



Species-dependent gelation mechanisms in traditional fish-based pempek: A multiscale FTIR–SEM–EDS study

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ABSTRACT

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Food systems in pempek have a unique fish-based gel whose properties represent cultural and technological versatility. This research aimed to understand how the multiscale structure and composition of pempek, a traditional Indonesian fish (*Channa striata*)-based gel, are influenced by fish species. It elucidated the hierarchical relationships linking ionic balance, protein secondary structure, and microstructural architecture that determine gel texture and stability using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray Spectroscopy (EDS). The multiscale structure of pempek involved spectral deconvolution of amide bands to quantify protein conformations, morphometric analysis of gel microstructure, and elemental mapping to evaluate ionic composition. *Channa striata* (snakehead) pempek exhibited β -sheet (44.1%) enrichment, higher Amide I/II ratios (1.28 ± 0.05), lower CH₂ ratios (1.32 ± 0.09), and a compact microstructure with thicker walls and lower porosity. In contrast, *Scomberomorus commerson* (mackerel) pempek displayed higher α -helix content (32.7%), increased lipid mobility (1.49 ± 0.08), and more open, porous networks. The Na/Cl atomic ratio was higher in snakehead samples, suggesting enhanced electrostatic stabilization and network cohesion. Correlation analysis confirmed a strong interdependence among ionic, molecular, and structural parameters, establishing a hierarchical mechanism from ionic milieu to gel microarchitecture. This study provides new mechanistic insights into species-specific gel formation and validates the FTIR–SEM–EDS framework as a powerful tool for understanding protein gelation, product authentication, and structural design. The research advances food material science by offering a reproducible, multiscale analytical model for optimizing texture, stability, and authenticity in traditional and modern protein-based food systems.

Contribution/Originality: The paper's contribution to cultural and technology could be represented by authenticity in traditional and modern protein-based food systems in fish species, pempek, species-specific gel formation, and validation using FTIR–SEM–EDS framework for understanding protein gelation, product authentication, and structural design.

1. INTRODUCTION

One of the most complex and valuable classes of food systems is fish-based gels, which have cultural value as well as technological flexibility. Among them, traditional foods like pempek or surimi food are unique in the worldwide culinary scene, especially in Southeast Asia. Myofibrillar proteins, which make up 70–80% of the total protein content of fish, are the major component of these gels. They have a significant impact on texture, elasticity, and sensory qualities (Liu et al., 2025; Xie et al., 2024). Because differences in protein composition, amino acid distribution, and lipid content between fish species have a significant impact on gelation behavior and network creation, there has been an increase in food technology industrial interest in recent years in optimizing fish-based gel properties in order to improve product quality and assure species authenticity (Sihono et al., 2024). Understanding these molecular and structural processes is critical for preserving the cultural integrity of traditional food systems in industrial processing worlds, as well as for enhancing product quality. Because pempek, a classic Indonesian fish gel, allows for the study of the interaction between species-specific composition and structural organization, it is a perfect model within this field. The two fish species most frequently used in pempek manufacturing are mackerel (*Scomberomorus commerson*) and snakehead (*Channa striata*). However, replacing one of these species with another frequently results in noticeable variations in the gel's texture, flavor, and palatability. This variation is strongly correlated with intrinsic differences in lipid distribution during gelation and myofibrillar protein conformations. The mechanical strength of the gel network is determined by the thermal denaturation and aggregation of these proteins, as well as by hydrophobic and electrostatic interactions (Singh et al., 2023). Additionally, the extent of network compression and stability can be impacted by the modulation of protein structure and hydration by mineral ions and natural additives found in fish muscle (Buamard, Singh, & Benjakul, 2024; Yang et al., 2025). Despite the significance of these processes, there are few organized comparative studies that use cutting-edge analytical methodologies to identify molecular and microstructural variances between the various fish species used in pempek.

The main research challenge in this area is developing a thorough, non-destructive analytical approach that can describe the molecular and microstructural features of complicated food matrices. Although helpful, conventional rheological, textural, or sensory evaluations just provide macroscopic data and do not show molecular-level conformational changes or microstructural distribution patterns. Therefore, Fourier Transform Infrared (FTIR) spectroscopy has gained popularity as a molecular probe that can detect changes in secondary structure, particularly in the amide I and II regions associated with gelation and denaturation events (Jiang et al., 2024; Sharma, Majumdar, & Mehta, 2023). In contrast, Scanning Electron Microscopy (SEM) offers visual proof of the gel network morphology, how molecular interactions affect structural compactness and porosity (Geng et al., 2025; Li et al., 2025).

Utilizing SEM alongside Energy Dispersive X-ray Spectroscopy, a strong method is available to analyze the fundamental components, focusing on elements with lower atomic numbers like sodium and chlorine. These elements are significant due to their involvement in maintaining electrostatic balance and forming connections inside protein structures of protein gelation (Jendrusch et al., 2025; Zhang et al., 2025). By incorporating these methods of analysis, scientists are able to connect the relationship between how molecules vibrate, what elements they consist of, and how their structures are arranged.

The lack of investigation into the interactions between protein structure, ionic environment, and microarchitecture is a major barrier to comprehending fish-based gels. Although some researchers have concentrated on the spectroscopic or morphological aspects of fish gels separately, few have taken a comprehensive strategy that integrates these data sets. Creating such a link is essential since it facilitates the mapping of hierarchical relationships that determine the entire gel structure and function. The impact of ionic components on protein folding and aggregation, which in turn affects textural qualities, can be seen using a single analytical approach. This method develops from simply explaining how protein gelation works to grasping the underlying mechanisms and making predictions for various materials and manufacturing conditions (Jiang et al., 2024; Sharma et al., 2023).

The significance of protein secondary structures in determining gel strength and water-holding capacity has been emphasized in prior research. A high proportion of α -helices increases hydrogen bonding and improves alignment, which in turn enhances elasticity and moisture retention (Chen et al., 2025). On the other hand, higher β -sheet architectures have been associated with rigidity and less flexibility, which are traits typically associated with harder but more fragile gels (Cao, Wang, Li, & Ren, 2025). The conformation of these structural motifs changes with heating and ionic conditions, changing the network's connectivity and stability (Lin et al., 2025). In addition, the ionic environment is crucial for controlling electrostatic interactions between charged residues, which affects protein solubility and aggregation kinetics (Zuazo-Gaztelu et al., 2025). As a result, the physical appearance of gelation and its sensory characteristics are determined by the combination of protein secondary structure and ionic equilibrium.

FTIR spectroscopy presents a dependable approach for assessing alterations in these structural arrangements, achieved through the analysis of shifts in unique bands associated with specific structural motifs (Du et al., 2024; Xiao et al., 2024). The amide I band ($1600\text{--}1700\text{ cm}^{-1}$) is very sensitive to the relative ratios of α -helix and β -sheet structures, while the amide II band ($1540\text{--}1560\text{ cm}^{-1}$) reflects N–H bending vibrations that aid in the interpretation of secondary structure. Data on lipid chain mobility and protein-lipid interactions are provided by variations in the CH_2 asymmetric and symmetric stretching bands at $2920/2850\text{ cm}^{-1}$. These molecular data are complemented by SEM observations, which enable us to see the physical organization of protein aggregates and make correlations between molecular conformation and observable network characteristics like pore size, wall thickness, and porosity (Jahangir et al., 2025; Kim, Kim, Seong, Kang, & Moon, 2025). Through the precise identification of spatial configurations within the elemental composition, focusing on sodium, chloride, calcium, and phosphorus, that affect the distribution of charge and equilibrium of hydration, the incorporation of EDS enriches this comprehension (Huang et al., 2024; Pandeya, Sah, Li, & Joshi, 2025). These three modalities work together to create an integrative analytical platform that can shed light on the hierarchical structure of fish-protein gels.

Even though many research papers have been written about gel systems made from fish, we still need more comparative research. These studies should use analytical techniques to investigate how pempek differs based on the species of fish used. Previous studies have mostly given attention to factors in processing such as temperature, amount of sodium chloride, and introduction of extra ingredients, without recognizing the inherent dissimilarities in biological chemistry from one type of fish to another (İşitan, 2025; Khan & Saleem, 2024). Consequently, the interconnections between molecular structure, ionic stabilization, and microarchitecture are still not well understood. The possibility of creating trustworthy spectral and morphological indicators for quality evaluation and species identification is hampered by the dearth of these integrated datasets. Furthermore, the combined use of FTIR and SEM with EDS in the context of conventional fish-based meals has not been well studied, even if each has been demonstrated to be helpful in assessing gel structure. Resolving these methodological problems could significantly improve both scientific knowledge and industry standards.

This research identifies the microcavity using a comprehensive analytical approach, combining FTIR, SEM, and EDS techniques. This integrated framework enables examination of pempek's structural and compositional characteristics at multiple scales, with pempek prepared from mackerel and snakehead fish. The study aims to develop a hierarchical model illustrating how protein secondary structures and ionic environments collectively influence gel microarchitecture by linking molecular vibrational features with microstructural characteristics and ionic distributions. It introduces a novel approach by employing a three-pronged method that integrates spectroscopic, microscopic, and elemental data to demonstrate a consistent correlation between structure and composition. Besides enhancing understanding of traditional fish-based gels, this strategy provides a transferable foundation for food authentication and structural optimization in modern food technology processing. Ultimately, the study contributes to establishing a scientifically sound basis for improving the quality, consistency, and authenticity of traditional food processes, especially fostering innovation in the field of food material science.

2. MATERIALS AND METHODS

This research utilized a comprehensive analytical strategy incorporating several techniques, including Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray Spectroscopy (EDS), to thoroughly examine how proteins form gels in pempek products. FTIR spectroscopy clarified protein secondary structures and lipid-related spectral indicators, while SEM imaging facilitated precise measurement of the gel networks' microstructure and design.

2.1. Sample Collection and Preparation

Pempek products available were thoroughly examined from various markets in Palembang, Indonesia, which is known for its rich culinary heritage from the era of the Sriwijaya Kingdom, to collect representative samples that describe different fish species. A total of forty-eight products were evaluated based on the specified fish species, ingredient lists, and production uniformity. Of these, eight samples were chosen for further analysis, four derived from snakehead fish and four from mackerel, after confirming their quality and conducting sensory assessments to guarantee their authenticity and formulation similarity. The two selected fish species were preferred because of their prevalent use in pempek recipes and their unique biochemical properties: *C. striata*, a freshwater species noted for its considerable myofibrillar protein content combined with a low-fat percentage; and *S. commerson*, a saltwater species that is abundant in polyunsaturated fatty acids.

Each sample was stored at -18°C until more tests could be done. To lower the chance of microbes or chemical changes, tests were done within two days after getting the samples, following the safety and cleanliness rules from food analysis standards (Dey et al., 2023; Widiasih et al., 2024). The number of samples used met the standards recommended for food composition studies, ensuring good statistical reliability without too much repetition (Anderson & Kelley, 2024).

2.2. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR spectroscopy was utilized to examine the characteristics of molecular vibrations and the secondary structures of proteins. The recordings were made with a Nicolet iS50 FTIR spectrometer from Thermo Fisher Scientific in the USA, which had an ATR diamond crystal. Spectra were gathered over the wavelength range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} , taking 50 scans for each sample. Each spectrum underwent baseline correction, normalization, and smoothing through Savitzky–Golay filtering to reduce noise and improve the clarity of signals (Ahlan & Hutami, 2025; Seredin et al., 2025).

Particular focus was placed on the amide I region ($1600\text{--}1700\text{ cm}^{-1}$), which primarily indicates C=O stretching vibrations that are sensitive to the secondary structures of proteins (Mancini et al., 2023). To separate overlapping spectral components linked to α -helix ($1648\text{--}1658\text{ cm}^{-1}$), β -sheet ($1615\text{--}1637\text{ cm}^{-1}$ and $1680\text{--}1695\text{ cm}^{-1}$), β -turn ($1660\text{--}1670\text{ cm}^{-1}$), and random coil ($1640\text{--}1648\text{ cm}^{-1}$) structures, deconvolution was performed using Gaussian–Lorentzian curve fitting. The area for each determined component was measured and represented as a proportion of the overall amide I absorbance, which helped in identifying the structural composition. Additionally, ratios of amide I to amide II ($1550\text{--}1570\text{ cm}^{-1}$) and CH_2 asymmetric/symmetric stretching bands ($2920/2850\text{ cm}^{-1}$) were calculated to assess protein conformation and lipid chain dynamics, respectively. These spectral indicators are well-known markers of gel structure and stability (Bujňáková et al., 2023; Jiang, Natarajan, & Margulies, 2024).

2.3. Scanning Electron Microscopy (SEM) Imaging and Morphometric Analysis

Microstructural analysis was performed using a JSM-IT700HR scanning electron microscope set to an accelerating voltage of $5\text{--}10\text{ kV}$. Fresh samples from each brand (PPB, PPPR, PPS, PPSM for Snakehead; PPCD, PPEL, PPNN, PPVC for Mackerel) were diced into small cubes (around $4 \times 4 \times 3\text{ mm}$) and immediately frozen at -20°C to prevent structural damage. The frozen samples were then freeze-dried at -50°C for 48 hours to thoroughly

eliminate moisture and avoid shrinkage during vacuum exposure (± 3.2 Pa). After the freeze-drying process, the specimens were affixed to aluminum stubs with double-sided conductive carbon tape, ensuring that the natural cross-section was oriented upward. To improve surface conductivity and minimize electron beam charging, all samples were coated with a gold-palladium (Au/Pd) alloy (approximately 10 nm thick) using a Quorum SC7620 sputter coater in an argon environment (20 mA for 90 seconds).

SEM images were obtained at magnifications between 1000 $\times$ and 5000 $\times$ to examine the internal structure of the gel network. The morphometric study concentrated on factors such as porosity, distribution of pore sizes, thickness of the walls, and consistency in surface texture (Lombardi, Gala, Esposito, & Tammaro, 2025; Peng et al., 2025). ImageJ software was utilized for quantitative morphometry. Porosity was calculated as the fraction of pore area compared to the entire image area, while measurements for pore diameter and wall thickness were derived from binary images through threshold segmentation and morphological filtering. Texture characteristics were evaluated using the gray-level co-occurrence matrix (GLCM), which assesses the uniformity and contrast of pixel intensity, reflecting the compactness and roughness of the gel network (Ciurzyńska, Janowicz, Karwacka, & Galus, 2024; Makame, Nolden, & Emmambux, 2023; Nemati & Takhar, 2025).

2.4. Energy Dispersive X-ray Spectroscopy (EDS) Mapping

Elemental composition was examined using EDS, integrated with the same SEM system, to assess the spatial arrangements of light elements (C, O, Na, Cl). A constant accelerating voltage of 10 kV was used, with a live-time recording of 120 seconds for each point. For quantitative assessment, ZAF correction was applied to consider atomic number, absorption, and fluorescence influences. The data analysis was conducted with Oxford INCA software for spectral separation and semi-quantitative evaluation of both elemental mass and atomic percentages.

EDS mapping was utilized to illustrate the distribution of elements within different microstructural areas, identifying sodium and chlorine as markers for ionic stabilization. The ratio of sodium to chlorine atoms was determined to serve as an index for electrostatic stability, based on earlier research establishing a connection between ionic balance and the integrity of gel structure as well as its ability to retain water (Morino & Kanada, 2023; Yoo, Joe, & Ko, 2025). Figure 6A and 6B showed representative EDS spectra and elemental maps for snakehead and mackerel pempek, indicating slight but consistent variations in ionic composition. These findings enhance the understanding of molecular and morphological aspects, allowing for a multi-dimensional analysis of structural phenomena.

2.5. Data Processing and Statistical Analysis

This research adopted a comprehensive analytical approach that integrated Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy, and Energy Dispersive X-ray Spectroscopy to investigate the molecular, structural, and elemental variations in pempek made from two fish species: snakehead and mackerel. Each phase of the methodology was structured to guarantee consistency, analytical precision, and adherence to recognized standards in food characterization studies. The experimental framework included sample collection, acquisition and preparation of spectral data, breakdown and measurement of protein secondary structures, imaging at the micro level, and analysis of elemental distribution.

All experimental measurements were performed in triplicate. Data were expressed as mean $\pm$ standard deviation. Statistical significance was evaluated using independent sample t-tests ($p < 0.05$). The correlation between FTIR-derived secondary structure components, SEM-derived morphometric indices, and EDS elemental ratios was analyzed using Pearson's correlation coefficient to elucidate relationships between molecular conformation, microstructural architecture, and ionic balance.

The methodological reproducibility was further ensured by adopting standardized procedures for spectral preprocessing, imaging calibration, and quantitative analysis. The FTIR and SEM-EDS combination allowed complementary insights into the hierarchical network from molecular conformations to microstructural topology,

thus strengthening interpretation validity. Moreover, adherence to rigorous sampling and statistical design protocols aligns this study with reproducible standards for compositional food analysis (Anderson & Kelley, 2024; Trott & Mulrennan, 2024).

3. RESULTS AND DISCUSSION

3.1. FTIR Spectral Analysis

The Fourier Transform Infrared (FTIR) spectra of pempek derived from *Channa striata* (Snakehead) and *Scomberomorus commerson* (Mackerel) displayed characteristic absorption features representing functional groups of proteins, lipids, and carbohydrates (Figure 1). Both species exhibited dominant bands around 3300–3150 cm^{-1} (O–H and N–H stretching), 2920/2850 cm^{-1} (asymmetric/symmetric CH_2 stretching of lipids), 1740 cm^{-1} (C=O ester), and the prominent Amide I ($\sim 1650 \text{ cm}^{-1}$) and Amide II ($\sim 1550 \text{ cm}^{-1}$) bands typical of protein backbones.

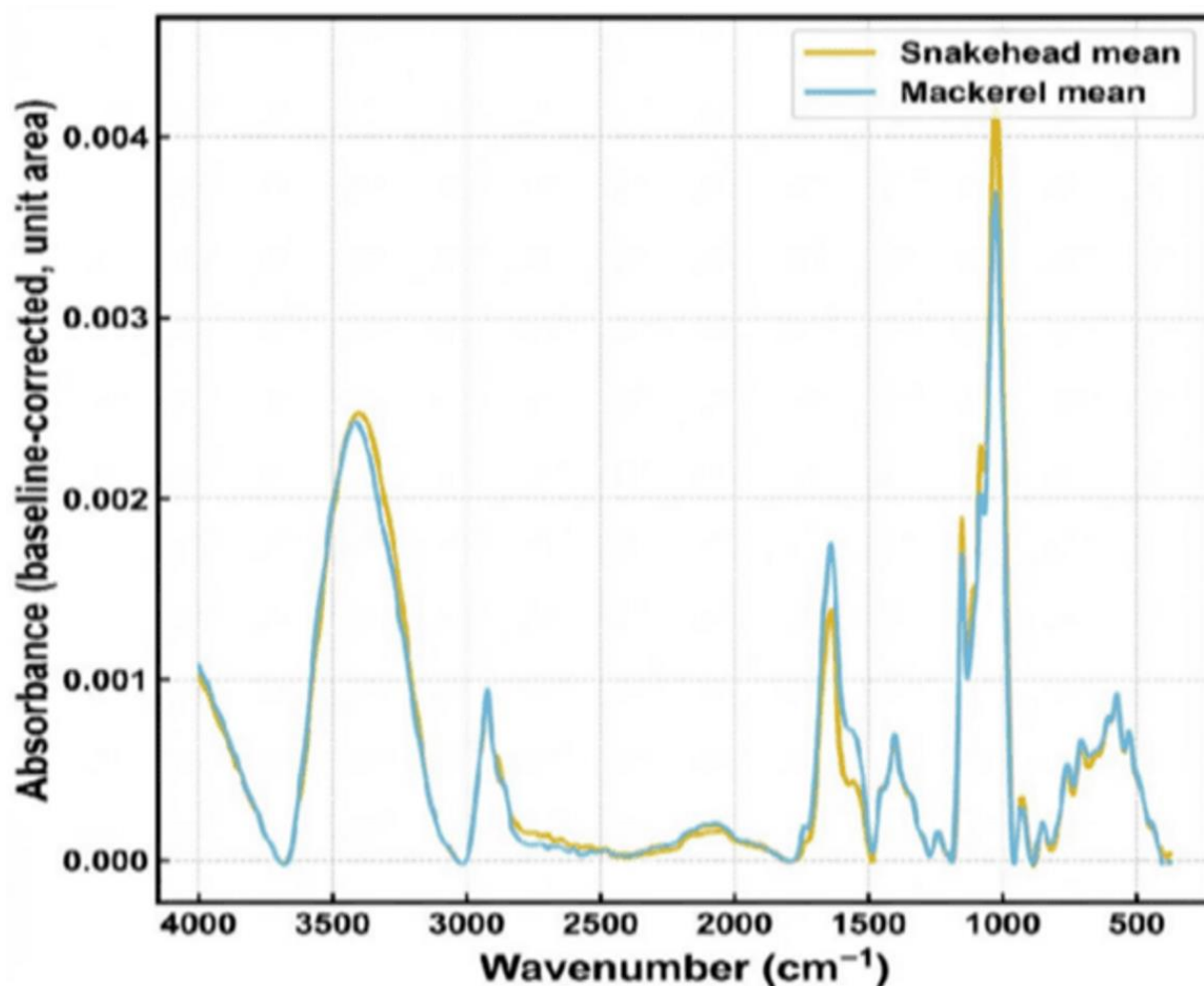


Figure 1. Baseline-corrected and normalized FTIR spectra of Snakehead (*C. striata*) and Mackerel (*S. commerson*) pempek, highlighting the main protein and lipid regions (4000–500 cm^{-1}).

Deconvolution of the Amide I region (1700–1600 cm^{-1}) identified four sub-bands corresponding to β -sheet, α -helix, β -turn, and random coil structures (Table 1). Snakehead pempek exhibited a β -sheet dominance (44.1%), followed by α -helix (24.5%), β -turn (16.8%), and random coil (14.6%). Mackerel pempek presented a higher α -helix (32.7%) and β -turn (23.4%) content, but a lower β -sheet fraction (36.2%).

Table 1. Secondary structure distribution (%) of Snakehead and Mackerel pempek derived from Amide I deconvolution.

Structure	Wavenumber	Snakehead	Mackerel	Structural role
β -sheet	1610–1638	44.1	36.2	Aggregation and strong hydrogen bonding
Random coil	1638–1648	14.6	7.7	Unfolded, disordered regions
α -helix	1648–1660	24.5	32.7	Compact, ordered structure
β -turn	1660–1695	16.8	23.4	Flexible domains, loop conformations

The greater β -sheet proportion in snakehead samples implies stronger intermolecular hydrogen bonding and higher gel compactness, corroborating earlier findings that link β -sheet dominance to enhanced gel strength (Delrue et al., 2025; Liao & Hung, 2023). Conversely, the elevated α -helix content in mackerel samples suggests a more flexible and hydrated protein conformation, consistent with less compact gel networks (Chiang et al., 2023; Roeters et al., 2023).

These differences reflect molecular-level species effects. Snakehead pempek forms stronger hydrogen-bonded β -sheet networks associated with rigidity, while Mackerel's higher α -helix content indicates flexibility and hydration (Figure 2).

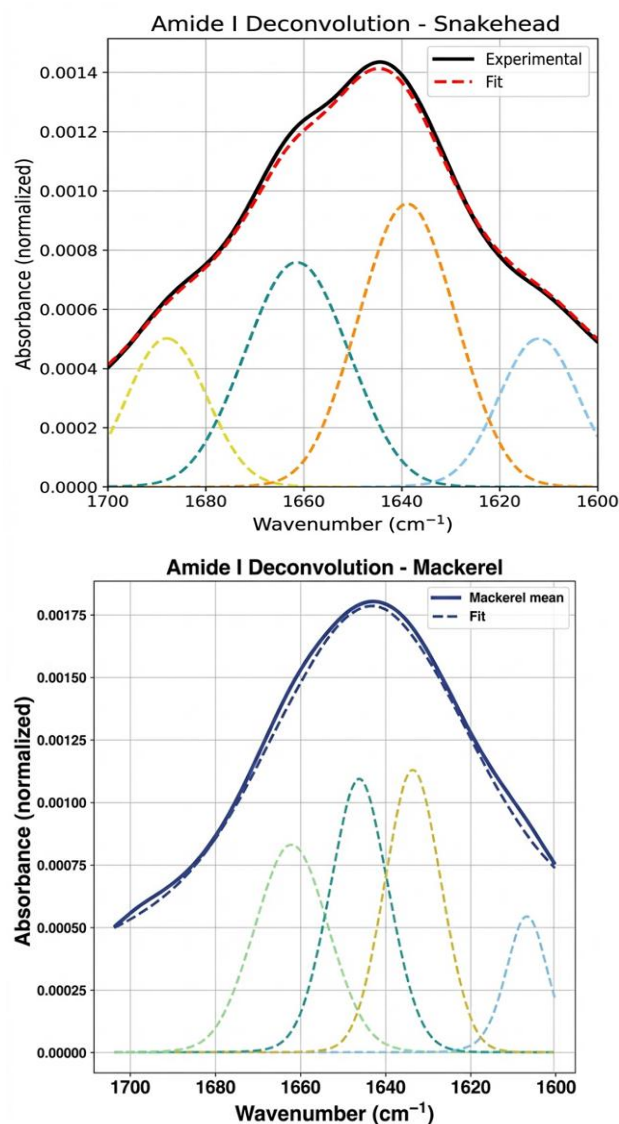


Figure 2. Gaussian deconvolution of Amide I envelope (1700–1600 cm^{-1}) showing contributions from α -helix, β -sheet, β -turn, and random coil structures in Snakehead and Mackerel pempek.

Overall, the FTIR results provide a molecular-level explanation for the textural and mechanical differences perceived between the two pempek types. The higher β -sheet dominance and lower CH_2 ratio in snakehead pempek correlate with increased molecular order, lower lipid mobility, and denser gel structures, whereas mackerel's higher α -helix and CH_2 ratios signify a more flexible and hydrated matrix (Figure 2). These molecular traits align with prior studies linking secondary structure composition to the functional properties of fish protein gels (Gunwant, Gahtori, Varanasi, & Pandey, 2024; Liao et al., 2025).

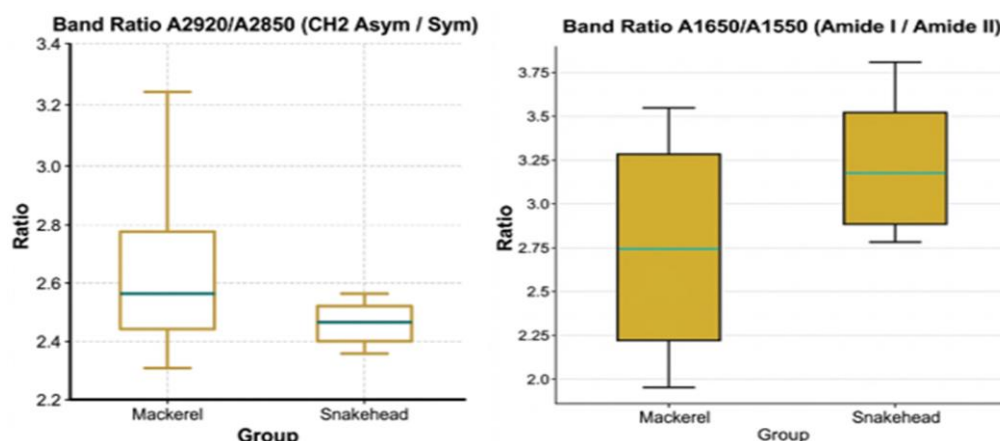
3.2. Diagnostic Band Ratios

Quantitative band ratios analysis further substantiated these distinctions. The amide I/II ratio averaged 1.28 ± 0.05 for snakehead and 1.11 ± 0.07 for mackerel pempek, indicating a higher degree of protein structural order and hydrogen bonding in snakehead formulations (Guleken et al., 2024). Meanwhile, CH_2 asymmetric/symmetric stretching ratios ($2920/2850 \text{ cm}^{-1}$) were 1.32 ± 0.09 and 1.49 ± 0.08 for snakehead and mackerel, respectively (Table 2). The higher CH_2 ratio in mackerel pempek implies greater lipid chain mobility, possibly contributing to softer texture and increased porosity (Gaschignard et al., 2024; Sharma et al., 2025). The combined evaluation of these ratios supports the interpretation that snakehead pempek forms a more stable and ordered protein network, whereas mackerel pempek displays a more fluid lipid–protein interaction matrix (Gunwant et al., 2024).

Table 2. Diagnostic FTIR ratios distinguishing structural order and lipid mobility between Snakehead and Mackerel pempek.

Band Ratio	Structural Meaning	Snakehead (Mean $\pm$ SD)	Mackerel (Mean $\pm$ SD)	p-value	Interpretation
A_{1650}/A_{1550}	Protein structural order	1.28 ± 0.05	1.11 ± 0.07	0.012	Higher in Snakehead (Ordered backbone)
A_{2920}/A_{2850}	Lipid chain mobility	1.32 ± 0.09	1.49 ± 0.08	0.021	Higher in Mackerel (Mobile lipid chains)

The FTIR evidence presented in this study indicates a species-dependent organization of the protein backbone in pempek gels, where snakehead samples exhibited higher β -sheet contributions alongside elevated Amide I/II ratios, and mackerel samples showed higher α -helix and β -turn contributions with increased CH_2 asymmetric/symmetric ratios (Figure 3). These vibrational fingerprints align with the broader literature in fish- and soy-based gels showing that secondary-structure transitions govern water retention, viscoelasticity, and gel cohesion (Gunwant et al., 2024; Płaczkiwicz et al., 2023; Srisutush et al., 2025). In tofu and gelatin systems, more ordered networks present as denser, less synergetic gels, supporting the conceptual link between secondary-structure enrichment and macroscopic firmness (Cosgun, Ceylan, Akdeniz, & Sarikaya, 2025; Derkach et al., 2024; Khoder et al., 2024; Zhang et al., 2024).



Within this framework, the relatively β -sheet-enriched signature of snakehead pempek suggests a gel matrix stabilized by interchain hydrogen bonding, whereas the higher α -helix content in mackerel points to a comparatively flexible backbone that accommodates lipid mobility and water, consistent with the elevated CH_2 ratio. In thermally induced fish gels, α -helix unfolding and concomitant β -sheet formation have been widely reported during gelation, and polysaccharides such as β -glucan can bias this conversion toward β -sheet content by reinforcing hydrophobic association (Fan et al., 2024). Though pH shifts may alter the balance among random coil, β -turn, and α -helix motifs, claims of a necessary return to β -sheet following α -helix increases remain context dependent and not generally supported (Zhang et al., 2023). The present results therefore favor a parsimonious interpretation: species-specific protein and lipid milieus steer α -helix-to- β -sheet progression to different equilibria, and these equilibria are measurable in the Amide I envelope and diagnostic band ratios.

3.3. SEM Microstructural Analysis

Scanning Electron Microscopy (SEM) revealed distinct differences in gel microarchitecture (Figures 4 & 5). In Table 3, Snakehead pempek exhibited a compact, continuous matrix with uniformly distributed micropores ($0.48 \pm 0.06 \mu\text{m}$) and thicker walls ($2.24 \pm 0.87 \mu\text{m}$). In contrast, Mackerel pempek displayed larger pores ($0.55 \pm 0.14 \mu\text{m}$), thinner walls ($1.58 \pm 0.36 \mu\text{m}$), and higher porosity ($34.76 \pm 20.24\%$).

Snakehead's denser morphology correlates with its β -sheet dominance, while Mackerel's higher porosity matches the elevated CH_2 ratio, confirming a structure–composition linkage. These results align with previous reports associating β -sheet enrichment with compact gel formation and α -helix/lipid interactions with structural looseness.

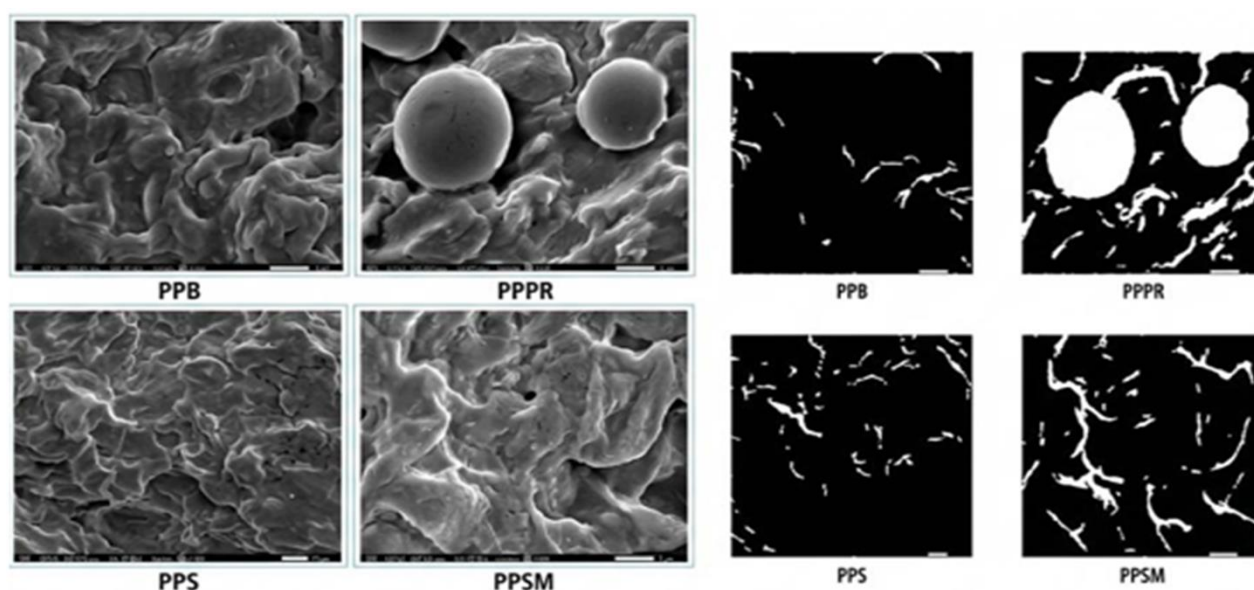


Figure 4. SEM micrographs of Snakehead pempek (PPB, PPPR, PPS, PPSM) at 3000 $\times$ magnification, showing dense, continuous networks with thick walls.

The observed microstructural disparities reflect the influence of protein–lipid interactions on gel formation. The denser network in snakehead pempek suggests stronger protein–protein aggregation, likely facilitated by a higher β -sheet content, which promotes compact network formation and reduced microcavity volume (Figure 4). This finding aligns with prior SEM-based analyses of surimi and gelatin gels, which reported inverse correlations between porosity and gel strength (Petcharat et al., 2025; Yin et al., 2025). Conversely, the open structure of mackerel pempek correlates with its higher lipid mobility (as indicated by FTIR CH_2 ratios), which can disrupt tight protein packing and yield less cohesive gels (Aussanasuwannakul & Singkammo, 2025).

Table 3. Morphometric parameters from SEM micrographs (mean $\pm$ SD, $n = 4$ per group).

Parameter	Snakehead	Mackerel	Description
Porosity (%)	22.48 ± 3.75	34.76 ± 20.24	Fractional void area
Pore diameter (μm)	0.48 ± 0.06	0.55 ± 0.14	Average pore size
Wall thickness (μm)	2.24 ± 0.87	1.58 ± 0.36	Thickness of protein walls
GLCM contrast	586.15 ± 68.10	660.91 ± 83.65	Textural heterogeneity index

Microtextural parameters derived from GLCM analysis further validated these morphological trends. Snakehead pempek exhibited a lower GLCM contrast (586.15 ± 68.10) compared to mackerel pempek (660.91 ± 83.65), implying greater homogeneity in the former and higher textural variability in the latter (Table 3). This pattern is significant given that Pearson's correlation shows that GLCM contrast positively correlates with textural roughness and softness perception in fish gels (Petcharat et al., 2025). Hence, the microstructural compactness of snakehead pempek likely modulates to firmer texture and higher mechanical resilience, while mackerel's looser matrix contributes to a softer, less elastic product (Figure 5).

In Figure 5, the micrographs of mackerel's pempek have more fractional void area due to lipid mobility, which made the texture more heterogeneous and bigger pore sizes. Known that mackerel's flesh has a higher lipid content than snakehead (Aussanasuwannakul & Singkammo, 2025). The interplay between microstructural and molecular parameters indicates a clear framework: the secondary structure conformation at the molecular level determines the extent of protein aggregation and network topology observed microscopically. This multiscale correspondence supports the hypothesis that species-specific compositional differences induce textural variation through structural mechanisms spanning from molecular to macroscopic levels.

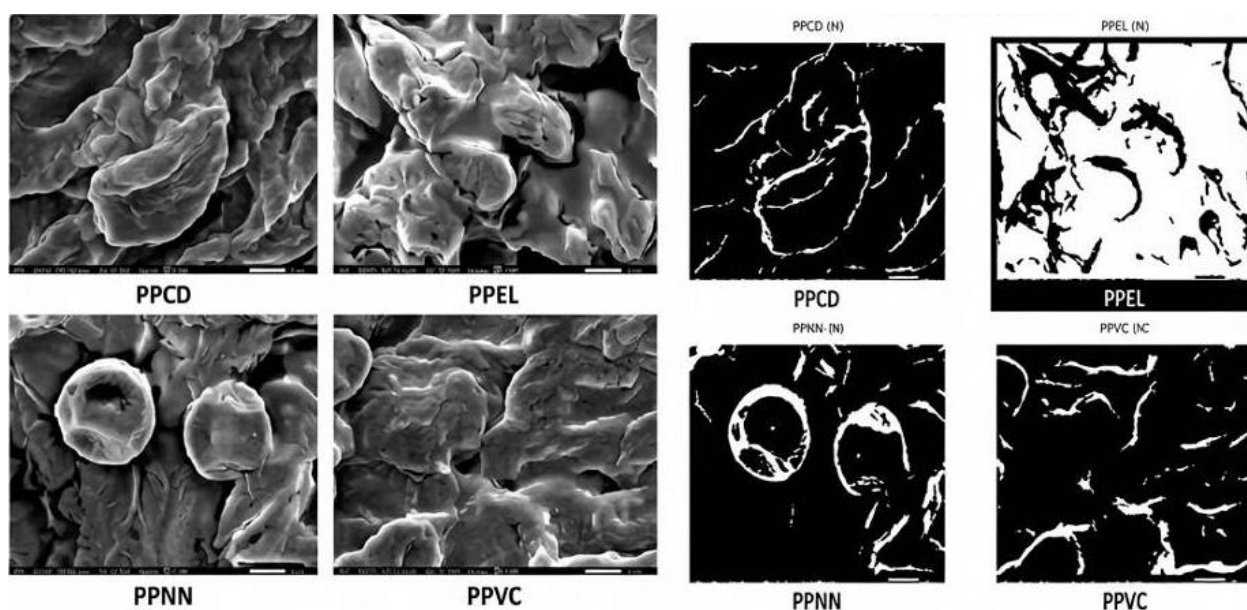


Figure 5. SEM micrographs of Mackerel pempek (PPCD, PPEL, PPNN, PPVC) at 3000 $\times$ magnification, showing open, irregular voids and disrupted structures.

3.4. EDS Elemental Analysis

Energy Dispersive X-ray Spectroscopy (EDS) identified the elemental composition of pempek, revealing dominant peaks for C, O, Na, and Cl (Figure 6A & 6B). Quantitative data confirmed uniform elemental distributions across all samples, with minor species differences (Table 4). The Na/Cl atomic ratio was higher in Snakehead (1.56) than in Mackerel (1.08), indicating stronger electrostatic stabilization and ionic cross-linking within Snakehead gels.

Although the differences in Na/Cl ratios between species were not statistically significant ($p = 0.297$), the trend suggests a slightly higher ionic stabilization in snakehead pempek, potentially contributing to stronger electrostatic protein–protein interactions. This observation aligns with prior studies reporting that adequate ionic balance,

particularly involving Na^+ and Cl^- , enhances gel network stability by reducing protein aggregation and promoting water retention (Kabil, Kol, Aydemir, & Oral, 2025; Morino & Kanada, 2023; Yoo et al., 2025). Furthermore, the uniform O distribution across all samples underscores its role in maintaining protein–water interactions that govern gel elasticity and water-holding capacity (Wang, Jiao, Wang, & Wang, 2025; Xia, Yang, Zhu, Sun, & Zhu, 2024).

Table 4. Mean elemental composition (Mass % and atomic %) of Snakehead and Mackerel pempek ($n = 4$ per group).

Element	Snakehead (Mass%)	Mackerel (Mass%)	Snakehead (Atom%)	Mackerel (Atom%)
C	55.08	59.92	62.86	67.39
O	41.24	36.55	35.36	30.96
Na	1.70	1.45	1.02	0.86
Cl	1.98	2.08	0.77	0.80

The EDS results also corroborate the SEM findings, as samples with higher Na/Cl ratios and more uniform oxygen distribution (snakehead pempek) exhibited denser microstructures and reduced porosity in Figure 6A. This supports the interpretation that ionic composition modulates microstructural stability through electrostatic and hydration mechanisms (Pablo, Klinkesorn, & Tongchitpakdee, 2025). The cross-validation among FTIR, SEM, and EDS analyses thus provides a consistent, multiscale understanding of structural and compositional differences.

The EDS results provide complementary insight into the ionic microenvironment that co-determines protein proximity and hydration behavior. Although Na and Cl were detected at low percentages, their atomic ratio trended higher in snakehead pempek (Figure 6A) than in mackerel (Figure 6B), suggesting subtle differences in charge screening within the protein network. In concentrated myofibrillar systems, Na^+ interacts with acidic residues to alleviate electrostatic repulsion, while Cl^- balances the ionic strength; optimized levels can promote cross-linking and compactness, but excessive concentrations may disrupt hydration shells and weaken gels (Liang, Chen, Fang, & Wu, 2024; Sun et al., 2024; Yang, Yang, Guan, & Bian, 2025).

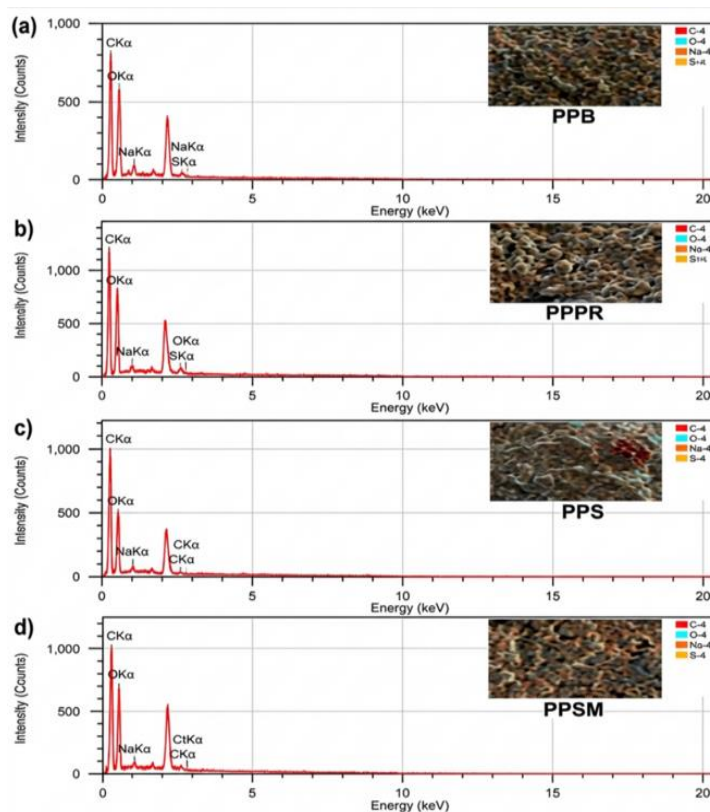


Figure 6A. EDS spectra of snakehead pempek showing major peaks for C, O, Na, and Cl. Insets display corresponding SEM fields used for acquisition.

The present Na/Cl trend, alongside the β -sheet-enriched snakehead spectra and denser SEM morphology, is consistent with an ion-mediated stabilization formulation in which modestly greater sodium availability favors closer chain packing. We emphasize that causality cannot be asserted from processing conditional; nevertheless, the co-variation across modalities coheres with earlier reports that ionic strength modulates α -helix unfolding and β -sheet accrual during thermal aggregation (Rajasekaran et al., 2023). In addition, the relatively uniform oxygen mapping across samples supports a broadly similar water–protein interaction framework, suggesting that ionic feature rather than bulk hydration differences contribute to the observed microstructural divergence.

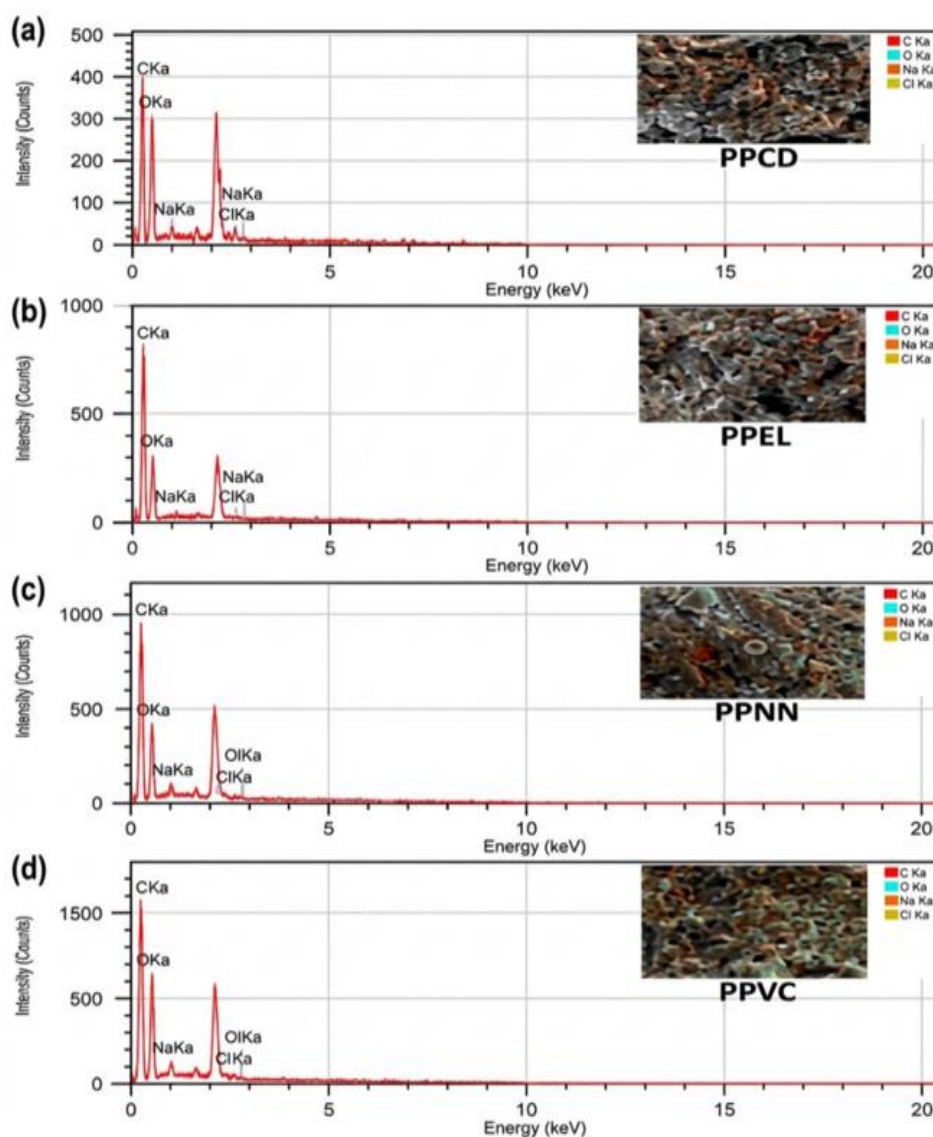


Figure 6B. EDS spectra of mackerel pempek showing major peaks for C, O, Na, and Cl. Insets display corresponding SEM fields used for acquisition.

3.5. Correlation Between Molecular, Structural, and Elemental Parameters

Correlation analysis integrated FTIR, SEM, and EDS datasets (Table 5). β -sheet content correlated positively with wall thickness ($r = 0.81$), while CH_2 ratio correlated negatively with wall thickness ($r = -0.74$) and positively with porosity ($r = 0.68$). The Na/Cl ratio showed a moderate positive correlation with β -sheet content ($r = 0.63$). These relationships confirm the hierarchical coupling of ionic environment, molecular conformation, and microarchitecture. They are consistent with previous observations that lipid–protein interactions influence gel rigidity and network homogeneity (Gul et al., 2025; Liang et al., 2024; Ma et al., 2025).

Table 5. Correlation coefficients (*r*) among key FTIR, SEM, and EDS parameters.

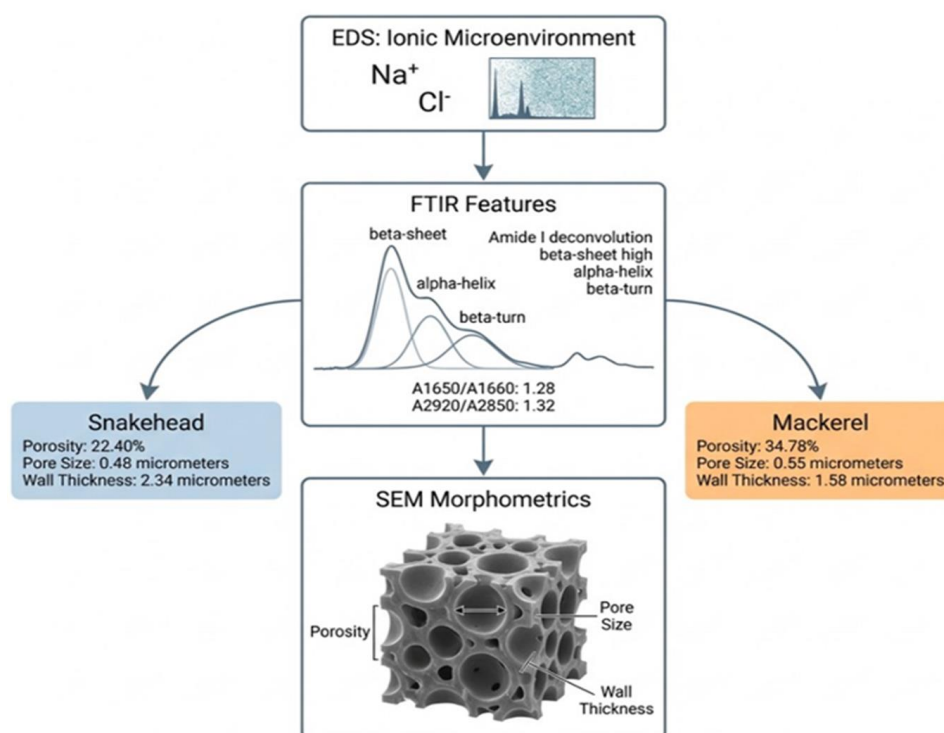
Variable pair	Correlation (<i>r</i>)	Interpretation
β-sheet (Wall thickness)	0.81	Structural order increases matrix density
CH ₂ ratio (Porosity)	0.68	Lipid mobility enhances pore formation
CH ₂ ratio (Wall thickness)	-0.74	Inverse relation: lipid plasticization thins walls
Na/Cl ratio (β-sheet)	0.63	Ionic stabilization promotes aggregation

Further, the Na/Cl ratio correlated positively with β-sheet content, supporting the role of ionic balance in promoting protein aggregation and gel firmness. This finding resonates with earlier reports emphasizing the function of sodium and chloride ions in enhancing electrostatic stabilization and cross-linking efficiency within protein networks (Pablo et al., 2025; Sakamut, Akkarapukjinda, Hongviangjan, & Sompongse, 2025). Collectively, these results illustrate a coherent structure–composition–function relationship in which ionic composition modulates protein conformation, thus determining microstructural network and textural outcomes.

3.6. Integrated Hierarchical Model

Synthesizing FTIR, SEM, and EDS data, a hierarchical structure–composition model (Figure 7) was developed. It elucidates how ionic balance (Na/Cl ratio) influences secondary-structure transitions (β-sheet vs. α-helix) that modulate gel microarchitecture. Snakehead pempek, with higher Na/Cl ratios and β-sheet content, forms denser networks with thicker walls and lower porosity. Conversely, mackerel pempek shows higher α-helix and CH₂ mobility, producing more open and heterogeneous matrices.

This multiscale interpretation demonstrates that Snakehead's β-sheet enrichment, ionic stabilization, and compact microstructure are mutually reinforcing, producing a firm, cohesive gel. In contrast, Mackerel's lipid-modulated flexibility leads to porous, elastic structures. These findings validate the integrated FTIR–SEM–EDS framework as a comprehensive tool for differentiating species-based gel architectures and informing quality design and authentication of traditional fish-based products.

**Figure 7.** Hierarchical model linking ionic composition, molecular conformation, and gel microstructure in pempek.

A central contribution of this work is the explicit triangulation of FTIR, SEM, and EDS to build a multiscale account of pempek structure–function relationships. FTIR isolates backbone conformation and lipid mobility through the Amide I/II and CH₂ metrics, SEM resolves the physical topology that confers mechanical performance, and EDS characterizes the light-element milieu associated with salt and hydration.

The literature affirms the value of such integration across food and biomaterial systems, where FTIR fingerprints functional groups and conformational shifts, SEM discloses morphology and porosity responsible for rheological behavior, and EDS adds elemental constraints that control interfacial physics (Akhtar et al., 2024; Peng et al., 2025; Rani, Ain, Sajid, Ibrahim, & Shah, 2025; Shelly, Nanda, & Mehta, 2023).

Cross-modal alignment provides protection against over-interpretation from any single technique and enhances reproducibility, since convergent evidence reduces the risk that findings are artifacts of band pattern fitting, thresholding, or spectral baseline options. In the present case, the co-occurrence of higher β -sheet fractions, lower CH₂ ratios, thicker walls, and slightly elevated Na/Cl atom ratios within snakehead samples constructs a cohesive explanatory chain from molecular vibration to network architecture and, by implication, to textural perception. Such triangulation also enables pragmatic analytics for authenticity, since each modality yields species-sensitive markers, Amide I partitions, porosity–wall thickness pairs, and Na/Cl ratios that can be deployed individually or jointly depending on instrument availability. The FTIR pipeline balanced robust preprocessing with conservative deconvolution to reduce overfitting while retaining spectral nuance. Area-normalized spectra with Savitzky–Golay smoothing and asymmetric least-squares baseline correction are documented to minimize concentration and scattering artifacts in complex biopolymers, and second-derivative guidance aids peak picking where starch bands overlap with protein signals (Ahlan & Hutami, 2025; Buiňáková et al., 2023; Mancini et al., 2023; Seregin et al., 2025).

Nevertheless, spectral pattern choices and initial parameterization remain sources of uncertainty; the correspondence with SEM and EDS partially mitigates this. SEM morphometrics were calibrated to micrometer units and averaged across multiple fields to improve representativeness; still, pore anisotropy and cut-plane orientation can bias thickness estimates, and GLCM contrast depends on acquisition settings. EDS quantification of light elements is sensitive to coating, accelerating voltage, and matrix effects; careful control of sputter thickness and matched acquisition conditions improved internal comparability, even if absolute concentrations must be interpreted cautiously (Awodi, Mohan, & Krishnan, 2025; Castro et al., 2024; De Lima et al., 2024; Perera, Jaiswal, & Jaiswal, 2023).

The statistical power is modest at $n = 4$ per group; however, the effect directions are consistent across independent modalities. Future studies should incorporate rheology, water-holding capacity, and low-field NMR to couple microstructure with viscoelastic response and water mobility, extending the multiscale chain of inference. The integrated structure composition model has immediate consequences for the design and authentication of traditional fish gels. For quality engineering, modulating ionic strength and lipid fraction offers a pathway to target textural profiles while preserving organoleptic identity. Ion-mediated stabilization principles are increasingly leveraged to replace or reduce synthetic additives in clean-label formulations by tuning electrostatic screening and coordinating protein–polysaccharide interactions (Liu et al., 2025; Perța-Crișan, Ursachi, Chereji, Tolan, & Munteanu, 2023).

In food authentication, FTIR can provide rapid, non-destructive screening of species identity through Amide I partitions and diagnostic ratios, while SEM-EDS can validate microstructural and ionic context where disputes arise (Ali, Tukiran, & Roslan, 2024; Cebi, Bekiroglu, Erarslan, & Rodriguez-Saona, 2023; Siska, Jumadil, Abdullah, Ramadon, & Mun'im, 2023). The present markers β -sheet enrichment with lower CH₂ ratios, denser wall architecture, and slightly higher Na/Cl atomic proportions compose a coherent signature for snakehead pempek that can be operationalized as a decision rule when paired with supervised classification. Beyond pempek, these findings generalize to fish-based gels whose textural attributes must be tuned for specific culinary applications; for example, increasing β -sheet formation through controlled thermal histories or ionic schedules may improve shear firmness and reduce syneresis, whereas favoring α -helix retention with higher lipid mobility can soften bite and enhance juiciness.

Fish gels are composite networks in which myofibrillar proteins co-assemble with starch and lipids to produce viscoelastic solids. The present data contribute to an emerging view that secondary-structure balance influences how proteins scaffold around starch granules and confine lipid domains during heating. Literature on lipid filling and water distribution suggests that fats can occupy or generate voids that mediate stress fields, with consequences for both porosity and wall continuity (Aussanasuwannakul & Singkammo, 2025; Gul et al., 2025; Ma et al., 2025).

The higher CH₂ ratio in mackerel pempek is consistent with increased lipid chain mobility and a wider distribution of void sizes that weaken load-bearing pathways, while the β -sheet-enriched snakehead matrix likely yields stronger confinement and fewer coalesced pores. Because SEM images capture a dried state, complementary cryo-SEM or confocal microscopy in hydrated gels would help validate the dynamic pore network under mechanical load and provide a more faithful mapping of moisture distribution. The hierarchical model proposed here, wherein ionic milieu influences secondary-structure partitioning that determines microstructural topology, is transferable across biopolymer systems. In gelatinous matrices, double-helix assembly drives network reinforcement; in tofu derived from marine mineral fortification, uniform cross-link density improves water binding and lowers syneresis (Cosgun et al., 2025; Derkach et al., 2024; Zhang et al., 2024). In surimi, pressure processing and transglutaminase can drive protein proximity and interchain linkages that mirror the densification attributed here to β -sheet accrual (Hongviangjan & Sompongse, 2024).

The cross-technique triangulation advocated in this work fosters such transferability: FTIR offers rapid screening for conformational endpoints, SEM supplies architectural constraints, and EDS provides a mechanistic lever for ion management. When integrated with rheological assays and sensory panels, this toolkit can become a pragmatic platform for formulation science, enabling data-driven design of heritage foods. The study's interpretive power is constrained by limited sample size, brand-to-brand variability, and the absence of direct measures of mechanical performance and water mobility. Although effect directions concur across FTIR, SEM, and EDS, broader sampling across seasons, fisheries, and processing lines would clarify the robustness of species signatures. Quantifying lipid classes and oxidation products could help explain CH₂ dynamics beyond chain mobility alone. In addition, time-temperature gelation profiles combined with in situ FTIR might resolve kinetic pathways linking α -helix unfolding to β -sheet accrual under ionic schedules. Advanced EDS standards or wavelength-dispersive spectroscopy would refine light-element quantification and mitigate coating artifacts. Finally, integrating chemometrics for supervised classification would formalize species discrimination and provide interpretable thresholds for authenticity testing in industry settings Figure 7. Schematic of the hierarchical structure–composition model integrating EDS, FTIR, and SEM findings. The ionic microenvironment (Na/Cl atomic ratio) modulates protein secondary-structure partitioning (β -sheet vs. α -helix/ β -turn), which determines gel microarchitecture (porosity, wall thickness, and textural homogeneity). Species-specific signatures are indicated for snakehead and mackerel pempek.

4. CONCLUSION

This study demonstrated that the hierarchical structure and compositional characteristics of pempek, a traditional Indonesian fish-based gel, are strongly influenced by fish species differences. Using an integrated FTIR–SEM–EDS analytical framework, the study revealed that *Channa striata* (snakehead) pempek possesses a β -sheet-enriched protein matrix with higher Amide I/II ratios, lower CH₂ ratios, and a compact microarchitecture characterized by thick walls and low porosity. In contrast, *Scomberomorus commerson* (mackerel) pempek exhibits a more α -helix-dominant structure, elevated CH₂ ratios indicating higher lipid mobility, and a porous, heterogeneous network. The Na/Cl atomic ratio in snakehead samples suggests stronger electrostatic stabilization, supporting denser molecular packing and enhanced gel firmness. These integrated findings confirm a hierarchical coupling among ionic composition, molecular conformation, and microstructural architecture, elucidating the multiscale mechanisms determining gel texture and stability. The study advances the current understanding of fish protein gelation and contributes a transferable analytical model for food material science, applicable to authentication, quality

control, and texture optimization of traditional and modern gel-based foods. Future research should extend this multiscale approach by incorporating rheological, sensory, and water mobility analyses to refine predictive models for structure–function relationships in protein-based systems.

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