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# Recycling Indonesian Native Chicken Eggshell Membrane Waste into Gelatine: A Comparative Characterisation Using SEM-EDX, FTIR, and XRF

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## ABSTRACT

Indonesian native chicken eggshells are an underutilised agricultural waste with potential for conversion into gelatine through sustainable recycling methods. This study evaluated and compared the physicochemical properties of gelatine extracted from Indonesian native chicken eggshell membranes (INCES), broiler chicken eggshell membranes (BCES), and bovine skin. Gelatine was extracted from each source and analysed in triplicate. Structural characteristics and elemental composition were investigated using Scanning Electron Microscopy combined with Energy Dispersive X-ray Spectroscopy (SEM-EDX), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Fluorescence (XRF) were used to elucidate structural features and elemental composition differences among the samples. The SEM photos show that BCES gelatine had larger, more irregular structures, while INCES gelatine had finer, more spherical particles. Bovine gelatine displayed a smoother, fibrous morphology. Nine significant elements were found by EDX elemental analysis (C, N, O, Na, Mg, Al, Si, Ca, and Au). While carbon elements predominated in bovine gelatine, calcium was present in larger amounts in eggshell protein (INCES & BCES). Typical amide bands (A, B, I, II, and III) were visible in the FTIR spectra. In contrast to BCES gelatine, which tended to

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exhibit more random coil properties, the spectra of ICES and bovine gelatine revealed β-sheet structures. Analysis using XRF revealed that INCES gelatine contained elevated calcium levels, most likely due to incomplete demineralisation during extraction. Overall, INCES represents a promising alternative gelatine source that supports the valorisation of poultry by-products. These findings demonstrate that INCES gelatine exhibits a distinctive mineral-rich, β-sheet-dominant profile relative to bovine gelatine, positioning it as a promising, halal-compliant alternative for functional food and nutraceutical applications, while further functional and safety validation is required before any biomedical application.

*Keywords:* Eggshell membranes, gelatine, physicochemical, poultry by-products

## INTRODUCTION

Gelatine is a versatile biopolymer widely applied in food, pharmaceutical, and cosmetic industries due to its distinctive functional attributes, such as gel formation, emulsifying capacity, and film-forming ability. It is typically produced through partial hydrolysis of collagen, which is commonly obtained from mammalian sources like bovine and porcine skin. The investigation of alternate sources, such as chicken by-products, has been prompted by the growing demand for halal and non-mammalian gelatine (Gómez-Guillén et al., 2011; Karim & Bhat, 2009). According to Herdiana et al. (2024) and Wahyuningsih et al. (2026), the public's growing awareness of ethics and raw material sustainability is correlated with the growing demand, especially when it comes to the exploration of poultry waste as a more morally and ecologically sound source of gelatine.

A waste product of the chicken business, eggshells worsen already-existing environmental problems. According to some research, eggshells contain amino acids, including substances that resemble collagen and can be used to make gelatine (Bootklad & Kaewtatip, 2013; Hamidi et al., 2017). An alternate source of eggshell membranes with a variety of characteristics is native Indonesian chicken. The composition and shape of Indonesian eggshell membrane (INCES) as a possible source of gelatine have been thoroughly studied. The composition and shape of Indonesian eggshell membrane (INCES) as a possible source of gelatine have been thoroughly investigated in earlier research by Wahyuningsih et al. (2026) and Permadi et al. (2024). They have effectively examined the composition and morphology of Indonesian eggshell membrane (INCES) as a potential source of gelatine. According to Wahyuningsih et al. (2026), the INCES membrane is more valuable in turning environmental waste into profitable gelatine goods because it has a higher content of important amino acids than the broiler chicken eggshell membrane (BCES). Furthermore, Wahyuningsih et al. (2026) reported that INCES contains approximately 25.3% crude protein, substantially higher than that of other poultry eggshell membranes, including indigenous chicken, laying hen, bakery waste, and hatchery waste eggshells. More recently, Wahyuningsih et al. (2026) demonstrated that gelatine extracted

from INCES achieved extraction yields of 47-51%, indicating that this material is not only protein-rich but also capable of producing gelatine with a high recovery. These findings provide a strong scientific basis for selecting INCES as the primary raw material in the present study. Examining protein development in gelatine synthesis aligns with the growing use of circular economy ideas and sustainable waste management (Hoerterer et al., 2022; Yuliatmo et al., 2017).

Currently, Scanning Electron Microscopy (SEM) combined with Energy Dispersive X-ray Spectroscopy (EDX) is widely employed to characterise the microstructure and elemental distribution of biomaterials such as gelatine, enabling clear differentiation in surface morphology and compositional features. In addition, spectroscopy-based techniques, including Fourier Transform Infrared (FTIR) and X-ray Fluorescence (XRF), provide powerful analytical approaches for identifying functional groups and assessing chemical composition, particularly in gelatine. These techniques have been used in earlier studies to evaluate gelatine generated from mammals and broilers (Al-Hassan & Norziah, 2017; Allegretta et al., 2020; Appoloni & Melquiades, 2014; Proietti et al., 2020; Rosa et al., 2024). Systematic investigations on gelatine derived specifically from INCES biomaterial remain limited. This scarcity highlights an important research gap in advancing the valorisation of native Indonesian poultry by-products.

To fill this research gap, the present work investigates gelatine obtained from INCES protein using SEM, EDX, FTIR, and XRF analyses For comparative purposes, gelatine extracted from broiler chicken eggshell membrane (BCES) was included to evaluate the influence of chicken breed on the structural and elemental characteristics of eggshell membrane-derived gelatine, while commercial bovine gelatine was used as a conventional reference material to benchmark the properties of INCES against an established industrial gelatine source. The novelty of this study lies in the application of a comprehensive multi-instrument analytical framework to INCES-based gelatine for the first time, thereby offering essential insights into its potential as a sustainable, culturally appropriate, and economically valuable alternative to conventional gelatine sources.

## MATERIALS AND METHODS

### Materials

Waste from native Indonesian chicken eggshells that were gathered from nearby farms in Yogyakarta City and Sleman Regency served as the study's samples. Membranes from Indonesian chicken eggshells were used to make gelatine. Samples of eggshells were sorted to make sure they were clean of sand and grime. Gelatine obtained from broiler chicken eggshell membranes and commercially available bovine skin served as control samples for comparative analysis. The auxiliary materials employed in this study included NaOH, acetic acid, and bovine gelatine, all of which were procured from Merck, Darmstadt, Germany. In this research, only analytical-grade compounds are used.

## METHODS

### Extraction Gelatine

With a few minor adjustments, the extraction technique is based on the research by Mohammadi et al. (2016). After being physically separated, the eggshell membrane was finely powdered. The eggshell was sliced into 1 cm pieces for the membrane extraction procedure, and it was then immersed in 0.1 M NaOH at a 1:40 (w/v) ratio for a full day at room temperature. The membrane samples were rinsed with running water until neutrality was achieved at pH 7. The membrane was then immersed in a second stage of 0.5 M acetic acid for 24 hours at 37°C in a water bath at a ratio of 1:40 (w/v). After filtering, the extracted material was dried in an oven for 48 hours. The residue was employed for proximate analysis, and a sample was taken for processing. After the extracted collagen was gathered, its structural and elemental characteristics were examined using SEM, EDX, FTIR, and XRF.

### Energy Dispersive X-Ray (EDX) and Scanning Electron Microscope (SEM) Analysis

The dried gelatine samples were finely ground and homogenised to minimise surface heterogeneity and obtain a more representative surface for elemental characterisation. Using a Hitachi MC 1000 sputter ion, the samples utilised in the SEM analysis were coated with gold (Au) to increase their conductivity, which is not a constituent of the gelatine sample. The coating was applied as a thin layer to minimise X-ray absorption and reduce potential interference during elemental analysis. Before the samples were examined using the SEM method, the ion sputter setting was changed to 20 mA for 20 seconds. The Hitachi SU 3500 (Tokyo, Japan) was used for SEM and EDX testing.

### Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The structural characteristics of the gelatine samples were examined spectroscopically using a PerkinElmer FTIR (Model Spectrum 100, PerkinElmer, Waltham, MA, USA). Potassium bromide (KBr) was combined with each sample (INCES gelatine, BCES gelatine, and bovine skin gelatine) to create pellets. FTIR spectra were recorded within the range of 4000-400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$. Distinctive absorption peaks associated with amide bands I, II, and III were identified and compared across the different gelatine types. Each sample was analysed in triplicate to ensure reproducibility of the results.

### X-Ray Fluorescence (XRF) Analysis

The elemental composition of the gelatine samples was determined using a Bruker S8 Tiger X-ray Fluorescence (XRF) spectrometer (Bruker AXS, Karlsruhe, Germany). Before being put in a dedicated container for XRF measurement, the gelatine samples were first

ground into a uniform powder. The intensity of distinctive X-ray emission peaks was used to identify the elements in the spectra, which were obtained over an energy range of 1-40 keV. Three technical replicates of each variety of gelatine were examined. A quantitative elemental composition profile was then obtained by processing and interpreting the data spectrum using Bruker S8 XRF software.

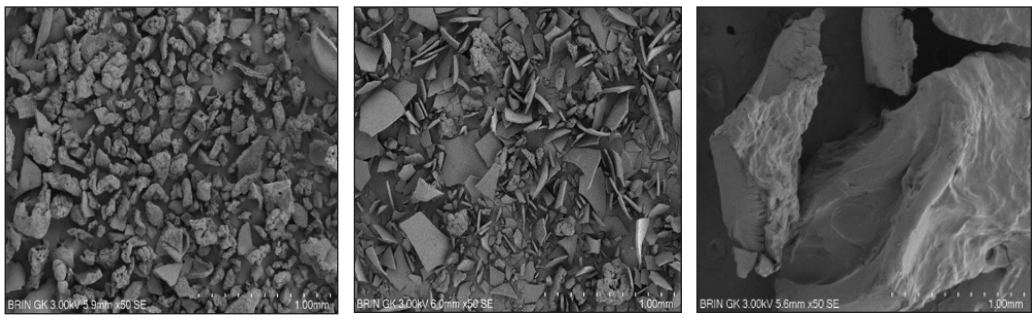
### Statistical Analysis

The surface shape and element distribution shown in the scanning pictures were visually interpreted to carry out descriptive SEM and EDX analyses. To ascertain the functional group features of each sample, the FTIR spectra were studied by locating and interpreting the absorption peaks in the wavelength range of 3000-450 $cm^{-1}$. In the meantime, Principal Component Analysis (PCA) was carried out using Unscrambler X10.4 software (Camo, Norway) to find relationships and patterns in the elemental data, and Analysis of Variance (ANOVA) with IBM SPSS Statistics 25 software was used to statistically analyse the elemental composition data obtained from XRF analysis to assess significant differences between gelatine types.

## RESULTS AND DISCUSSION

### Comparative Scanning Electron Microscope (SEM) Analysis of Microstructural Properties in Gelatine Extracted from Various Sources

Significant morphological variations between gelatine from INCES, BCES, and bovine skin sources are revealed by the SEM measurements as shown in Figure 1. All things considered, SEM offers a micro-level visual representation of gelatine's physical structure, demonstrating its status as a naturally occurring polymer capable of forming intricate networks. In addition to the structure being somewhat porous or hollow, the finer structure of INCES gelatine is defined by small particles of uniform size, with each edge being blunt and loosely bound in the matrix. These morphological findings demonstrate that the collagen hydrolysis process in INCES gelatine is operating efficiently since it can be removed nearly flawlessly. This result supports the hypothesis that gelatine with smoother structures and smaller particle sizes tends to be more soluble (Li et al., 2023). The presence of porosity probably promotes faster dissolution by enabling improved water diffusion and swelling. These microstructural characteristics are linked to higher levels of collagen denaturation and triple-helical domain rupture, indicating that the extraction conditions used on INCES raw materials were adequate to break intermolecular cross-links. On the other hand, a lower degree of hydrolytic breakdown is implied by the relatively compact and coarse morphology seen in BCES and bovine skin gelatine, which could result in decreased solubility and functional performance.




(a) (b) (c)

*Figure 1.* SEM micrograph of gelatine with different sources: (a) Gelatine from Indonesian native chicken eggshell membrane (INCES), (b) Gelatine from broiler chicken eggshell membrane (BCES), (c) Gelatine from bovine skin

In contrast, BCES gelatine has a larger, rough, glass-like structure, is non-uniform, and non-porous. Furthermore, the particles remain strongly bound to the matrix, with irregular shapes, and each particle tip is pointed. This indicates incomplete collagen degradation during extraction, or it can be said that the collagen in the sample is not completely extracted. The cause of this problem is likely influenced by the characteristics of the raw material, which is different from the raw material for INCES gelatine, the solvent concentration used, and the soaking time during extraction, which is not long enough, so that the extraction process does not run optimally, and reduces its solubility in water. Previous research (Xu et al., 2025) reported that rough structures in gelatine could negatively impact its mechanical properties and solubility.

In contrast, bovine skin gelatine has a smooth structure with thin fibres and big, spherical aggregates. Higher levels of hydrolysis and a more regulated extraction process are reflected in this morphology, which produces a more uniform network. This structure supports the notion that, in comparison to poultry gelatine, bovine gelatine has historically been recognised for having more stable physicochemical characteristics (Karim and Bhat, 2009). Variations in solubility among the three gelatine types can be linked to residual protein content and the molecular interactions within their networks. The uniform morphology observed in INCES gelatine and bovine skin gelatine suggests that such structures facilitate more effective interactions with water. In contrast, the comparatively rough morphology of broiler chicken gelatine appears to restrict this capacity (Usman et al., 2024). Furthermore, drying or gelation pathways, temperature profiles, cooling rates, drying techniques, the presence of solvents or additives, bloom strength, and pH all have a significant impact on the morphological variations among gelatines. These factors together determine whether gelatine forms fibrous structures, solid films, porous matrices, or particulate morphologies.

## Comparative Energy Dispersive X-Ray Spectroscopy (EDX) Analysis of Gelatine Derived from Different Sources

The elemental mapping patterns and elemental spectra of INCES, BCES, and bovine skin gelatine surface were revealed by analysis utilising Energy Dispersive X-ray Spectroscopy (EDX), as shown in Figure 2 and Table 1. Nine elements, carbon (C), nitrogen (N), oxygen (O), sodium (Na), magnesium (Mg), aluminium (Al), silicon (Si), and calcium (Ca) were successfully identified in all samples using this method, which is based on the idea that each

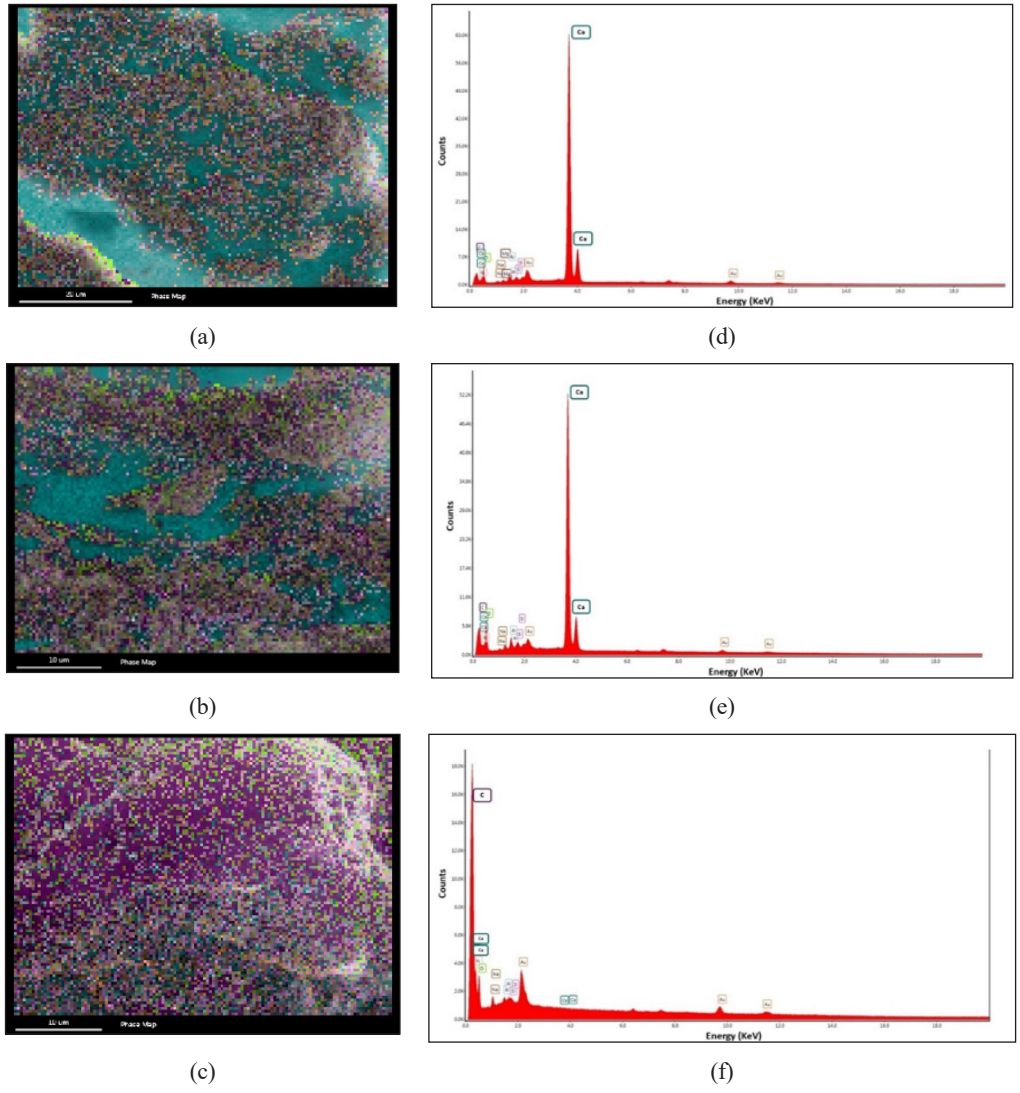



*Figure 2.* (a) EDX elemental mapping of gelatine from Indonesian native chicken eggshell membrane (INCES), (b) broiler chicken eggshell membrane (BCES), (c) bovine skin and (d) EDX spectrum of gelatine from INCES, (e) BCES, (f) bovine skin

Table 1
*Elements composition by EDX analysis (%)*

| No. | Elements | Gelatin Source | | |
|---|---|---|---|---|
| | | INCES | BCES | Bovine Skin |
| 1 | Carbon (C) | 3.06 | 5.15 | 47.83 |
| 2 | Nitrogen (N) | 1.02 | 3.09 | 10.87 |
| 3 | Oxygen (O) | 5.10 | 8.25 | 9.78 |
| 4 | Natrium (N) | 1.02 | 2.06 | 6.52 |
| 5 | Magnesium (Mg) | 2.04 | 0.00 | 0.00 |
| 6 | Aluminium (Al) | 4.08 | 4.12 | 8.70 |
| 7 | Silicon (Si) | 3.06 | 4.12 | 9.78 |
| 8 | Calcium (Ca) | 80.61 | 73.20 | 6.52 |

*Note.* INCES: Gelatine from Indonesian native chicken eggshell membrane; BCES: Gelatine from broiler chicken eggshell membrane (BCES)

element emits a distinctive emission spectrum according to its atomic configuration. While these elemental constituents are common to all gelatine types, the relative abundance of major elements varies considerably depending on the raw material source. Such differences suggest potential variations in chemical composition, which the intrinsic collagen structure and the extraction methodology employed may shape.

SEM-EDX analysis showed that calcium (Ca) was the predominant element detected on the surface of INCES and BCES gelatine, accounting for 79% and 71% of the detected surface elemental composition, respectively. In contrast, the commercial bovine-based gelatine displayed a markedly higher proportion of carbon (C), approximately 44%. The predominance of calcium in gelatine derived from chicken eggshell membranes may be associated with residual mineral components remaining after the demineralisation process. However, the present analytical techniques (SEM-EDX and XRF) do not allow differentiation between residual eggshell-derived calcium and calcium naturally associated with the eggshell membrane matrix. During bovine gelatine production, the demineralisation step is typically carried out intensively to lower mineral levels, with particular emphasis on reducing calcium content. However, in this study, the eggshell samples only underwent a relatively short demineralisation treatment (1 day), which may have contributed to the retention of certain mineral elements, particularly calcium. These findings suggest that the demineralisation process may influence the elemental composition of eggshell membrane-derived gelatine. Calcium contributes to the reinforcement of gelatine structure through ionic interactions, influencing key properties such as gel strength, viscosity, and melting point. By contrast, carbon represents the fundamental constituent of gelatine, exerting a profound impact on its molecular framework, physical attributes, and functional performance. Accounting for approximately 50.5% of the composition, carbon

is indispensable to the protein backbone of gelatine, and its unique gelling and binding capacities would not exist without this element.

This observation is consistent with the findings of Mohammadi et al. (2016), who reported that poultry-derived gelatine often retains elevated calcium levels due to suboptimal demineralisation. In contrast, the predominance of carbon in bovine gelatine agrees with the results of Karim and Bhat (2009), who demonstrated that bovine skin collagen contains a higher proportion of carbon. Similarly, Rigueto et al. (2022) emphasised that the mineral composition of gelatine is strongly influenced by both the raw material source and the processing method, thereby corroborating the elemental differences identified in this study. Such variations directly affect the functional properties and application potential of gelatine. The elevated calcium content observed in INCES and BCES gelatine suggests possible utility in biomedical applications; however, excessive mineral levels may compromise solubility and other physicochemical attributes, indicating the need for further optimisation if the material is to be applied in food or pharmaceutical contexts. Conversely, the high carbon content in bovine gelatine reflects its suitability for applications requiring a more stable organic matrix, such as film-forming material or gelling agent. Calcium contributes to the reinforcement of gelatine structure through ionic interactions, thereby influencing critical properties such as gel strength, viscosity, and melting point. In contrast, carbon constitutes the principal and indispensable element of gelatine, exerting a profound influence on its molecular framework, physical attributes, and functional performance. Representing approximately 50.5% of the composition, carbon is integral to the protein backbone of gelatine, and its distinctive gelling and binding capacities would not exist without this element.

## Comparative Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Gelatine Derived from Different Sources

The FTIR on gelatine serves to identify typical functional groups and characterise the molecular structure of gelatine. The spectra of INCES, BCES, and bovine skin gelatine are presented in the accompanying Figure 3 and Table 2. Each gelatine sample exhibited five characteristic amide regions: amide A, B, I, II, and III. Specifically, amide A was observed in the range of 3196.32-3276.61 $cm^{-1}$ with a representative hydrogen bond stretched by NH, amide B in 2109.06-2112.36 $cm^{-1}$ with a representative asymmetric stretching vibration of CH and $NH3^{+}$, amide I in 1602.86-1648.91 $cm^{-1}$ with a representative COO is stretched by the C=O bond, amide II in 1527.54-1539.06 $cm^{-1}$ with a representative the peptide group out-of-phase combination of in-plane NH deformation and CN stretch modes is responsible for the fluctuation mode, and amide III in 1410.41-1448.61 $cm^{-1}$ with a representative variation in the glycine CH2 group or variation in the plane of the boned amide C, N, and h groups. Mohammadi et al. (2016) reported that the amide regions of

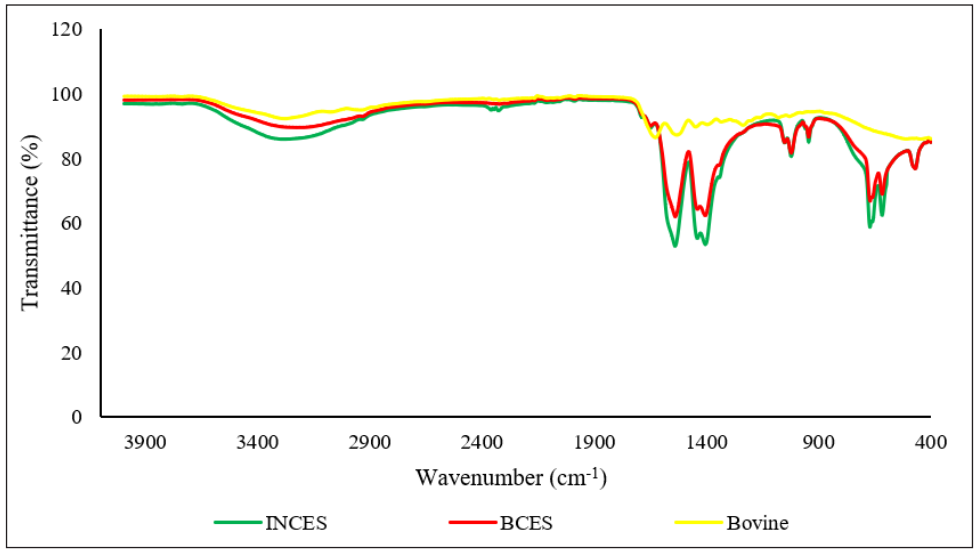



*Figure 3*. FTIR study of gelatin from bovine skin, broiler chicken eggshell membrane (BCES), and Indonesian native chicken eggshell membrane (INCES)

gelatine spectra from eggshell membrane appeared at 3385.9 $cm^{-1}$ (amide A), 2946.2 $cm^{-1}$ (amide B), 1654.1 $cm^{-1}$ (amide I), 1552.5 $cm^{-1}$ (amide II), and 1239.1 $cm^{-1}$ (amide III). According to Pulidori et al. (2023), the amide I region (1600-1700 $cm^{-1}$) corresponds to the secondary structure of gelatine. Within this spectral region, peaks at 1610-1642 $cm^{-1}$ are indicative of β-sheet structures, while those at 1642–1650 $cm^{-1}$ correspond to random coil conformations. Absorptions between 1650–1660 $cm^{-1}$ reflect α-helix structures, peaks at 1660–1680 $cm^{-1}$ denote β-turns, and those observed at 1680–1700 $cm^{-1}$ represent β-antiparallel arrangements. Accordingly, the distribution of amide I bands provides meaningful insight into the relative abundance of secondary structures in INCES, BCES, and bovine skin gelatine, thereby elucidating their functional and physicochemical properties. These FTIR findings are supported by previous SDS-PAGE analysis of collagen extracted from the same INCES raw material, which identified the characteristic α1, α2, β, and γ chains of type I collagen (Febrisiantosa et al., 2024). This complementary evidence confirms the molecular characteristics of the collagen precursor from which the gelatine was derived. However, the functional characterisation is highly recommended to validate the relationship between these functional groups and the mechanical strength of the gelatine

From these observations, it can be concluded that all three samples exhibit secondary structures characteristic of gelatine. β-sheet conformations dominate the INCES and bovine gelatine samples, whereas the BCES sample is characterised mainly by a random coil structure. Gelatine, a hydrolysed form of collagen, possesses β-sheet structures that impart mechanical strength, structural stability, and pronounced gel-forming capacity.

Table 2
*The Amida region of the peak wave number ($cm^{-1}$) of gelatine source*

| Amida Region | Gelatine SourceS | | | Assignment |
|---|---|---|---|---|
| | INCES | BCES | Bovine skin | |
| A | 3196.32 | 3227.47 | 3276.61 | Hydrogen bond stretched by NH |
| B | 2112.36 | 2112.12 | 2109.06 | Asymmetric stretching vibration of CH and $NH3^{+}$ |
| I | 1602.86 | 1648.91 | 1628.20 | COO is stretched by the C=O bond |
| II | 1527.54 | 1539.06 | 1532.46 | The peptide group out-of-phase combination of in-plane NH deformation and CN stretch modes is responsible for the fluctuation mode |
| III | 1410.41 | 1440.65 | 1448.61 | Variation in the glycine CH2 group or variation in the plane of the boned amide C, N, and h groups |

*Note.* INCES: Gelatine from Indonesian native chicken eggshell membrane; BCES: Gelatine from broiler chicken eggshell membrane (BCES)

These attributes underpin its widespread application as a tablet binder, capsule former, emulsifier, and film former across the food and pharmaceutical industries. In addition, the random coil conformation of gelatine provides a molecular arrangement that facilitates polypeptide aggregation and interaction, a process essential for reversible gel formation. This structural flexibility enables gelatine molecules to adapt their shape and establish interactions that promote gelation under varying temperature conditions.

Raw material characteristics and extraction parameters, including temperature, heating duration, and acid or alkali treatment, strongly influence variations in gelatine structure. These factors determine the extent of collagen chain degradation, average molecular weight, and amino acid composition. Consequently, the molecular architecture shaped by raw material composition and processing conditions governs key physical properties such as gel strength, viscosity, and solubility. For BCES gelatine, the eggshell membrane was separated from the shell through heating, whereas no comparable pre-treatment was applied to BCES and bovine skin gelatine. Exposure to temperatures exceeding 60 °C likely induced protein denaturation, disrupting certain bonds and thereby modifying the secondary structure of gelatine. This observation is consistent with earlier reports demonstrating that thermal treatment exerts a significant influence on the molecular arrangement and secondary structure of gelatine (Karim & Bhat, 2009).

## Comparative X-Ray Fluorescence (XRF) analysis of gelatine derived from different sources, one-way analysis of variance (ANOVA)

The results of the ANOVA analysis showed that gelatine from various sources (INCES, BCES and bovine skin) had significantly different mineral element profiles as shown in Table 3. The calcium content (Ca) in INCES and BCES gelatine was similar (around 50.36%) and much higher than bovine (0.13 %; $p<0.05$), indicating that gelatine from

Table 3
*Macro and micromineral composition by XRF analysis*

| Elements | Gelatine Source | | |
|---|---|---|---|
| | **INCES** | **BCES** | **Bovine Skin** |
| **Micromineral** | | | |
| Mg (%) | 0.50±0.01a | 0.24±0.13b | nd |
| P (%) | 0.51±0.01a | 0.45±0.08a | 0.24±0.04b |
| S (%) | 0.12±0.01b | 0.06±0.01b | 0.95±0.09a |
| Ca (%) | 50.36±0.04a | 50.36±1.19a | 0.13±0.01b |
| **Micromineral** | | | |
| Cl (ppm) | 114.33±0.01c | 371.05±37.55b | 596.80±74.80a |
| Fe (ppm) | 402.50±0.10a | 276.65±0.35b | 122.90±16.81c |
| Zn (ppm) | 450.25±4.35a | 439.45±3.85b | 9.27±3.58c |

*Note.* INCES: Gelatine from Indonesian native chicken eggshell membrane; BCES: Gelatine from broiler chicken eggshell membrane (BCES); nd: not detected; Notations without similar letters indicate significant differences ($p < 0.05$)

bovine skin underwent a more effective demineralisation process during the raw material preparation stage. This is due to the theory that the demineralisation process in gelatine production is often more intensive in bovine skin raw materials, considering that its denser collagen structure requires longer chemical treatment to soften the collagen fibres (Amalia et al., 2023). In contrast, the high calcium levels detected in INCES and BCES gelatine suggest that demineralisation of poultry skin was incomplete, most likely due to variations in collagen density, tissue composition, and processing parameters between poultry and bovine sources.

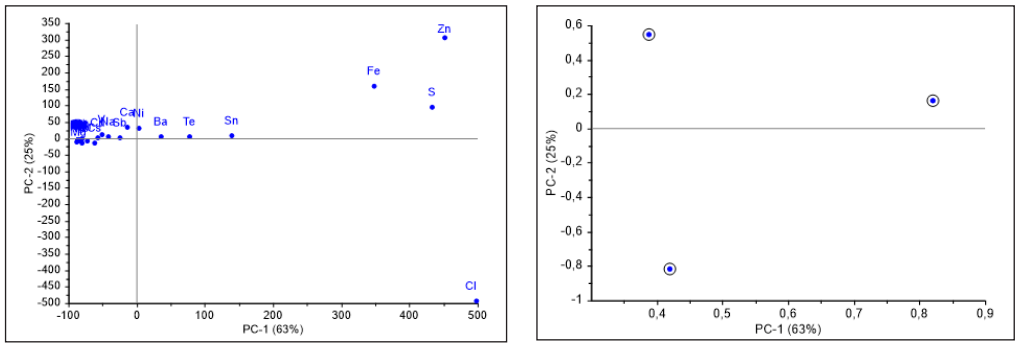
The iron (Fe) concentration in INCES gelatine (402.50 ppm) was markedly higher than that observed in BCES (276.65 ppm) and bovine gelatine (122.90 ppm) ($p < 0.05$). This elevated level is likely attributable to residual haemoglobin or tissue fragments from poultry skin that were not fully eliminated during processing. Arnesen and Gildberg (2007) similarly noted that incomplete removal of residual tissues during extraction may result in increased iron content in gelatine. In contrast, bovine gelatine exhibited the highest sulphur (S) concentration (0.95 ppm), compared with INCES (0.12 ppm) and BCES (0.06 ppm) ($p < 0.05$). This suggests a higher presence of keratin or sulphur-containing proteins, which are known constituents of bovine skin. Mariod and Adam (2013) reported that sulphur compounds originating from keratin may affect the mechanical behaviour of gelatine, particularly its elasticity and gel-forming capacity. In addition, the relatively high zinc (Zn) concentrations in INCES (450.25 ppm) and BCES gelatine (439.45 ppm) indicate the physiological role of zinc as a structural component of the skin and supporting tissues. In contrast, the lower zinc content observed in bovine gelatine (9.27 ppm) reflects

a more efficient mineral removal process. According to Jongjareonrak et al. (2006), zinc plays a pivotal role in sustaining collagen stability, which subsequently governs the physicochemical attributes of gelatine, notably gel strength and viscosity.

## Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is primarily employed to reduce the dimensionality of gelatine XRF data and to identify distinctive patterns or features that facilitate the differentiation or classification of gelatine sources. By projecting the data onto two or three principal components, scatterplot visualisation reveals natural groupings and trends among the samples. As shown in Figure 4, PC-1 accounts for 63% of the total variation, while PC-2 explains 25%, together representing 88% of the overall variability. These results indicate that the first two principal components captured most of the variation in the XRF dataset, providing an effective representation of the elemental composition among the gelatine samples. The PCA results showed differences in the characteristics of the elements in gelatine derived from INCES, BCES, and bovine skin. This analysis also revealed the influence of certain elements, such as Cl, Zn, and S, contributing to the differentiation of gelatine sources, as described in the scores plot (Figure 4a) and loading plot (Figure 4b). In the INCES gelatine sample, chlorine (Cl) was identified as the dominant element, contributing substantially to the variability captured by PC-2.

The occurrence of Cl is commonly associated with inorganic compounds such as calcium chloride, which may form during the extraction or processing of eggshell-derived gelatine (Huang et al., 2021). Its presence may also reflect the use of an acidic extraction medium, such as acetic acid, in this study. Comparable findings were reported by Mohammadi et al. (2016), who observed that incomplete demineralisation of eggshells can result in gelatine with relatively high residual mineral content. In the case of BCES gelatine, Zn and sulphur S were the principal contributors to PC-1. Zinc in gelatine typically




(a) (b)

*Figure 4.* (a) Elemental scores plot based on PCA; (b) Sample loadings plot based on PCA for gelatine types

originates from bone or connective tissue components (Liu et al., 2021), while Sarbon et al. (2013) noted that poultry-derived gelatine generally contains higher concentrations of essential elements compared with mammalian sources. The presence of sulphur reflects sulphur-containing amino acids, such as cysteine, which are characteristic of poultry-based gelatine. This suggests that BCES gelatine may have a protein structure enriched in sulphur-containing residues relative to the other samples.

Bovine skin gelatine contains trace elements such as tin (Sn) and tellurium (Te), which, although not predominant, distinguish it from other gelatine types. The presence of these elements may be associated with specific commercial processing methods or inherent characteristics of bovine skin. Karim and Bhat (2009) noted that bovine gelatine typically exhibits lower mineral content than gelatine derived from bones or eggshells, a difference attributed to the more intensive demineralisation procedures used during its production. These findings align with the theory of gelatine structure and composition, which states that the presence of trace mineral elements is strongly influenced by both the raw material source and the extraction process employed (Liu et al., 2021; Mohammadi et al., 2016). The presence of elements such as Zn and Cl highlights the potential functional role of gelatine as a supplementary mineral source, particularly in nutritional and biomedical contexts. Similarly, S, which is linked to protein structure via sulphur-containing amino acids, can influence the physicochemical properties of gelatine, including solubility and viscosity attributes essential for its industrial applications (Muyonga et al., 2004).

Differences in elemental composition among the gelatine types suggest their potential suitability for distinct application areas as shown in Figure 3. Bovine skin gelatine, with its relatively high Cl content, may possess functional properties advantageous for biomedical formulations. Conversely, INCES gelatine, characterised by elevated Zn and sulphur levels, appears more appropriate for nutritional or functional food applications, given the essential roles of these elements in human health. Overall, bovine skin gelatine, along with certain minor components, exhibits stable properties that support its use across diverse industrial sectors. Statistical analysis further confirmed significant variations in the mineral profiles of INCES, BCES, and bovine skin gelatine. Notably, the mineral composition of INCES and BCES gelatine was superior to that of bovine gelatine, underscoring their promise as candidates for targeted applications in functional foods and pharmaceutical products, where trace mineral content can influence product quality and functionality (Liu et al., 2009).

ANOVA analysis demonstrated significant differences in the mineral element profiles of gelatine obtained from INCEM, BCEM, and bovine skin. These results were further supported by PCA, which highlighted Fe, Ca, and Zn as the principal elements differentiating the gelatine sources. Collectively, the more mineral-rich compositions of INCEM and BCEM gelatine relative to bovine gelatine underscore their potential for specialised applications, particularly in functional foods and pharmaceutical products, where trace elements play a critical role in determining product quality and functionality.

## CONCLUSION

The study establishes that gelatine produced from Indonesian native chicken eggshell membranes exhibits distinct morphological, elemental, and structural features, reinforcing its potential as a sustainable substitute for traditional gelatine sources. SEM observations revealed that INCES gelatine contained finer and more homogeneous particles than BCES and bovine skin gelatine. Consistently, EDX analysis detected higher calcium levels in INCES and BCES, attributable to incomplete demineralisation during processing. FTIR spectra indicated that INCES and bovine gelatines predominantly displayed β-sheet structures, while BCES gelatine was dominated by random coil conformations. XRF analysis confirmed significant calcium enrichment in INCES gelatine, emphasising its unique mineral composition. Overall, these results underscore the promise of INCES-derived gelatine as a functional biomaterial, while simultaneously supporting the valorisation of eggshell membrane waste and the development of ethical, sustainable gelatine alternatives.

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