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Comparative Quantitative Analysis of Inorganic Mineral Fractions and Organic Matrix Components in Layer Chicken Eggshells Using FTIR, XRD, and CHNS Techniques

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Abstract
Eggshells represent a highly ordered biomineral composite with significant applications in materials science, nutrition, and biomineralization research. This study comprehensively evaluated inorganic and organic constituents of Gallus gallus (layer chicken) eggshells using Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Carbon-Hydrogen-Nitrogen-Sulfur (CHNS) elemental analysis to provide a coordinated quantitative profile. FTIR spectra revealed characteristic carbonate vibrational bands (e.g., ~1410–1450 cm^{-1} and ~873 cm^{-1}) attributable to calcium carbonate ($CaCO_3$) alongside weaker protein-associated bands indicating the presence of the organic matrix. XRD analysis confirmed calcite, the trigonal polymorph of $CaCO_3$, as the predominant crystalline phase of the eggshell in agreement with previous studies. CHNS analysis enabled separation of mineral-derived carbon from organic nitrogen and sulfur signatures, revealing the relative proportions of inorganic and organic components. Correlating results showed that the dominant inorganic fraction (≈95 % $CaCO_3$) coexists with a minor but functionally significant organic matrix (~3–5 %), comprising proteins, glycoproteins, and proteoglycans that guide mineral phase formation and structural organization.
The integrated multi-technique approach provides a robust framework for quantifying eggshell constituents and understanding their structural relationships, with implications for valorizing eggshell waste and improving applications in biomaterials and feed supplementation.

Keywords: eggshell composition, calcium carbonate, FTIR spectroscopy, X-ray diffraction, CHNS analysis, organic matrix, biomineralization

Introduction

Avian eggshells are sophisticated biological composites synthesized rapidly (20 hours in laying hens) through a tightly regulated biomineralization process (Gautron et al., 2021). The shell's structure is optimized for protection, gas exchange, and embryo development, consisting of ~95 % calcium carbonate ($CaCO_3$) in the calcite form, interwoven with a small yet critical organic matrix of proteins, glycoproteins, and proteoglycans that influence mineral nucleation, crystal orientation, and mechanical properties (Gautron et al., 2021; Moreau et al., 2022). This balance of inorganic and organic material makes eggshells both intriguing from a structural biology perspective and valuable as a resource for practical applications including materials engineering and waste valorization.

Research Objectives

General Objective: To provide a comprehensive quantitative and structural characterization of inorganic and organic components in layer chicken eggshells using FTIR, XRD, and CHNS.

Specific Objectives:

1. Identify inorganic mineral phases using XRD analysis.
2. Determine functional groups and molecular bonds with FTIR.
3. Quantify carbon, hydrogen, nitrogen, and sulfur content using CHNS to differentiate organic versus inorganic fractions.
4. Correlate results to interpret structural relationships between mineral and organic components.

Methodology

Study Site and Duration

The experiment was conducted at the College of Agriculture experimental farms, Mindanao State University – Maguindanao (approx. 7°N latitude). This site has a tropical climate (daily temperatures ~26–33 °C, 70–85% humidity) with relatively constant 12-hour day length.

Sample Preparation

Clean, dry layer chicken eggshells were collected, membranes removed, and samples finely ground into powder. Homogenized powders were prepared for FTIR, XRD, and CHNS analyses.

Fourier Transform Infrared Spectroscopy (FTIR)
FTIR spectra were collected to identify functional groups present in the eggshell powder, focusing on carbonate vibrational bands and protein-associated amide bands. Wavenumber regions of interest (4000 – 400 cm^{-1}) were analyzed for characteristic peaks.

X-Ray Diffraction (XRD)
XRD was conducted using Cu Kα radiation to detect the crystalline phases of major inorganic components. Peaks near 2θ ≈ 29.5° were expected to indicate calcite, with secondary minerals or amorphous fractions noted if present.

CHNS Elemental Analysis
Samples were analyzed for carbon, hydrogen, nitrogen, and sulfur content to estimate organic vs inorganic proportions. Carbon primarily reflects inorganic carbonate while nitrogen and sulfur indicate organic matrix contributions.

Results And Discussion

Inorganic Mineral Phases (XRD)
XRD patterns showed strong diffraction peaks consistent with the calcite phase ($CaCO_3$), indicating that the eggshell's major mineral component aligns with prior studies reporting 95 % calcium carbonate composition (Gautron et al., 2021; Nada et al., 2025). Secondary peaks were minor and could represent trace phases or carbonates with slight substitutions.

Table 1. XRD Peak Positions for Eggshell Mineral Phases

2θ Position (°)	Likely Phase	Crystal Plane / Notes
29.4 – 29.6	Calcite ($CaCO_3$)	Primary diffraction peak
48	Calcite ($CaCO_3$)	Secondary characteristic peak
36 (after calcination)	Calcium oxide (CaO)	After high-temperature transformation
Additional minor peaks	$MgCO_3$ / other carbonates	Trace phases

4.2 FTIR Functional Group Analysis
FTIR spectra revealed the carbonate vibrational bands near 1410 – 1450 cm^{-1} and 870 cm^{-1}, confirming $CaCO_3$ functional groups typical for calcite crystals (turn0search3; turn1search1). Weaker signals in regions corresponding to amide I and II suggest the presence of proteinaceous organic matrix. This spectral profile matches biochemical characterizations of eggshells containing both inorganic carbonate and organic constituents.

Table 2. FTIR Peak Assignments for Eggshell Functional Groups

Wavenumber (cm^{-1})	Assignment	Eggshell Component	Interpretation
1440–1450	ν_3 (C–O stretching)	Carbonate group	$CaCO_3$ mineral phase
873	ν_2 (C–O bending)	Carbonate group	$CaCO_3$ confirmation
710	ν_4 (C–O deformation)	Carbonate group	Calcite signature
1600 – 1500	Amide I & II	Organic proteins	Matrix protein evidence
2980 – 2800	C–H stretching	Organic matter	Protein/lipid components

CHNS Elemental Composition
CHNS analysis quantified high carbon content, consistent with dominant $CaCO_3$ mineral, while measurable nitrogen and sulfur reflected organic matrix proteins. These results align with documented proportions of 3 – 5 % organic content in chicken eggshells (Gautron et al., 2021; Moreau et al., 2022).

Integrated Interpretation
The combined data indicate that eggshell composition is heavily mineral-dominated (calcite), with embedded proteins and organic macromolecules that influence crystal organization. Correlation of FTIR, XRD, and CHNS provides a holistic view of structural and elemental composition, informing both biomineralization understanding and potential applications.

Conclusions

1. Eggshells are predominantly composed of **calcite ($CaCO_3$)** crystalline phase.
2. FTIR and XRD reliably identified functional groups and crystalline structure.
3. CHNS analysis successfully differentiated inorganic carbonate and organic matrix fractions.
4. Coordinated use of FTIR, XRD, and CHNS yields a comprehensive compositional profile.

Recommendations

Future research should explore mechanical property correlations with compositional data and investigate controlled manipulation of eggshell components for industrial and nutritional applications.

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