness, springiness, and gel strength, together with enhanced rheological properties and water-holding capacity. These improvements were accompanied by a more homogeneous and compact protein network, greater surface hydrophobicity, and

enhanced sulfhydryl/disulfide-mediated interactions, whereas FTIR analysis indicated no significant changes in overall protein secondary structure. LF-NMR further suggested improved water immobilization within the AMBM gel matrix. In contrast, AMWB exhibited substantially impaired gel properties, characterized by coarse and discontinuous microstructures, reduced viscoelasticity, lower Ca^{2+} -ATPase activity and surface hydrophobicity, and altered sulfhydryl/disulfide profiles, indicating extensive protein structural disruption and disordered aggregation during whole-fish comminution. AMWB also showed the highest TVB-N and TBARS values, suggesting greater freshness deterioration and lipid oxidation. Overall, finely processed fish bone meal functioned as both a calcium-rich functional ingredient and a structural filler, facilitating protein interactions and the formation of a compact gel network, whereas direct whole-fish comminution with bones caused excessive structural damage.

Keywords: anchovy mince; fish bone meal; gel properties; protein conformation; disulfide bonds; water holding capacity; resource utilization

Introduction

The valorization of low-value small pelagic fish has attracted increasing attention for improving aquatic resource utilization and promoting sustainable seafood processing. Anchovies are rich in high-quality proteins and polyunsaturated fatty acids, but their small body size, fine skeletal structure, and relatively low economic value limit their utilization in high-value food products. Processing anchovies into mince or surimi-type products provides an effective approach to improving their utilization efficiency; however, their relatively weak gel-forming ability remains a major limitation. Surimi gelation involves protein unfolding, aggregation, and intermolecular association through hydrophobic interactions, disulfide bonds, hydrogen bonds, and other molecular forces, resulting in the formation of a three-dimensional protein network (Walayat et al., 2022; Xie et al., 2024). Yi et al. (2020) reported that blending anchovy (*Engraulis japonicus*) mince with silver carp (*Hypophthalmichthys molitrix*) surimi significantly improved gel strength,

water-holding capacity, and network compactness. However, this improvement relied on a relatively high proportion of high-gelling surimi, which increases raw-material requirements and may alter the characteristic properties of anchovy-derived products. Therefore, improving the intrinsic gel-forming functionality of anchovy mince is of greater significance for its high-value utilization.

Fish bones are major seafood-processing by-products and contain abundant calcium-containing minerals, proteins, and other valuable components. Their conversion from low-value by-products into functional food ingredients has received increasing attention, particularly because fish bone can provide a natural calcium source while potentially contributing to the structural properties of protein-based food systems (Pérez et al., 2024; Jimenez-Champi et al., 2024). Importantly, the functionality of fish bone in surimi depends not only on calcium content but also on particle size and its interactions with the protein matrix. Yin et al. (2014) demonstrated that fish-bone particle size significantly affected surimi gel properties, while subsequent work showed that reducing particle size could enhance the physicochemical availability and calcium release of fish bone (Yin et al., 2015). Gao et al. (2022) further demonstrated that calcium derived from nano fish bone underwent changes in distribution during myosin gelation, suggesting its participation in protein gel formation. More recently, micron-scale fish bone was shown to embed within the myofibrillar protein network and promote protein unfolding and covalent cross-linking, thereby improving gel properties. Submicron- and nanoscale tuna fish bone meal has likewise been reported to enhance surimi texture, water retention, hydrophobic interactions, disulfide cross-linking, and β -sheet formation (Wei et al., 2024; Liu et al., 2024). These findings indicate that appropriately processed fish bone can function simultaneously as a calcium-rich ingredient and a particulate gel-reinforcing phase.

Despite these advances, previous studies have predominantly focused on adding pre-processed fish bone powder to deboned mince or surimi, whereas the influence of the fish-bone incorporation strategy on protein functionality and gel formation remains unclear. This issue is particularly relevant to small fish because mechanical

separation of muscle and bone is difficult and may increase processing complexity. Direct whole-fish comminution could enable simultaneous utilization of muscle and skeletal tissues and reduce processing steps, but intensive mechanical processing may also expose myofibrillar proteins to greater shear and thermal stress, potentially affecting their subsequent gel-forming ability. Thus, whether direct whole-fish processing can provide the same functional benefits as controlled incorporation of finely processed fish bone remains unresolved.

Therefore, this study systematically compared the effects of three fish bone utilization strategies, namely AM, AMBM, and AMWB, on the physicochemical properties, gelation behavior, protein structure, and quality characteristics of anchovy mince. The results demonstrated that AMBM exhibited superior gel-forming ability, water-holding capacity, viscoelasticity, and textural properties compared with AM and AMWB. In contrast, AMWB showed weaker gel properties, impaired protein functionality, a less continuous gel network, and greater protein deterioration and lipid oxidation. These results demonstrate that the mode of fish-bone incorporation is a critical determinant of anchovy-mince gelation, providing a practical basis for the synergistic high-value utilization of small fish and their skeletal by-products.

Materials and methods

Preparation of anchovy mince and gel

Fresh anchovies were harvested from the South China Sea, transported on ice, eviscerated, headed, washed, frozen at $-20\text{ }^{\circ}\text{C}$, and thawed overnight at $4\text{ }^{\circ}\text{C}$ before use. The water-retention agent consisted of sodium pyrophosphate, sodium hexametaphosphate, and sodium tripolyphosphate at a ratio of 2:1:2. TGase was obtained from Sdic Dongsheng Biotech (Taizhou, China).

After thawing, the anchovies were washed three times with cold water (solid-to-liquid ratio, 1:2, w/v) and dewatered. AMWB was obtained by grinding whole fish using a meat grinder (TG 1001) followed by a bone pulverizer (GN-110), whereas AM was prepared by filleting and grinding the muscle at $4\text{ }^{\circ}\text{C}$. Fish bone meal was prepared by treating bones with 10% (w/v) NaHCO_3 for 20 min, followed

by rinsing, drying at 105 °C for 10 h, grinding, and sieving through a 100-mesh screen. AMBM was prepared by mixing AM with bone meal at a mass ratio of 40:1.

Each mince formulation was mixed with 1% NaCl and 0.5% sucrose and chopped at 4 °C for 2 min. Subsequently, 0.5% water-retention agent and 0.16% TGase were added sequentially, with chopping for 90 and 30 s, respectively. The resulting pastes were transferred into cubic molds (1.5 × 1.5 × 1.5 cm) and heat-set at 45 °C for 30 min, followed by 90 °C for 20 min.

Determination of nutritional composition

Crude fat was determined by Soxhlet extraction using petroleum ether, followed by solvent evaporation and gravimetric determination. Crude protein was determined by the Kjeldahl method using sulfuric acid digestion with a copper sulfate–potassium sulfate catalyst, followed by distillation and titration with standardized HCl. Mineral elements were determined by ICP-MS according to GB 5009.268–2025 (National Health Commission of the People's Republic of China & State Administration for Market Regulation, 2025). Briefly, samples were microwave-digested with nitric acid and hydrogen peroxide, diluted to volume with ultrapure water, and analyzed by external calibration with internal standard correction.

Determination of texture profile analysis and mince gel strength

The TPA and gel strength of anchovy mince were measured using a TA-XT texture analyzer (Stable Micro Systems, Godalming, UK). A P/36R cylindrical probe was used for the TPA and the testing parameters were configured as follows: a strain level of 50%; pre-test, mid-test, and post-test speeds of 2 mm/s, 1 mm/s, and 1 mm/s, respectively; a lift distance of 20.0 mm; a trigger force of 5 g; and a 5 s pause between two consecutive compression cycles.

A spherical probe (P/5S) was used for the gel strength test under the following parameters: a test speed of 1 mm/s, a trigger force of 10 g, and a compression distance of 10 mm. The gel strength was calculated as follows:

$$\text{Gel strength (g} \times \text{cm)} = \text{Distance to Rupture (cm)} \times \text{Breaking Force (g)}$$

Determination of dynamic rheological properties

The storage modulus (G') and loss modulus (G'') were measured using a

Discovery HR-30 rheometer (TA Instruments, USA). Approximately 2–3 g of mince paste was placed between parallel plates with a 1 mm gap. Temperature-sweep measurements were conducted from 25 to 95 °C at 2 °C/min. Frequency-sweep measurements were performed from 10 to 0.1 Hz at 25 °C and a strain of 0.5%.

Water holding capacity (WHC) and LF-NMR measurement of mince gels

The mince gels were cut into 5 mm slices and weighed (m_1). After being wrapped with the filter paper, the slices were centrifuged at 3000 r/min for 10 minutes and then weighed (m_2). The WHC was calculated as follows:

$$\text{WHC (\%)} = m_2 / m_1 \times 100$$

For LF-NMR analysis, gel samples ($1.5 \times 1.5 \times 1.5$ cm) were analyzed using an NMI20-060H-I analyzer (Suzhou Nuomai Analytical Instruments Co., Ltd.) with a CPMG pulse sequence. The resonance frequency was 21 MHz, with a repetition time of 8000 ms, four signal accumulations, an echo time of 0.2 ms, and 20,000 echoes.

Microstructure of mince gel and fish bone meal

The microstructures of the three mince gels and fish bone meal were observed using a scanning electron microscope (JSM-IT500, JEOL Ltd., Japan). The mince gel samples were cut into cubes measuring $5 \times 5 \times 2$ mm and fixed in 4% paraformaldehyde solution for 24 h. After fixation, the samples were washed four times with deionized water. All samples were subjected to vacuum freeze-drying to preserve their microstructural integrity. Prior to imaging, all samples were sputter-coated with a layer of gold for 30 seconds to enhance conductivity.

Determination of Ca^{2+} -ATPase activity in anchovy mince

AM, AMBM, and AMWB (7.0 g) were homogenized with 30 mL of ice-cold purified water for 30 s and centrifuged at 10,000 r/min for 10 min at 4 °C. Protein concentration was determined using the Lowry method. Ca^{2+} -ATPase activity was determined according to Li et al. (2022). The protein extract was diluted to 2 mg/mL, and 2 mL was incubated with 0.5 mL of 0.5 M Tris-HCl buffer (pH 7.5), 8 mL of 10 mM CaCl_2 , and 0.5 mL of 20 mM ATP at 25 °C for 8 min. The reaction was terminated with 5 mL of 15% (w/v) TCA, followed by centrifugation at 6000 r/min for 5 min. An aliquot (2 mL) of the supernatant was mixed with 2 mL of ammonium

molybdate–sulfuric acid reagent, 1 mL of 2% sodium sulfite, and 1 mL of 0.5% hydroquinone and diluted to 10 mL with deionized water. After 30 min at room temperature, absorbance was measured at 660 nm.

Surface hydrophobicity measurements

Surface hydrophobicity was determined using 8-anilino-1-naphthalenesulfonic acid (ANS) according to Li et al. (2023). ANS solution (20 μ L, 8 mM) was added to protein solutions at concentrations of 0.2–0.6 mg/mL and incubated in the dark for 10 min. Relative fluorescence intensity was measured at excitation and emission wavelengths of 390 and 470 nm, respectively, using a F7100 spectrophotometer (Hitachi, Tokyo, Japan). Surface hydrophobicity was expressed as the initial slope of relative fluorescence intensity versus protein concentration obtained by linear regression.

Determination of total and active sulfhydryl content of gel

Total sulfhydryl content was determined according to Ellman (1959). Protein solution (1 mL, 3 mg/mL) was mixed with 8 mL of Ellman's reaction buffer and centrifuged at 7000 r/min for 10 min at 4 ° C. The supernatant (4.5 mL) was reacted with 0.5 mL of 0.01 M DTNB for 1 min at room temperature, and absorbance was measured at 412 nm. Reactive sulfhydryl content was determined using the same procedure, except that urea was omitted from the reaction buffer. Sulfhydryl content was calculated as:

$$\text{SH (mmol/kg)} = (A_{412} \times \epsilon) / C$$

Where A_{412} is absorbance value at 412 nm, ϵ is the dilution factor and C is the concentration of protein samples.

The disulfide bond content is calculated as total sulfhydryl groups minus reactive sulfhydryl groups.

Secondary structure of anchovy protein

Freeze-dried mince samples were ground and mixed with KBr at a ratio of 1:100 (w/w), followed by compression into transparent pellets. A KBr pellet was used for background correction. FTIR spectra were recorded from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per sample. Three independent measurements were

performed for each treatment. The amide I region was subjected to second-derivative analysis and peak fitting using PeakFit v4.12 (Systat Software Inc.).

TVB-N content and TBARS of anchovy mince

TVB-N and TBARS were determined according to GB 5009.228–2016 and GB 5009.181–2016 (National Health Commission of the People's Republic of China & State Administration for Market Regulation, 2016a, 2016b). For TVB-N, 2 g of sample was extracted with 15 mL of distilled water for 30 min, followed by addition of 0.2 g MgO and distillation using an automatic Kjeldahl analyzer. Boric acid solution (30 mL) was used as the receiving solution, and the distillate was titrated with 0.005 mol/L HCl using a methyl red–bromocresol green mixed indicator. The TVB-N content was calculated as follows:

$$X_1 (\text{mg} / 100 \text{ g}) = V_1 \times c_1 \times 14 \times 100 / m_1$$

where X_1 is the TVB-N content, V_1 is the volume of HCl consumed, c_1 is the concentration of HCl, and m_1 is the sample mass.

For TBARS, 2 g of sample was extracted with 20 mL of trichloroacetic acid solution at 50 °C for 30 min. After filtration, 5 mL of the extract was mixed with 5 mL of TBA solution and heated at 90 °C for 30 min. Absorbance was measured at 532 nm against a trichloroacetic acid blank. The TBARS value was calculated using the following formula:

$$X_2 (\text{mg} / \text{kg}) = V_2 \times c_2 / m_2$$

where X_2 is the TBARS value, V_2 is the volume of the sample solution, c_2 is the MDA concentration, and m_2 is the sample mass.

Statistical analysis

All experiments were performed in triplicate. Data were analyzed using SPSS 26.0 (IBM Corp., USA), and figures were prepared using GraphPad Prism 9.0 (GraphPad Software Inc., USA). Statistical significance was defined at ($p < 0.05$).

Results and discussion

Nutritional composition of anchovy

As shown in Table 1, anchovies exhibited a high protein content, reaching 17.40

g/100 g. Sodium was the most abundant mineral, followed by calcium, potassium, and magnesium, which was consistent with the characteristic mineral profile reported for marine fish (Karşlı et al., 2025). Significant differences in calcium content were observed among the three processing treatments. Compared with the AM group, both bone-in processing methods significantly increased the calcium content of the surimi. The calcium content of the AMBM group was approximately 5400.00 mg/kg, which was close to the calcium content of raw anchovy and 3.4 times higher than that of the AM group, while the AMWB group showed a slightly lower calcium content of 4356.33 mg/kg. The higher calcium content in AMBM compared with AMWB may be attributed to the addition of finely pulverized fish bone meal, as finer bone particles may be more uniformly dispersed within the mince matrix, potentially reducing particle aggregation and facilitating more efficient recovery of bone-derived calcium during analysis. Overall, these findings suggest that fish bone incorporation can effectively enhance the calcium content of anchovy mince while promoting the valorization of fish-processing by-products, thereby offering a potential strategy for improving the nutritional value and resource efficiency of anchovy-based products.

Gel properties of minced anchovies

The top view and cross-section of the surimi gel are shown in the Figure 1. The AMBM gel exhibited a slightly more yellow appearance, which may be attributed to the intrinsic color of the fish bone meal generated during high-temperature dehydration. In addition, the AMBM gel showed a relatively smooth and uniform surface with fine particles uniformly dispersed throughout the protein matrix. The AM and AMWB matrix, however, exhibited pronounced aggregation of large particulate clusters together with relatively large cavities. The matrix appeared fractured and loosely organized, with disruption of the continuous protein network and a greater number of structural defects. Collectively, these observations indicate that the incorporation of finely ground fish bone meal promoted the formation of a more homogeneous and compact gel microstructure, whereas direct grinding of whole anchovy resulted in greater structural heterogeneity and disruption of matrix continuity.

In order to investigate the effects of three processing methods on the gel properties of anchovy mince, gel strength and TPA were determined. As shown in Figure 2, compared with AM group, AMBM group exhibited the highest value for gel strength and breaking force, reaching 196.71 g·cm and 200.30 g, respectively. While the AMWB group showed the lowest gel strength and breaking force. As shown in Table 2, the AMBM group exhibited the highest values for most texture parameters, indicating a superior gel texture compared with the other groups. Specifically, the hardness, gumminess and chewiness of AMBM reached about 2444.42 g, 874.06 g and 746.99 g, respectively, which were markedly higher than those of AM group. In contrast, AMWB group showed a significant decrease in texture parameters, especially in hardness, gumminess and chewiness. These results indicated that the addition of fish bone meal improved the gel texture of the anchovy mince (Wei et al. 2024). According to previous studies, fish bone could act as a filler embedded into the gel network, leading to a stronger and more elastic structure (Gao et al. 2022). However, the increasement of the gel strength by bone meal depends on particle size and proportion. The addition of nano-scaled fish bone at 1 g/100 g could effectively increase gel breaking force and penetration distance while micro-scaled fish bone did not (Yin et al. 2014). On the other hand, the greater shear forces experienced by AMWB during the grinding process led to damage of myofibrillar proteins, resulting in weaker texture properties. During the grinding process, AMWB was subjected to intense shear forces that likely caused severe damage to myofibrillar proteins, particularly myosin heavy chains, which play a crucial role in gel network formation, and the accompanying temperature rise further promoted protein oxidation. This dual mechanism of mechanical damage and thermal oxidation resulted in irreversible protein denaturation that severely limited the gel-forming ability of AMWB (Li et al. 2019), explaining its significantly inferior performance compared to AMBM and AM in gel strength.

Rheological properties

The rheological properties of the samples were characterized using the storage modulus (G') and loss modulus (G''), which describe the deformation and flow

behavior of a material under external stress. The temperature sweep is a fundamental rheometric procedure used to characterize the evolution of a material's viscoelastic properties (Yuan et al. 2019). As shown in Figure 3A and 3B, the G' values of the three fish mince samples were consistently higher than the G'' values, indicating that all samples exhibited predominantly elastic solid-like behavior (Liang et al. 2025). Furthermore, these values increased in the range of 40–45 °C. During this temperature interval, protein chains, particularly myofibrillar proteins, underwent unfolding, which facilitated the initial formation of a gel network through hydrogen bonding and hydrophobic interactions, thereby contributing to enhanced gel elasticity (Miller et al. 2020). When the temperature increased to 75–85 °C, the G' and G'' values of the three mince gels reached their maximum levels, followed by a rapid decline. This trend may be attributed to enhanced cross-linking interactions among protein aggregates and the incorporation of denatured proteins into the network, which strengthened the gel matrix (Xiong 2017). Furthermore, as the temperature further increased, the synergistic effects of heating and TGase activity promoted protein denaturation and exposure of reactive sites, thereby facilitating gel network formation through covalent and non-covalent interactions (Ding et al. 2024). Frequency sweep analysis is a technique used to evaluate the viscoelastic response of a material by varying oscillation frequency at a constant temperature. Fig. 3C and D showed that the G' values of the three mince gels were higher than the corresponding G'' values. Both G' and G'' increased only slightly with increasing frequency, exhibiting weak frequency dependence. Furthermore, the G' values remained higher than the G'' values throughout the entire frequency range, indicating that all samples exhibited predominantly elastic behavior (Wang et al. 2019). Regardless of whether temperature sweep or frequency sweep analysis was performed, AMBM exhibited the highest G' values, whereas AMWB showed the lowest values. A higher G' value generally indicates a stronger elastic structure and improved gel strength. This trend was consistent with the measured gel strength and TPA results, suggesting that AMBM formed the strongest and most elastic gel network. The lower gel elasticity of AMWB may be attributed to the higher shear forces generated during grinding, which likely

induced excessive denaturation of myofibrillar proteins and impaired the formation of an optimal gel structure. Based on the findings of Wang et al. and the frequency sweep results, all samples were classified as weak gels, and the weak frequency dependence suggested that the gel-like network was primarily maintained by non-covalent physical cross-links (Tang and Liu 2013; Wang et al. 2022).

Water holding capacity and water distribution

WHC was determined to evaluate the ability of the gels to retain water during processing, as WHC is a critical factor influencing the final texture and product yield of mince gels (Yin et al. 2014; Zhang et al. 2023). To minimize the influence of differences in moisture content on the determination of water-holding capacity, gel strength, and other parameters, the moisture content of all samples was adjusted and maintained within the range of 73–74% during the experiments. As shown in Figure 4A, AMBM exhibited a significantly higher WHC than AM and AMWB. The higher WHC of AMBM may be attributed to its denser and more homogeneous gel structure, which likely provided greater capacity for water retention under external forces.

Furthermore, low-field nuclear magnetic resonance (LF-NMR) was utilized to reveal the water status and distribution in mince gels by detecting water proton relaxation behavior. Water in mince gels can generally be categorized into three populations based on their T_2 relaxation times. The relaxation time T_{21} is typically interpreted as bound water, which is tightly associated with hydrophilic groups on protein molecules via hydrogen bonds. The relaxation time T_{22} component is designated as immobilized water, which is entrapped within the micropores of the mince protein gel network. Conversely, the relaxation time T_{23} represents free water, which is unbound and resides in the larger voids or cracks of the network. The free water is highly susceptible to loss under external force. The proportion of different water states in the mince gel can be calculated from the relative area of their respective peaks in the T_2 relaxation time spectrum (Ullah et al. 2019). As shown in Figure 4B and Table 3, the T_{21} peak of AMWB shifted leftward, indicating that the bound water in its gel matrix was more tightly associated with the protein matrix than that in AM and AMBM. Although the T_{21} peak of AMWB shifted leftward within the

bound-water region, its peak area was the smallest among the three groups, indicating the lowest proportion of bound water. Meanwhile, the increased T_{22} peak area in AMWB suggested a greater proportion of immobilized water, potentially reflecting the redistribution of water from the bound-water fraction to the immobilized-water fraction. This redistribution of water may be associated with protein oxidation and denaturation during processing, which can alter the availability of hydrophilic binding sites and the microenvironment of the protein matrix, thereby affecting the mobility and distribution of water (Zheng et al. 2021). Following protein unfolding, protein aggregation occurred through hydrophobic interactions, disulfide bonding, and other intermolecular interactions, causing some hydrophilic sites originally exposed on the protein surface to become buried within aggregates (Li et al. 2014). This reduced the number of available sites for hydrogen bonding with water, thereby decreasing the proportion of water detected as the bound-water component (T_{21}). Although the overall proportion of bound water decreased, the remaining bound water may have exhibited more restricted mobility due to its stronger association with the newly formed dense protein aggregates, resulting in a leftward shift of the T_{21} peak toward shorter relaxation times (Guo et al. 2023). There was no significant change in moisture content in the AMBM group compared to the AM group, indicating that the addition of fish bone meal had little effect on moisture content.

Microstructure of mince gel and fish bone meal

Scanning electron microscopy (SEM) was employed to characterize the microstructure of the mince gels as well as the morphology and particle size distribution of the fish bone materials. As shown in Figure 5A, AM did not exhibit a typical continuous gel network, instead, the anchovy muscle appeared as dense, sheet-like structures, which may be attributed to the relatively limited gel-forming ability of anchovies. In our previous work, we found that anchovy fish paste has inherent limitations in gel formation and exhibits low gel strength (Ma et al. 2026). Both AMBM and AMWB formed certain gel networks, and the structure of AMBM was the most developed, exhibiting a three-dimensional and uniform porous architecture. While AMWB exhibited a relatively shallow porous structure, with pore

characteristics intermediate between those of AM and AMBM. These pores appeared to arise primarily from protein aggregation rather than from the formation of a uniform and finely interconnected network, leading to the lower gel strength and reduced water-holding capacity in AMWB. As shown in Figure 5E, the bone particles in AMWB predominantly exhibited a short-rod morphology, with particle sizes mainly ranging from 20 to 70 μm . As shown in Figure 5D, the fish bone meal in was primarily irregular and granular in shape, with a broader size distribution of approximately 50 to 100 μm . These findings suggest that not only particle size but also particle morphology plays a critical role in determining their compatibility with the protein gel matrix. Irregular granular particles with a wider size distribution may be more effectively embedded within the protein network, whereas rod-like particles are more likely to act as structural discontinuities, thereby weakening the overall gel structure.

Ca^{2+} -ATPase activity in anchovy mince

Ca^{2+} -ATPase activity is widely recognized as a sensitive indicator of the structural integrity and functional state of myofibrillar proteins, with higher activity generally reflecting better preservation of protein conformation and quality (Jiang et al. 2025). As shown in Fig. 6A, significant differences were observed among the three groups, with AM exhibiting the highest Ca^{2+} -ATPase activity, followed by AMBM, whereas AMWB showed the lowest activity. These results suggest that the conventional processing method used for AM resulted in better preservation of the native conformation and enzymatic functionality of myofibrillar proteins. The reduced Ca^{2+} -ATPase activity observed in AMBM may be partly associated with the release of calcium ions from the added fish bone meal. Ca^{2+} can influence the structural integrity of myofibrillar proteins by altering electrostatic interactions and inducing conformational changes, thereby affecting protein stability and functionality (Tan et al. 2025). This controlled level of structural modification may even be beneficial for subsequent gel formation, as partial unfolding can expose reactive groups involved in intermolecular interactions (Zhu et al. 2024). In contrast, the substantial decrease in Ca^{2+} -ATPase activity observed in AMWB indicates extensive protein denaturation.

This can be attributed to the intense mechanical shear forces generated during whole-fish comminution, which are likely accompanied by localized temperature increases. These combined mechanical and thermal stresses can lead to irreversible conformational changes, aggregation, and loss of enzymatic activity. Such severe structural damage may have impaired the ability of myofibrillar proteins to participate in ordered gel network formation, thereby contributing to the inferior gel properties observed in AMWB.

Surface hydrophobicity of mince protein

Surface hydrophobicity reflects the extent to which hydrophobic groups, originally buried within the interior of protein molecules, become exposed on the molecular surface as a result of structural alterations. An increase in the exposure of these hydrophobic residues generally leads to higher surface hydrophobicity values (Li et al. 2023). A moderate increase in surface hydrophobicity during processing is considered beneficial, as it enhances hydrophobic interactions among protein molecules, thereby promoting protein aggregation and the formation of a stable gel network. These interactions play a critical role in stabilizing the three-dimensional structure of protein gels, particularly in myofibrillar protein systems (Xie et al. 2024). As shown in Fig. 6A, AMBM exhibited significantly higher surface hydrophobicity compared to both AM and AMWB, which is consistent with its superior gel strength. This result suggests that the incorporation of fish bone meal promoted a moderate degree of protein unfolding, thereby exposing hydrophobic groups that could facilitate intermolecular interactions. Consequently, the enhanced protein–protein association may have contributed to the formation of a more cohesive and elastic gel matrix. In addition, calcium ions released from the fish bone meal may alter the hydration layer surrounding the protein surface, thereby contributing to the increased surface hydrophobicity (Das Mahanta et al. 2017). In contrast, the relatively low surface hydrophobicity observed in AMWB may be associated with excessive mechanical processing, which may have induced severe protein denaturation and aggregation, potentially accompanied by increased Ca^{2+} release. Such rapid and extensive aggregation may cause a substantial proportion of previously exposed hydrophobic

groups to become buried again within disordered protein aggregates, thereby limiting the increase in surface hydrophobicity detectable by fluorescent probes. Additionally, the excess Ca^{2+} can shield negatively charged protein surfaces, sharply reducing electrostatic repulsion and triggering intense, uncontrolled hydrophobic aggregation. This phenomenon ultimately limits the formation of an ordered and continuous gel network, consistent with the inferior gel properties observed in AMWB. These findings are consistent with the Ca^{2+} -ATPase activity and ultrastructural analyses, collectively suggesting that the proteins in AMWB underwent extensive conformational disruption and aggregation.

Molecular forces in anchovy mince protein

Total and active sulfhydryl contents are important indicators of the gelation behavior of mince proteins, as they reflect the availability and accessibility of thiol groups involved in protein cross-linking. During gelation, heating can promote the exposure of reactive sulfhydryl groups, which may subsequently undergo oxidation to form disulfide bonds that contribute to the formation of a stable and strong gel matrix (Liu et al. 2022). Sulfhydryl groups in proteins mainly come from the amino acid cysteine. These groups can also reflect the level of protein oxidation and influence the formation of disulfide bonds (Wu et al. 2021). As shown in Fig. 6B, AM and AMBM showed higher total sulfhydryl content than AMWB. Furthermore, the AMWB group had the highest disulfide bond content. This difference may be associated with the intense mechanical processing of AMWB, which likely promoted protein denaturation and aggregation. This process could mask sulfhydryl groups within the protein structure, making them less detectable and leading to the lower measured content (Gao et al. 2019). Such extensive protein denaturation and aggregation may occur before the formation of a well-developed gel network. Under these conditions, the increased proximity of reactive thiol groups may facilitate disulfide bond formation during protein aggregation. Such cross-linking may be relatively disordered, potentially involving excessive intramolecular interactions or the formation of discontinuous protein aggregates rather than a continuous, interconnected three-dimensional network (Vischers and de Jongh 2005). As shown in Fig. 6B, the

AMBM group had the highest levels of free sulfhydryl groups and disulfide bonds. This effect may be partly related to the calcium ions provided by the fish bone meal, which can activate transglutaminase (TGase) and promote protein cross-linking through the formation of ϵ -(γ -glutamyl) lysine bonds (Benjakul and Visessanguan 2003). Ca^{2+} also can promote the SH/SS exchange reaction, thereby accelerating the cross-linking of protein particles during gelation. These interactions may promote the formation of disulfide bonds during gelation while maintaining a relatively high proportion of accessible sulfhydryl groups in the partially unfolded protein structure (Xiao et al. 2021). Furthermore, the increase in disulfide bond content suggests enhanced covalent cross-linking, which stabilizes the gel matrix. The interplay between hydrophobic interactions and disulfide bonding plays an important role in the development of strong and elastic gel networks.

Secondary structure of anchovy mince protein

The secondary structures of anchovy mince proteins subjected to different processing treatments were characterized using Fourier-transform infrared spectroscopy (FTIR). The amide I region ($1600\text{--}1700\text{ cm}^{-1}$), which is mainly associated with the C=O stretching vibration of the peptide backbone, was selected for secondary structure analysis. The spectra were further resolved into component peaks using second-derivative analysis, and the relative proportions of α -helix, β -sheet, β -turn, and random coil structures were quantified using PeakFit software. As presented in Fig. 7, no significant differences ($p > 0.05$) were observed in the secondary structure composition among the three mince samples subjected to different processing treatments. The comparable secondary structure profiles between AM and AMBM suggest that the improved gel properties of AMBM were not associated with changes in the protein backbone conformation. Instead, the incorporation of finely ground fish bone may have enhanced the gel network through physical filling and protein–particle interactions, which is consistent with the higher surface hydrophobicity and more compact microstructure observed for AMBM. Similarly, although AMWB experienced greater mechanical processing, its secondary structure remained relatively unchanged, suggesting that the processing-induced deterioration

of gel properties was more likely related to changes in protein integrity and higher-order intermolecular interactions rather than substantial disruption of the peptide backbone (Wei et al. 2018). Overall, these results indicate that the differences in gel properties among AM, AMBM, and AMWB were mainly associated with protein interactions and gel network organization rather than changes in protein secondary structure.

TVB-N content and TBARS value of anchovy mince

Total volatile basic nitrogen (TVB-N) and thiobarbituric acid reactive substances (TBARS) are widely used indicators to evaluate protein degradation and lipid oxidation in fish products, respectively. TVB-N generally reflects the accumulation of volatile nitrogenous compounds associated with protein degradation and freshness deterioration, whereas TBARS primarily reflects the extent of lipid oxidation (Piranavatharsan et al. 2023; Ramadhan et al. 2025). As shown in Fig. 8, the TVB-N values of the three samples followed the order: AMWB > AMBM > AM. Specifically, AM exhibited the lowest TVB-N value, indicating a relatively lower accumulation of volatile nitrogenous compounds and better freshness preservation under the processing conditions used. In contrast, AMWB exhibited the highest TVB-N value, suggesting greater accumulation of volatile nitrogenous compounds and more pronounced freshness deterioration, which may be associated with the intensive whole-fish comminution process. This processing involves intense mechanical shear and potential localized heating and may also increase the release or exposure of metal ions and heme-containing components from the whole-fish matrix, potentially imposing additional oxidative stress on proteins. These factors can accelerate protein denaturation and enzymatic or non-enzymatic degradation, leading to increased production of volatile nitrogenous compounds. AMBM, on the other hand, showed an intermediate TVB-N value. This suggests that, although the incorporation of fish bone meal induced some degree of processing-related structural change, protein stability was better preserved in AMBM than in AMWB. Thus, finely processed bone meal appears to cause less pronounced freshness deterioration than direct whole-fish comminution. A similar trend was observed for TBARS values (Fig. 8), which reflect

lipid oxidation levels. AMWB exhibited significantly higher TBARS values than the other two groups, indicating that lipid oxidation was most pronounced in this sample. This result may be partly associated with the release of heme-containing components during whole-fish comminution. Iron released from heme-containing components can catalyze free-radical formation, thereby promoting the peroxidation of unsaturated fatty acids and increasing TBARS accumulation (Carlsen et al. 2005). In contrast, AM and AMBM exhibited relatively low TBARS values, with no significant difference between the two groups. This suggests that the addition of finely processed fish bone meal does not markedly promote lipid oxidation under the conditions used in this study.

Conclusions

This study systematically evaluated the effects of different processing strategies on the physicochemical and functional properties of anchovy mince. AMBM significantly improved gel strength, water-holding capacity, and rheological properties while also increasing calcium content. These improvements were likely associated with the dual role of bone meal as a calcium source and structural filler, which may facilitate protein cross-linking and gel network formation. In contrast, AMWB, although beneficial for improving raw material utilization, was associated with extensive protein denaturation, likely resulting from excessive mechanical shear and thermal effects, thereby compromising gel properties and overall product quality. Overall, the results suggest that controlled incorporation of finely processed fish bone meal is a more effective strategy than direct whole-fish comminution with bones for improving the nutritional and functional properties of anchovy mince.

This study provides a scientific basis for the high-value utilization of anchovy resources and highlights the importance of optimizing bone processing strategies for industrial applications.

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draft and Funding acquisition; Hu Hou: Project administration.

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Conflicts of Interest: The authors declare that Pingjiang Jinzai Food Co., Ltd. provided anchovy raw materials, partial experimental equipment, and covered the article processing charges, but was not involved in the study design, data collection, analysis, interpretation, manuscript writing, or submission decision. All experiments were conducted at Changsha University of Science and Technology with financial support solely from the above joint foundation project. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics, Consent to Participate, and Consent to Publish declarations: not applicable.

References

- Benjakul, S., & Visessanguan, W. (2003). Transglutaminase-mediated setting in bigeye snapper surimi. *Food Research International*, 36, 253–266. [https://doi.org/10.1016/S0963-9969\(02\)00167-9](https://doi.org/10.1016/S0963-9969(02)00167-9).
- Carlsen, C. U., Møller, J. K. S., & Skibsted, L. H. (2005). Heme-iron in lipid oxidation. *Coordination Chemistry Reviews*, 249, 485–498. <https://doi.org/10.1016/j.ccr.2004.08.028>.
- Das Mahanta, D., Samanta, N., & Mitra, R. K. (2017). Decisive role of hydrophobicity on the effect of alkylammonium chlorides on protein stability: A terahertz spectroscopic finding. *The Journal of Physical Chemistry B*, 121, 7777–7785. <https://doi.org/10.1021/acs.jpcb.7b04088>.
- Ding, Y., Feng, R., Zhu, Z., Xu, J., & Xu, Y. (2024). Effects of different protein cross-linking degrees on physicochemical and subsequent thermal gelling properties of silver carp myofibrillar proteins sol subjected to freeze-thaw cycles. *Food Chemistry: X*, 22, 101448. <https://doi.org/10.1016/j.fochx.2024.101448>.

- Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82, 70–77. [https://doi.org/10.1016/0003-9861\(59\)90090-6](https://doi.org/10.1016/0003-9861(59)90090-6).
- Gao, X., Zhang, M., Xiong, S., Hu, Y., Yin, T., & Liu, R. (2022). Speciation changes and distribution of calcium in added nano fish bone during myosin gelation. *Food Science*, 43, 24–29. <https://doi.org/10.7506/spkx1002-6630-20211108-082>.
- Gao, W., et al. (2019). Effect of soluble soybean polysaccharides on freeze-denaturation and structure of myofibrillar protein of bighead carp surimi with liquid nitrogen freezing. *International Journal of Biological Macromolecules*, 135, 839–844. <https://doi.org/10.1016/j.ijbiomac.2019.05.186>.
- Guo, Z., et al. (2023). LF-NMR determination of water distribution and its relationship with protein-related properties of yak and cattle during postmortem aging. *Food Chemistry*: X, 20, 100891. <https://doi.org/10.1016/j.fochx.2023.100891>.
- Jiang, X., He, Y., Li, X., Huang, Y., Liu, Y., & Wang, F. (2025). Triple gel enhancement, antioxidant and cryoprotective effects of the enzyme-assisted extracted surimi by-product proteins on unwashed silver carp surimi. *International Journal of Biological Macromolecules*, 309, 143167. <https://doi.org/10.1016/j.ijbiomac.2025.143167>.
- Jimenez-Champi, D., Romero-Oregon, F. L., Muñoz, A. M., & Ramos-Escudero, F. (2024). The revalorization of fishery by-products: Types, bioactive compounds, and food applications. *International Journal of Food Science*, 2024, 6624083. <https://doi.org/10.1155/2024/6624083>.
- Karslı, B., Öğretmen, Ö. Y., & Emanet, M. (2025). Proximate composition, fatty acid characteristics, and amino acid profile of European anchovy (*Engraulis encrasicolus* L. 1758): Monthly and seasonal influences. *Frontiers in Nutrition*, 12, 1684958. <https://doi.org/10.3389/fnut.2025.1684958>.
- Li, J., Niu, L., Yu, J., Wang, F., Li, X., Huang, Y., & Liu, Y. (2022). Effects of frozen temperature and multiple freeze–thaw cycles on gel structure, protein and lipid oxidation and formation of advanced glycation end-products in unwashed silver carp surimi. *International Journal of Food Science & Technology*, 57, 6191–6200.

<https://doi.org/10.1111/ijfs.15987>.

Li, X., Fu, L., He, Z., et al. (2023). Effect of protein-glutaminase on calcium sulphate-induced gels of SPI with different thermal treatments. *Molecules*, 28, 1752. <https://doi.org/10.3390/molecules28041752>.

Li, F., Wang, B., Kong, B., Shi, S., & Xia, X. (2019). Decreased gelling properties of protein in mirror carp (*Cyprinus carpio*) are due to protein aggregation and structure deterioration when subjected to freeze-thaw cycles. *Food Hydrocolloids*, 97, 105223. <https://doi.org/10.1016/j.foodhyd.2019.105223>.

Li, Y., et al. (2014). Fluctuated low temperature combined with high-humidity thawing to reduce physicochemical quality deterioration of beef. *Food and Bioprocess Technology*, 7, 3370–3380. <https://doi.org/10.1007/s11947-014-1337-3>.

Li, J., Dai, Z., Chen, Z., Hao, Y., Wang, S., Mao, X., et al. (2023). Improved gelling and emulsifying properties of myofibrillar protein from frozen shrimp (*Litopenaeus vannamei*) by high-intensity ultrasound. *Food Hydrocolloids*, 135, 108188. <https://doi.org/10.1016/j.foodhyd.2022.108188>.

Liang, Y., Jiang, M., Yin, C., Xiao, N., Xia, Q., Yin, Y., & Liu, S. (2025). Coordinated improvement of frozen shrimp surimi gel quality by soybean protein isolate and transglutaminase: The role of building a bridge. *Food Research International*, 221, 117515. <https://doi.org/10.1016/j.foodres.2025.117515>.

Liu, H. H., Li, Z. W., Li, Y., et al. (2024). Preparation of tuna submicron-nano-scale fish bone meal and its effect on the quality of surimi gel. *Journal of Nuclear Agricultural Sciences*, 38, 502–511. <https://doi.org/10.11869/j.issn.1000-8551.2024.03.0502>.

Liu, Y., et al. (2022). Insight into the correlations among rheological behaviour, protein molecular structure and 3D printability during the processing of surimi from golden pompano (*Trachinotus ovatus*). *Food Chemistry*, 371, 131046. <https://doi.org/10.1016/j.foodchem.2021.131046>.

Ma, X., Chen, S., Bai, J., Yin, S., Rong, Z., Hou, H., & Kang, W. (2026). Optimization of anchovy–threadfin bream composite surimi: I-optimal mixture

design for sensory enhancement and impact assessment of three exogenous proteins. *Foods*, 15, 1417. <https://doi.org/10.3390/foods15081417>.

Miller, D. K., Acevedo, N. C., Lonergan, S. M., Sebranek, J. G., & Tarté, R. (2020). Rheological characteristics of mechanically separated chicken and chicken breast trim myofibril solutions during thermal gelation. *Food Chemistry*, 307, 125557. <https://doi.org/10.1016/j.foodchem.2019.125557>.

Pirnavatharsan, U., Jinadasa, B. K. K., & Jayasinghe, C. V. L. (2023). Validation of thiobarbituric acid reactive substances (TBARS) method for measuring secondary lipid oxidation products in fresh Indian mackerel (*Rastrelliger kanagurta*). *Food Humanities*, 1, 1194–1199. <https://doi.org/10.1016/j.foohum.2023.09.009>.

Pérez, A., Ruz, M., García, P., Jiménez, P., Valencia, P., Ramírez, C., Pinto, M., Nuñez, S. M., Park, J. W., & Almonacid, S. (2024). Nutritional properties of fish bones: Potential applications in the food industry. *Food Reviews International*, 40, 79–91. <https://doi.org/10.1080/87559129.2022.2153136>.

Ramadhan, A. H., et al. (2025). Effects of packaging and storage time on lipid and protein oxidation and modifications in texture characteristics of refrigerated grass carp (*Ctenopharyngodon idellus*) fish muscles. *International Journal of Food Science & Technology*, 60, vvaf082. <https://doi.org/10.1093/ijfood/vvaf082>.

Tan, Z., Yang, X., Yu, X., Wang, Y., Liu, Z., & Dong, X. (2025). Investigating the role of calcium ions in modulating the physicochemical and gel properties of myofibrillar protein-soybean protein isolate binary composites. *Food Research International*, 214, 116605. <https://doi.org/10.1016/j.foodres.2025.116605>.

Tang, C. H., & Liu, F. (2013). Cold, gel-like soy protein emulsions by microfluidization: Emulsion characteristics, rheological and microstructural properties, and gelling mechanism. *Food Hydrocolloids*, 30, 61–72. <https://doi.org/10.1016/j.foodhyd.2012.05.008>.

Ullah, I., Hu, Y., You, J., Yin, T., et al. (2019). Influence of okara dietary fiber with varying particle sizes on gelling properties, water state and microstructure of tofu gel. *Food Hydrocolloids*, 89, 512–522.

<https://doi.org/10.1016/j.foodhyd.2018.11.006>.

Vischers, R. W., & de Jongh, H. H. J. (2005). Disulphide bond formation in food protein aggregation and gelation. *Biotechnology Advances*, 23, 75–80.

<https://doi.org/10.1016/j.biotechadv.2004.09.005>.

Walayat, N., Xiong, H., Xiong, Z., Moreno, H. M., Nawaz, A., Niaz, N., & Randhawa, M. A. (2022). Role of cryoprotectants in surimi and factors affecting surimi gel properties: A review. *Food Reviews International*, 38, 1103–1122.

<https://doi.org/10.1080/87559129.2020.1768403>.

Wang, Y., Eastwood, B., Yang, Z., de Campo, L., Knott, R., Prosser, C., Carpenter, E., & Hemar, Y. (2019). Rheological and structural characterization of acidified skim milks and infant formulae made from cow and goat milk. *Food Hydrocolloids*, 96, 161–170. <https://doi.org/10.1016/j.foodhyd.2019.05.020>.

Wang, H., Yang, Z., Yang, H., Xue, J., Li, Y., Wang, S., Ge, L., Shen, Q., & Zhang, M. (2022). Comparative study on the rheological properties of myofibrillar proteins from different kinds of meat. *LWT*, 153, 112458.

<https://doi.org/10.1016/j.lwt.2021.112458>.

Wei, Y., Wang, C., Guo, X., Wang, Z., Deng, X., & Zhang, J. (2024). Enhancement of micron fish bone on gelling and 3D printing properties of Northern pike (*Esox lucius*) low-salt surimi. *LWT*, 208, 116709.

<https://doi.org/10.1016/j.lwt.2024.116709>.

Wei, W., et al. (2018). Analysis of protein structure changes and quality regulation of surimi during gelation based on infrared spectroscopy and microscopic imaging. *Scientific Reports*, 8, 5566. <https://doi.org/10.1038/s41598-018-23645-3>.

Wu, M., et al. (2021). Role of disulfide bonds and sulfhydryl blocked by N-ethylmaleimide on the properties of different protein-stabilized emulsions. *Foods*, 10, 3079. <https://doi.org/10.3390/foods10123079>.

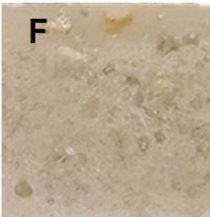
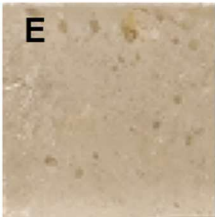
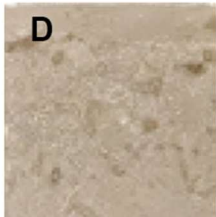
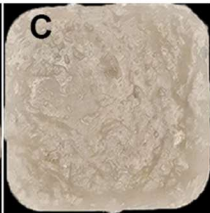
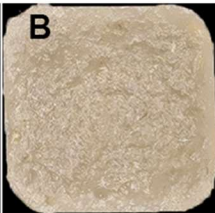
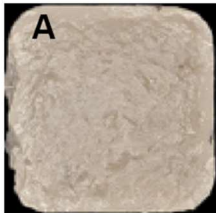
Xiao, Y., Kang, S., Liu, Y., et al. (2021). Effect and mechanism of calcium ions on the gelation properties of cellulose nanocrystals-whey protein isolate composite gels. *Food Hydrocolloids*, 111, 106401.

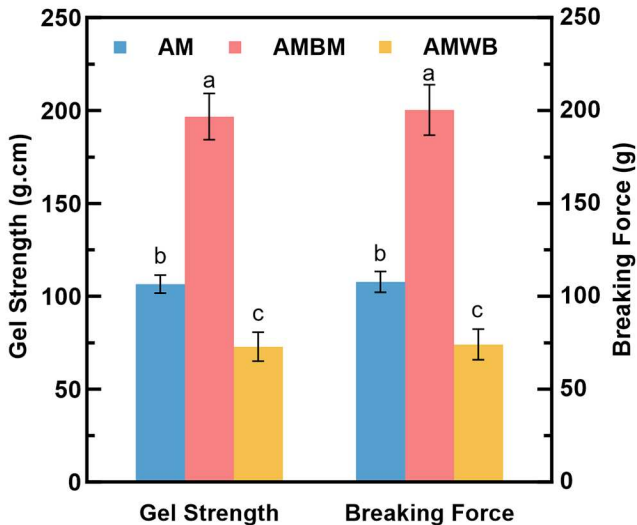
<https://doi.org/10.1016/j.foodhyd.2020.106401>.

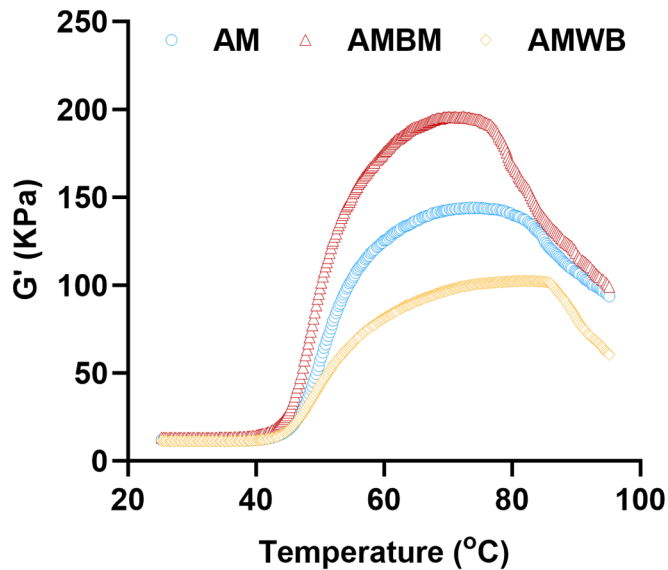
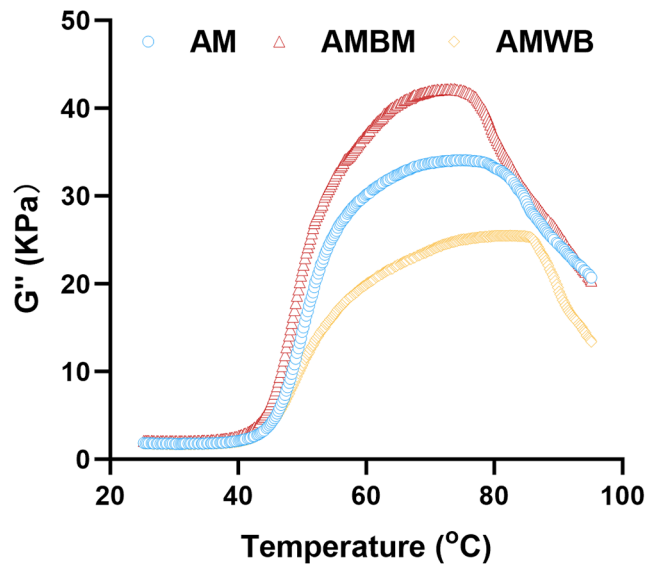
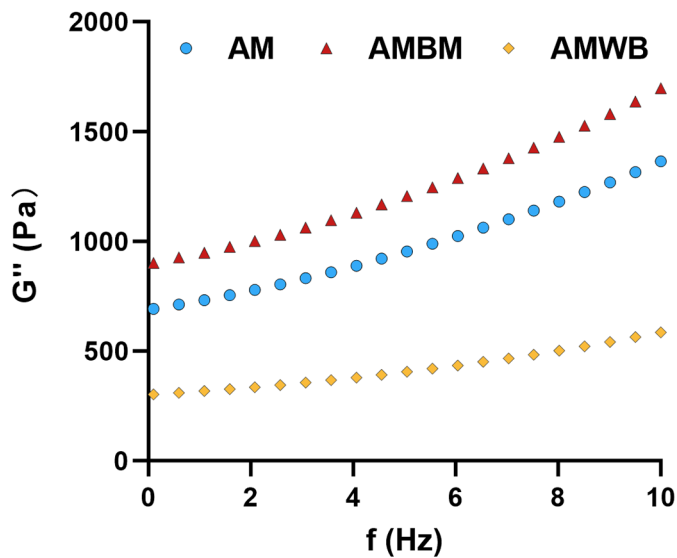
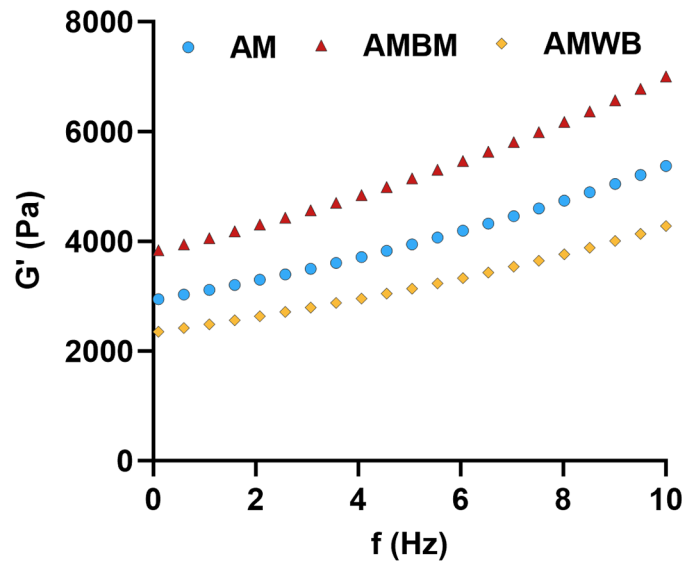
- Xie, D., Tang, Y., & Dong, G. (2024). Various factors affecting the gel properties of surimi: A review. *Journal of Texture Studies*, 55, e12847. <https://doi.org/10.1111/jtxs.12847>.
- Xie, Y., Zhao, K., Yang, F., Shu, W., Ma, J., Huang, Y., Cao, X., Liu, Q., & Yuan, Y. (2024). Modification of myofibrillar protein structural characteristics: Effect of ultrasound-assisted first-stage thermal treatment on unwashed silver carp surimi gel. *Ultrasonics Sonochemistry*, 107, 106911. <https://doi.org/10.1016/j.ultsonch.2024.106911>.
- Xiong, Y. L. (2017). Structure-function relationships of muscle proteins. In S. Damodaran & A. Paraf (Eds.), *Food proteins and their applications* (pp. 341–392). CRC Press. <https://doi.org/10.1201/9780203755617>.
- Yi, S., Ji, Y., Guo, Z., Zhu, J., Xu, Y., Li, X., & Li, J. (2020). Gel properties and flavor characteristics of blended anchovy (*Engraulis japonicus*) mince and silver carp (*Hypophthalmichthys molitrix*) surimi. *RSC Advances*, 10, 6563–6570. <https://doi.org/10.1039/C9RA10847E>.
- Yin, T., Reed, Z. H., & Park, J. W. (2014). Gelling properties of surimi as affected by the particle size of fish bone. *LWT - Food Science and Technology*, 58, 412–416. <https://doi.org/10.1016/j.lwt.2014.03.037>.
- Yin, T., Park, J. W., & Xiong, S. B. (2015). Physicochemical properties of nano fish bone prepared by wet media milling. *LWT - Food Science and Technology*, 64, 367–373. <https://doi.org/10.1016/j.lwt.2015.06.007>.
- Yuan, L., Yu, J., Mu, J., Shi, T., Sun, Q., Jin, W., & Gao, R. (2019). Effects of deacetylation of konjac glucomannan on the physico-chemical properties of surimi gels from silver carp (*Hypophthalmichthys molitrix*). *RSC Advances*, 9, 19828–19836. <https://doi.org/10.1039/C9RA03517F>.
- Zhang, X., Guo, Q., & Shi, W. (2023). Ultrasound-assisted processing: Changes in gel properties, water-holding capacity, and protein aggregation of low-salt *Hypophthalmichthys molitrix* surimi by soy protein isolate. *Ultrasonics Sonochemistry*, 92, 106258. <https://doi.org/10.1016/j.ultsonch.2022.106258>.
- Zheng, Y., Zhou, F., Zhang, L., Wang, H., & Wang, X. (2021). Effect of different

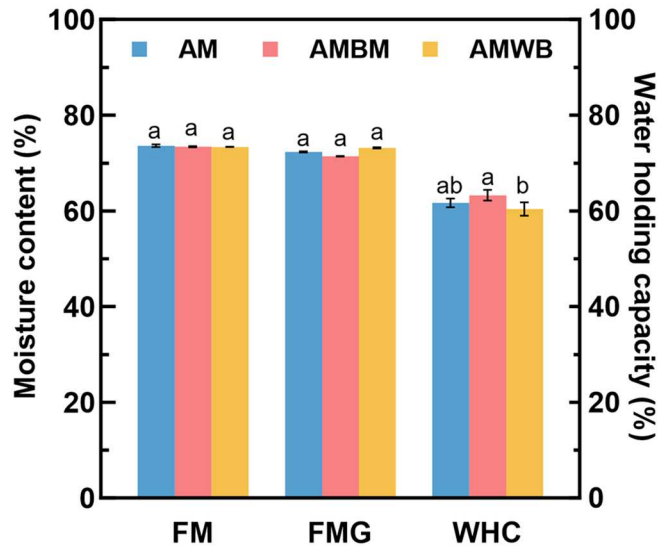
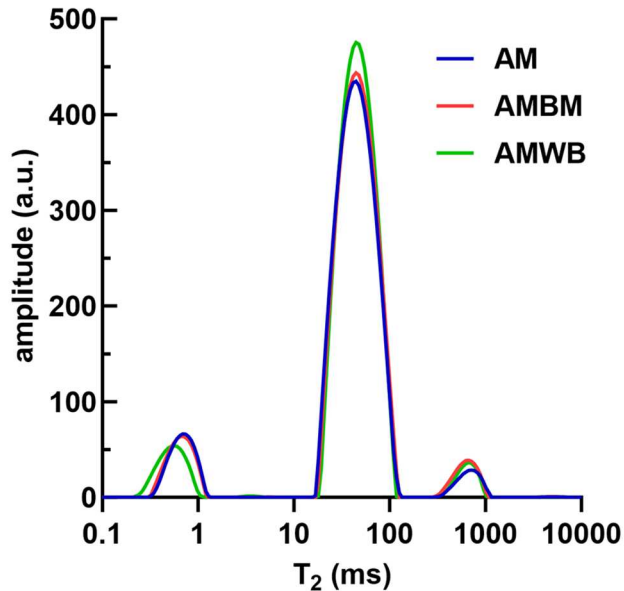
744 extent of protein oxidation on the frozen storage stability of muscle protein in
745 obscure pufferfish (*Takifugu obscurus*). *LWT*, 137, 110416.
746 <https://doi.org/10.1016/j.lwt.2020.110416>.

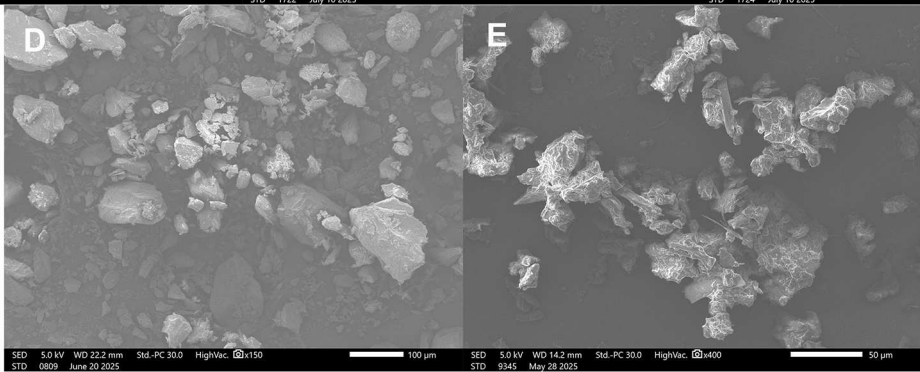
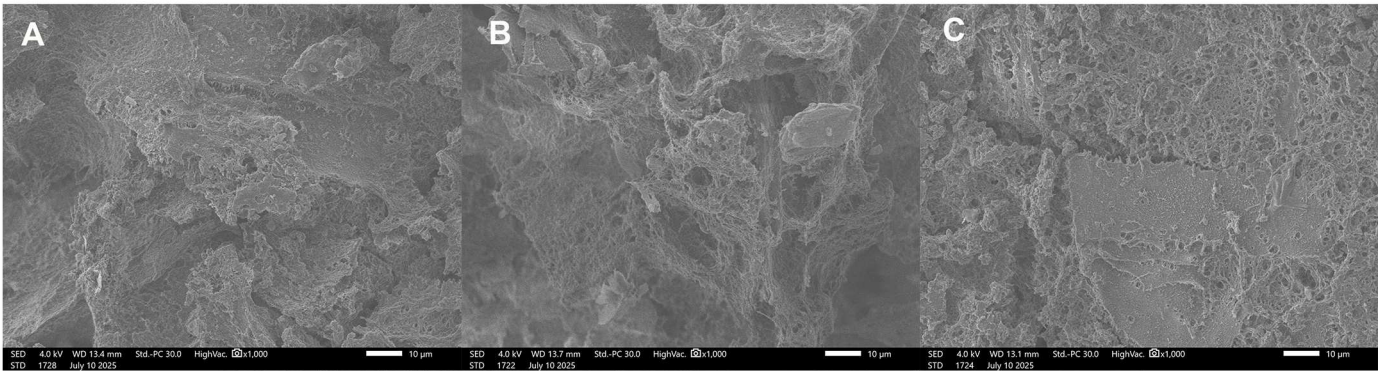
747 Zhu, H., Zhang, Q., Ding, Y., Liu, S., Zhu, S., & Zhou, X. (2024). Myofibrillar protein
748 oxidation associated with surimi processing and emerging control techniques: A
749 review. *Trends in Food Science & Technology*, 149, 104560.
750 <https://doi.org/10.1016/j.tifs.2024.104560>.

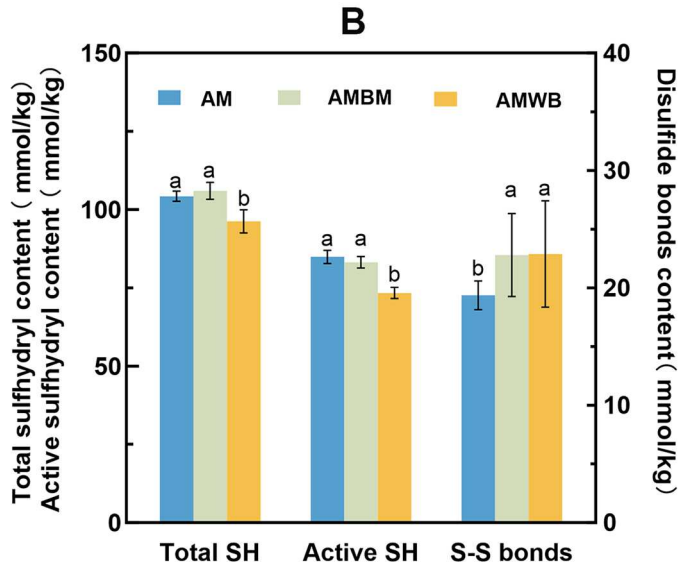
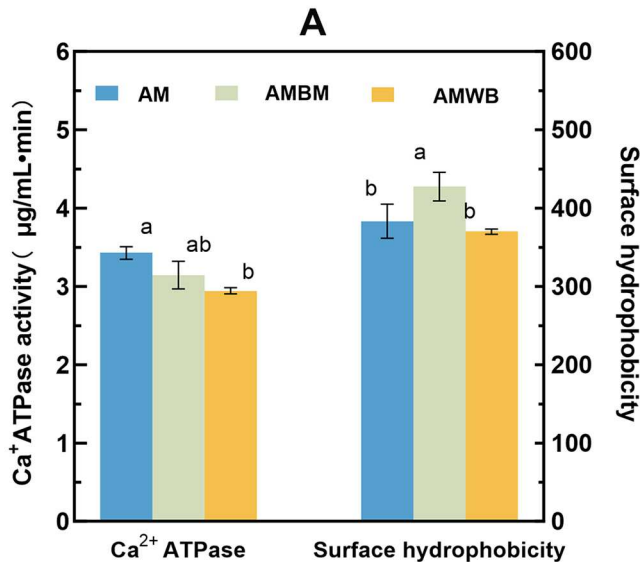


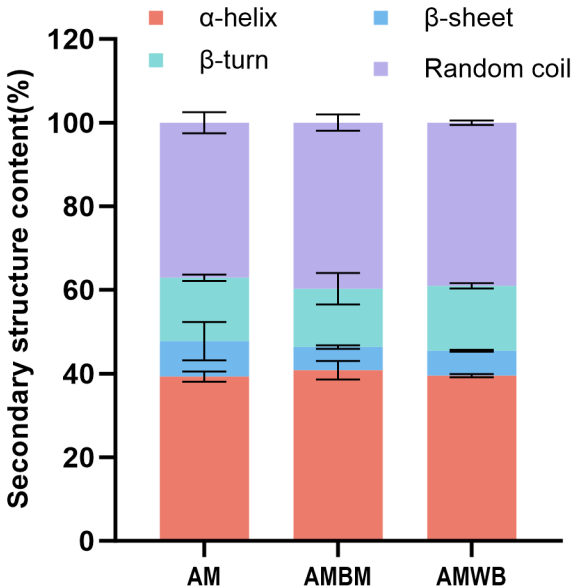


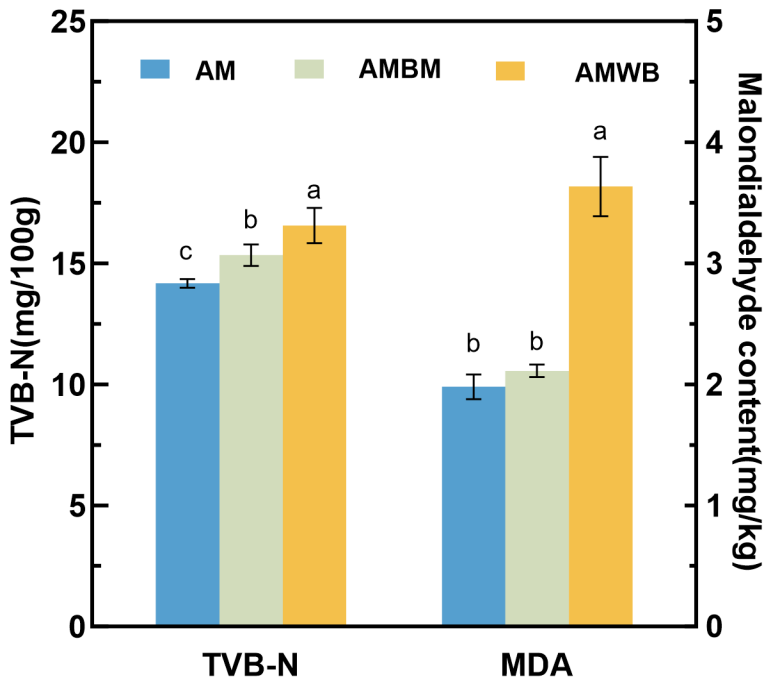
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