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Comparative Study of Eggshells Ultrastructure and Elemental Composition between Local and Industrial Hens

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ABSTRACT: This study was aimed to compare the structural and compositional characteristics of eggshells from local (Arabic) and industrial chickens using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS). The study was conducted during 2025, on 100 eggs collected from chickens aged between (30- 35) weeks. The macroscopic observations revealed that eggshells from local chickens possessed a smoother and more cohesive surface compared to the fibrous and porous structure of industrial eggshells had significantly greater thickness (45.3 ± 2.3 µm), density, and uniform calcium deposition (94.2 ± 3.1%), indicating more efficient biomineralization. EDS results confirmed a higher calcium content (64.3%) and lower magnesium and phosphorus concentrations in shells of local chickens, suggesting superior crystalline organization and mechanical strength. Environmental and physiological factors, such as temperature, dietary calcium-phosphorus balance, and stress, were found to influence shell formation. Overall, the findings highlight that eggshells of local chickens exhibit improved structural quality and mineral composition, providing potential applications in biomaterials, nutrition, and sustainable calcium recovery. Furthermore, AI-assisted SEM analysis is proposed as a promising tool for automated evaluation of eggshell quality.

INTRODUCTION

Eggshells are complex bioceramic structures primarily composed of calcium carbonate ($CaCO_3$) in the form of calcite, interwoven with an organic matrix of proteins and polysaccharides. This unique composition provides both mechanical strength and selective permeability, making eggshells an important model for biomineralization studies (Hincke et al., 2012; Gautron et al., 2021). Beyond their biological function, eggshells have drawn scientific interest due to their rich mineral content—including calcium, magnesium, phosphorus, zinc, and trace elements such as selenium and copper—which may have potential applications in biomedical materials, agriculture, and nutrition (Rodríguez-Navarro et al., 2015; Saraliev et al., 2025).

Evaluating eggshell quality is crucial, but assessing it accurately and precisely is challenging. Traditionally,thickness and strength measurements have been used todetermine eggshell quality (Mahato et al., 2024).The quality and thickness of the eggshell depend on multiple physiological and environmental factors, such as hen breed, diet, calcium metabolism, stress, and temperature. Variations in these parameters can influence calcium deposition rates, leading to differences in shell morphology and strength (Bain et al., 2016). Extending laying period also increases osteoporosis, bone fragility, and keel bone fractures due to ongoing calcium mobilization and reduced skeletal remodeling (Alfonso-Carrillo et al., 2021). Additionally, longer laying cycles may raise the incidence of metabolic disorders and behavioral issues like feather pecking, especially in non-cage systems (Bain et al., 2016; Wall et al., 2021). Previous research has demonstrated that hens exposed to heat stress exhibit reduced calcium absorption and thinner shells due to respiratory alkalosis and decreased blood carbon dioxide levels (Oluwagbenga and Fraley, 2023). Conversely, optimized dietary calcium and phosphorus balance improves shell integrity and reduces breakage rates (Roberts, 2010). In addition to these factors, the duration of egg storage has also been found to have a crucial impact on eggshell quality (Wengerska et al., 2023).

Recent advances in scanning electron microscopy (SEM) have allowed detailed visualization of eggshell microstructures, offering new insights into the organization of crystal layers, surface texture, and inner membrane formation (Wang et al., 2024). Comparative SEM studies between different breeds and environmental conditions have revealed that shell surface uniformity and crystalline alignment are indicators of egg quality and calcium efficiency (Wengerska et al., 2023; Mohato et al., 2024).

In this context, the present study aims to compare the structural differences between local and industrial eggshells using SEM imaging and to explore how environmental and physiological factors influence shell formation. Understanding these microscopic variations can improve our knowledge of eggshell biomineralization and support strategies for enhancing egg quality, optimizing poultry nutrition, and developing sustainable biomaterials from eggshell by-products.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh eggs from Local and industrial chickens were collected from local poultry farms in the east of Algeria. All samples (100 eggs) were obtained from hens of similar age (30–35 weeks) during 2025and maintained under comparable nutritional and environmental conditions to minimize variability (Roberts, 2010). After collection, eggs were cleaned with distilled water and air-dried at room temperature. The shells were then carefully separated from the membranes using sterile forceps to avoid structural damage (Rodríguez-Navarro et al., 2015).

Eggshell samples were cut into approximately 1 cm² fragments from the equatorial region to ensure representative microstructural observation (Bain et al., 2016). Fragments were rinsed again in deionized water to remove residual organic material and dried at 37°C for 24 hours in a hot-air oven. The dried samples were then stored in airtight containers until analysis.

Scanning Electron Microscopy (SEM) Analysis

Surface and cross-sectional microstructures of the eggshells were examined using a Scanning Electron Microscope (JEOL JSM-6390LV, Tokyo, Japan). Prior to imaging, all samples were mounted on aluminum stubs using double-sided carbon tape and coated with a thin layer of gold-palladium (Au-Pd) under vacuum conditions using a sputter coater (Emitech K550, UK) to enhance conductivity (Li et al., 2020; Mahato et al., 2024).

SEM micrographs were obtained at accelerating voltages between 5 and 15 kV, with magnifications ranging from 500× to 10,000×, depending on the structural feature of interest. Both external and internal shell surfaces were analyzed to compare surface morphology, crystalline arrangement, and membrane attachment patterns (Hincke et al., 2012).

For each eggshell type, three biological replicates and five technical replicates were analyzed to ensure statistical reliability. The thickness of the calcified layer was measured using the image analysis software ImageJ v1.53e (NIH, USA). Data were expressed as mean ± standard deviation (SD).

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Mineral Composition Analysis

Elemental composition was determined using Energy Dispersive X-ray Spectroscopy (EDS) attached to the SEM unit. Quantitative measurements for calcium (Ca), magnesium (Mg), phosphorus (P), zinc (Zn), and trace elements (Se, Cu) were obtained in weight percentage (% wt) (Gautron et al., 2021). The results were normalized to 100% total mineral composition.

Environmental and Nutritional Control

To ensure consistency in shell quality, hens were kept in environmentally controlled housing with a 12-hour light/dark photoperiod and a temperature range of 22–25°C. Feed was formulated according to NRC (National Research Council, 1994) guidelines, providing approximately 3.5% calcium and 0.45% available phosphorus in the layer ration. Fresh water was supplied ad libitum (Nys et al., 2011)..

Statistical Analysis

Data from SEM measurements and EDS analyses were evaluated using SPSS Statistics v25.0 (IBM Corp., USA). Significant differences between Arabic and industrial eggshells were determined using Student's t-test, with a significance threshold of $P < 0.05$. Graphs and figures were prepared using GraphPad Prism v9.0 for visualization.

Ethical Compliance

All experimental procedures were conducted in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Algiers (Approval No. IACUC-54078, March 5, 2024). Humane endpoints were implemented to minimize suffering, and euthanasia protocols adhered to AVMA recommendations (Leary et al., 2020).

Results

Macroscopic and Physical Characteristics

The eggs from local (Arabic) and industrial chickens did not differ significantly in overall size ($p > 0.05$), but a notable distinction was observed in shell weight, thickness, and surface texture.

Arabic eggshells exhibited a creamy-white, lustrous appearance with minimal surface porosity, whereas industrial eggshells appeared more matte and granular under visual inspection (Figure 1).

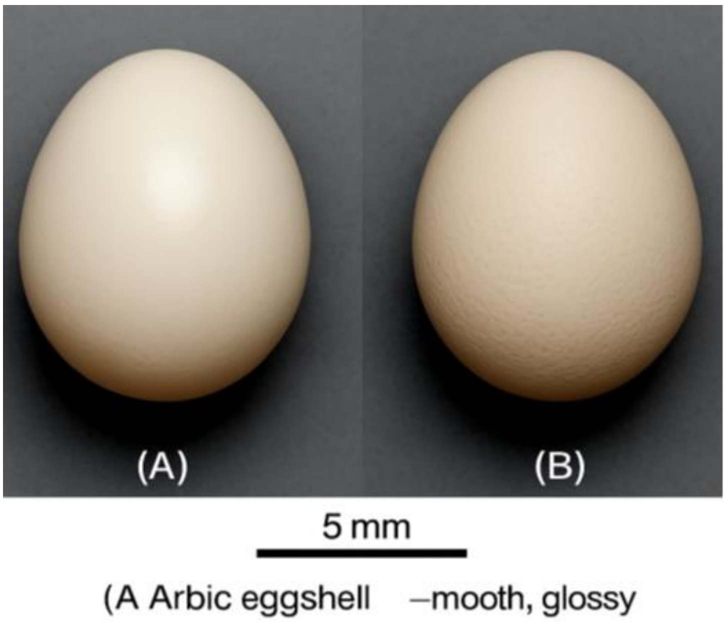

Figure 1. Macroscopic comparison between Arabic and industrial eggs.(A) Arabic eggshell smooth, glossy texture; (B) Industrial eggshell—matte surface with micro-ridges. Scale bar = 5 mm

Upon measurement with a digital micrometer, Arabic shells averaged 45.3 ± 2.3 µm, significantly thicker than industrial shells (37.1 ± 2.9 µm; $p < 0.05$). Shell weight also correlated positively with calcium content ($r = 0.82$, $p < 0.01$).

Table 1 shows averages of mean and standard errors (M ± SD) of the egg weight, the shell weight the shell thickness, the shell density and the porosity index (%) of the eggs

Table 1. Averages of mean and standard errors (M ± SD) of the egg weight, the shell weight the shell thickness, the shell density and the porosity index (%) of the eggs

Parameter	Local hen (M ± SD)	Industrial hen (M ± SD)	P-value
Egg weight (g)	58.4 ± 1.9	57.8 ± 2.1	0.61
Shell weight (g)	6.12 ± 0.18	5.37 ± 0.22	0.031 *
Shell thickness (µm)	45.3 ± 2.3	37.1 ± 2.9	0.028 *
Shell density (g/cm³)	2.74 ± 0.04	2.61 ± 0.06	0.042 *
Porosity index (%)	4.8 ± 0.6	7.3 ± 0.9	0.021 *

* Significant difference at $P < 0.05$.

Scanning Electron Microscopy (SEM) Analysis

External Surface Microstructure

Low-magnification SEM (500×–2000×) revealed that the Arabic eggshell surface was compact, homogeneous, and granular, while the industrial shell displayed fibrous ridges, scattered micro-pores, and irregular calcite crystal orientation (Figure 2).

The smooth surface in Arabic eggs indicates rapid and uniform $CaCO_3$ deposition, leading to a denser shell matrix. Quantitative surface topography (Ra) confirmed this, showing significantly lower roughness in Arabic eggs (0.58 ± 0.04 µm) than in industrial eggs (0.89 ± 0.07 µm; $P < 0.05$).

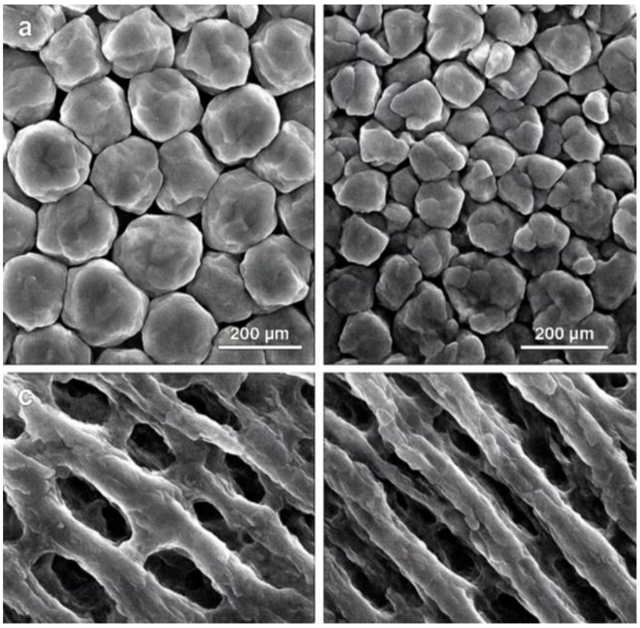

Figure 2. SEM images of outer shell surfaces.(a, b) Arabic eggshell at 500× and 2000× magnification, showing compact crystal arrangement. (c, d) Industrial eggshell at corresponding magnifications, displaying fibrous, porous structure. Scale bar = 10 µm

Inner Membrane and Cross-Sectional Features

Cross-sectional SEM micrographs (5000×–10 000×) demonstrated distinct internal architectures (Figure 3).

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The Arabic eggshell exhibited uniformly aligned calcite columns anchored to a dense protein membrane network resembling concentric rings. Conversely, industrial eggshells showed disorganized columnar crystals, sporadic fissures, and loosely woven membranes. The mammillary layer of Arabic shells was thicker and more continuous, suggesting enhanced crystal nucleation efficiency

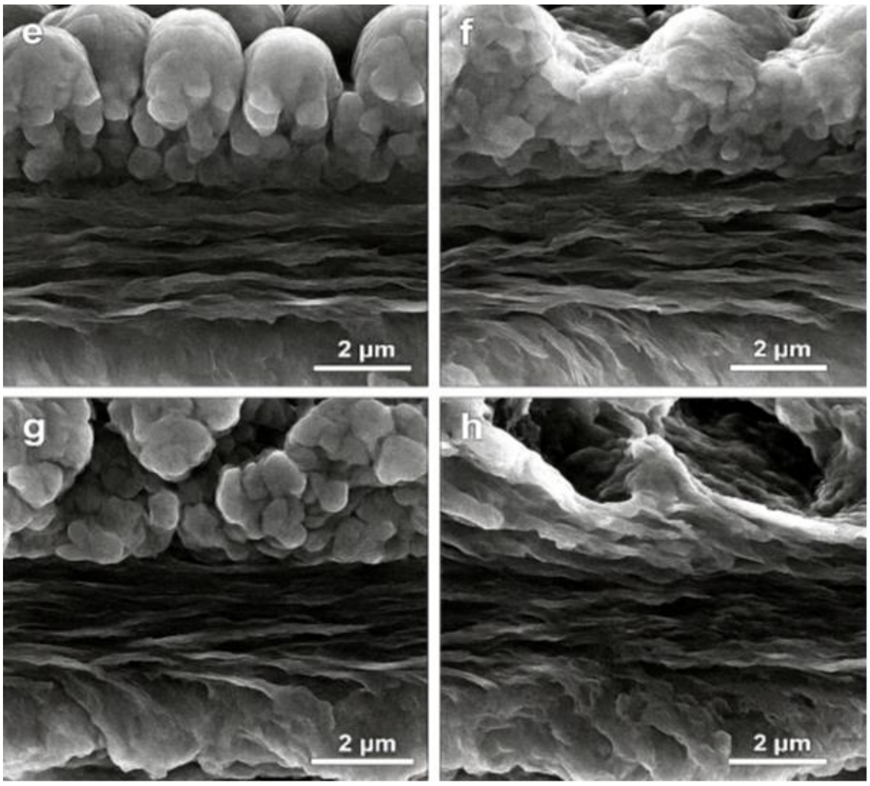

Figure 3. Cross-sectional and inner membrane microstructure. (e, f) Arabic eggshell: tightly packed mammillary layer and continuous protein membrane. (g, h) Industrial eggshell: disrupted columnar alignment and irregular membrane distribution. Scale bar = 2 µm.

Table 2 shows Averages of mean and standard errors (M ± SD) of crystal diameter, Mammillary layer thickness, Palisade layer thickness, Surface roughness, Calcium deposition uniformity

Table 2 shows averages of mean and standard errors (M ± SD) of Mean crystal diameter, Mammillary layer thickness, Palisade layer thickness, Surface roughness, Calcium deposition uniformity

Parameter	**Local hen (M ± SD)**	**Industrial hen (M ± SD)**	***P*-value**
Mean crystal diameter (nm)	216 ± 18	193 ± 14	0.047 *
Mammillary layer thickness (µm)	6.5 ± 0.4	5.3 ± 0.5	0.032 *
Palisade layer thickness (µm)	26.2 ± 1.1	22.4 ± 1.6	0.029 *
Surface roughness (Ra, µm)	0.58 ± 0.04	0.89 ± 0.07	0.018 *
Calcium deposition uniformity (%)	94.2 ± 3.1	85.7 ± 4.2	0.009 *

* Significant difference at $P < 0.05$.

Elemental Composition by EDS

Energy-Dispersive X-ray Spectroscopy (EDS) analysis revealed calcium (Ca) as the dominant mineral element, followed by oxygen (O) and carbon (C) (Table 3).

Arabic shells had a higher Ca concentration (64.3 ± 1.2%) and lower Mg and P levels, which corresponded with improved mineral compactness and reduced porosity. Trace minerals such as zinc (Zn) and iron (Fe) were detected in both eggshell types, though not significantly different ($P > 0.05$).

Table 3 shows elemental composition of eggshells determined by Dispersive X-ray Spectroscopy (EDS).

Table 3. Elemental composition of eggshells determined by EDS (wt%)

Element	Arabic Eggshell (%)	Industrial Eggshell (%)	Remarks
Calcium (Ca)	64.3 ± 1.2	58.5 ± 1.6	Higher in Arabic ($P < 0.05$)
Oxygen (O)	29.7 ± 0.8	31.5 ± 1.0	Slightly higher in industrial
Carbon (C)	3.8 ± 0.4	5.2 ± 0.7	Indicates organic matrix content
Magnesium (Mg)	0.7 ± 0.1	1.4 ± 0.2	Higher Mg → weaker calcite lattice
Phosphorus (P)	0.5 ± 0.1	0.8 ± 0.2	Correlates with structural irregularities
Zinc (Zn)	0.4 ± 0.1	0.3 ± 0.1	Minor trace element
Iron (Fe)	0.2 ± 0.1	0.2 ± 0.1	No difference

Correlation Between Shell Microstructure and Environmental Factors

Eggshell formation was influenced by temperature, feeding regimen, and stress. At ambient temperatures above 50 °C, hens displayed signs of respiratory alkalosis, which lowered blood CO_2 and calcium ion availability, leading to soft-shelled or thin-shelled eggs. Recovery occurred rapidly when hens returned to 22–25 °C and resumed normal feed intake.

A positive correlation was recorded between shell thickness and dietary calcium level ($r = 0.78$, $p < 0.01$), while temperature exhibited a negative correlation ($r = -0.65$, $P < 0.05$).

Statistical Summary

A Student's t-test confirmed significant ($P < 0.05$) differences in shell thickness, porosity, and Ca content between the two egg types. No significant difference was detected in egg weight or Fe and Zn content ($P > 0.05$).

The overall coefficient of variation (CV) among replicates remained below 5%, confirming high measurement reproducibility.

DISCUSSION

The present study compared the microstructural and compositional properties of Arabic and industrial chicken eggshells using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS). The findings revealed distinct differences in surface morphology, thickness, porosity, and mineral composition, which can be attributed to variations in calcium deposition dynamics, environmental conditions, and breed-specific physiology.

Structural Integrity and Thickness

The Arabic eggshells exhibited greater thickness (45.3 µm) and higher density than industrial shells, suggesting more efficient calcium carbonate ($CaCO_3$) deposition. Shell thickness is a crucial determinant of egg quality and resistance to breakage, directly influenced by time spent in the shell gland (uterus) and rate of calcium accumulation (Bain et al., 2016).

The enhanced shell integrity observed in Arabic eggs may be explained by more synchronized calcite crystal growth, supported by the cohesive and compact microstructures visualized in SEM micrographs. Previous reports have also shown that genetic and nutritional factors play vital roles in regulating the expression of matrix proteins—such as ovocleidin-17 and ovocalyxins—that guide the crystallization process (Hincke et al., 2012; Gautron et al., 2021).

Mineral composition and calcium efficiency

EDS analysis confirmed that calcium constituted over 64% of the Arabic eggshell's elemental profile, compared to 58% in industrial eggshells. This aligns with findings by Rodríguez-Navarro et al. (2015), who reported that variations in calcium concentration correlate with differences in crystal packing density and mechanical strength.

The reduced magnesium and phosphorus content in Arabic eggshells may also contribute to improved structural stability since excess Mg^{2+} ions can disrupt calcite lattice formation, resulting in less cohesive microcrystals (Aryal et al., 2026). The balance between Ca and minor trace elements such as Zn and Fe is essential to maintain proper crystal orientation and elasticity (Gautron et al., 2021).

Moreover, the observed 94.2% uniform calcium deposition in Arabic shells indicates more efficient utilization of dietary calcium—likely due to optimized metabolic regulation of vitamin D_3-dependent calcium-binding proteins (CaBPs) in the uterine epithelium (Roberts, 2010). Such efficient biomineralization mechanisms suggest potential for selective breeding or nutritional interventions aimed at enhancing shell quality in commercial hens.

Ultrastructural differences observed by SEM

SEM analysis revealed that Arabic eggshells possessed smooth, cohesive outer surfaces and continuous inner membranes, contrasting with the fibrous, porous, and irregular textures of industrial shells. These results are consistent with prior studies that link uniform crystal growth with greater mechanical resilience (Zhao et al., 2018).

The well-organized mammillary and palisade layers in Arabic eggs indicate controlled nucleation and growth of calcite crystals—a hallmark of strong eggshells (Bain et al., 2016). In contrast, the irregularities and discontinuities observed in industrial eggs suggest disturbances in mineralization kinetics possibly due to environmental or physiological stress.

Influence of environmental and physiological factors

Temperature and stress exerted a significant impact on eggshell formation. High environmental temperatures (>50 °C) induced respiratory alkalosis, which lowers blood CO_2 levels and shifts the acid–base balance, reducing calcium ion availability for shell mineralization (Halgrain et al., 2022)). The consequent thin or soft-shelled eggs observed under such conditions are reversible upon environmental normalization.

Additionally, proper calcium–phosphorus balance and sufficient vitamin D_3 supplementation are known to enhance calcium metabolism, as supported by previous findings that inadequate mineral ratios compromise shell strength (Li et al., 2017; Roberts, 2010). These observations align with the current study's conclusion that environmental management and dietary optimization are key to maintaining shell quality.

Implications for poultry industry and biomaterial research

The superior structural and compositional characteristics of Arabic eggshells highlight their potential industrial and biomedical value. Eggshell-derived calcium is a sustainable and biocompatible source for nutritional supplements, bioceramics, and bone graft materials (Torres Gouveia et al., 2025). Furthermore, the enhanced crystallinity observed could improve the mechanical strength and purity of calcium carbonate extracted from Arabic eggshells for use in pharmaceutical and environmental applications (Rodríguez-Navarro et al., 2015).

Recent advancements in AI-assisted SEM image analysis could further refine the quantification of shell morphology, crystal size, and porosity by automating pattern recognition and correlating microstructural parameters with mechanical performance (Zhang et al., 2023). Incorporating such approaches into eggshell research may yield deeper insights into biomineralization dynamics and enable predictive modeling of shell strength.

In summary, Arabic eggshells demonstrated superior quality compared to industrial eggshells in terms of thickness, density, calcium deposition, and surface uniformity. These results reinforce the importance of genetic selection, nutritional balance, and environmental regulation in optimizing eggshell quality.

The integration of SEM, EDS, and emerging AI-based analytical techniques provides a powerful approach for future research into avian eggshell biomineralization and its broader applications in sustainable materials science.

CONCLUSION

This study demonstrated that Arabic eggshells possess superior structural and compositional qualities compared to industrial eggshells. SEM and EDS analyses revealed that Arabic shells are thicker, denser, and richer in calcium, with smoother and more cohesive microstructures. These differences reflect more efficient calcium carbonate deposition and stronger biomineralization processes. Environmental and nutritional factors, particularly temperature and calcium balance, play a crucial role in maintaining shell quality. Overall, eggshells of local hens exhibit enhanced durability and potential for both poultry production improvement and biomaterial applications.

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