



## More than just a meal: The fascinating link between egg origins and lysosomal content a study from Rapides Parish, Louisiana

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### Abstract

Lysosomes are vital eukaryotic organelles, essential for cellular degradation, nutrient recycling, and antimicrobial defense. In biological systems, including avian eggs, they contribute significantly to internal stability and protection against microbial degradation. This study investigated whether the origin of eggs (organic, cage-free, and conventionally farmed) influences their lysosomal content. Using a differential centrifugation and pH adjustment protocol, egg white samples from these three backgrounds were processed to isolate lysosomal components. Results indicated that organic eggs yielded the highest relative concentration of lysosomal fluid, followed by cage-free and then conventionally farmed (store-bought) eggs. This suggests a potential correlation between egg production methods and the intrinsic antimicrobial capacity of eggs, potentially impacting their shelf life. While further quantitative analysis is warranted, these findings highlight the intricate link between an egg's background and its biochemical composition, with implications for egg quality and preservation.

**Keywords:** Lysosomes; Egg production methods; Antimicrobial capacity; Egg quality; Shelf life

### 1. Introduction

Lysosomes are fundamental organelles in eukaryotic cells, playing crucial roles in maintaining cellular homeostasis through the breakdown of waste materials, cellular debris, and macromolecules via a diverse array of hydrolytic enzymes (Mousavi et al., 2020). Beyond their general cellular functions, lysosomes, and specifically the enzyme lysozyme a prominent lysosomal enzyme are particularly abundant and significant in avian eggs. Lysozyme constitutes a major protein component of the hen's egg vitelline membrane, contributing approximately 37% of its total protein content (De Boeck and Stockx, 1986).

Within eggs, lysosomes are instrumental in establishing and maintaining an acidic microenvironment through the action of proton pumps. This acidic milieu is known to inhibit the proliferation of various bacteria, thereby contributing to the egg's innate defense mechanisms and extending its natural shelf life (Magar, 2021; Sani et al., 2023). The antibacterial properties of eggs are further enhanced by the ability of lysosomal enzymes to eliminate cellular components or entire microbial cells that could serve as substrates for bacterial activity (Mousavi et al., 2020).

While the role of lysosomes in egg preservation is established, the extent to which varying hen rearing conditions and egg production methods (often referred to as "backgrounds") influence the quantity or concentration of these protective organelles and their associated enzymes remains an area warranting investigation. Conventional agricultural practices, including dietary regimens and housing environments, differ significantly across organic, cage-free, and conventionally farmed systems. These differences could potentially impact the physiological state of the laying hens and, consequently,

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the biochemical composition and intrinsic defensive capabilities of the eggs they produce (Alig et al., 2024; Tumova et al., 2024).

This study hypothesizes that eggs produced under organic farming conditions will exhibit higher lysosomal concentrations compared to cage-free and conventionally farmed (store-bought) eggs. This hypothesis stems from the premise that potentially more consistent and favorable physiological conditions for hens in organic systems might translate to enhanced innate egg quality. To test this, a comparative analysis of lysosomal fluid yield from eggs obtained from diverse backgrounds within Rapides Parish, Louisiana, was conducted.

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## 2. Materials and Methods

### 2.1. Materials

- Centrifuge tubes
- Beakers (various sizes)
- Acetic acid (0.5 M)
- Centrifuge
- Distilled water
- pH meter
- Table salt (NaCl)
- Filter paper or cheesecloth (optional)
- Dilute base solution (e.g., dilute NaOH, if needed for pH adjustment)
- Organic eggs (Rapides Parish, Louisiana origin)
- Cage-free eggs (Rapides Parish, Louisiana origin)
- Conventionally farmed (store-bought) eggs (Rapides Parish, Louisiana origin)

### 2.2. Procedure

The following procedure was adapted for the relative isolation of lysosomal components from egg white:

- **Egg White Separation:** Carefully separate the egg white from the yolk for each egg sample. Discard the yolk.
- **Initial Homogenization and Salting Out:** For each egg white sample, add table salt to achieve a 5% (w/v) concentration. Stir the mixture continuously for 15-20 minutes to ensure thorough dissolution of the salt and initial protein precipitation.
- **First Centrifugation:** Transfer the salted egg white mixture into centrifuge tubes. Centrifuge the samples at 5000 x *g* for 15 minutes. This step aims to "salt out" and pellet larger protein aggregates and cellular debris, leaving soluble components, including lysosomal elements, in the supernatant.
- **Supernatant Collection and pH Adjustment:** Carefully collect the supernatant from each centrifuged sample. Slowly add 0.5 M acetic acid dropwise to the supernatant, continuously monitoring the pH with a pH meter, until the pH is adjusted to between 4.5 and 5.0. This pH range is optimal for the precipitation and concentration of certain lysosomal proteins, including lysozyme.
- **Second Centrifugation:** Place the pH-adjusted samples back into the centrifuge tubes and perform a second centrifugation run at 10000 x *g* for 15 minutes. This higher centrifugal force further concentrates the lysosomal components into a pellet.
- **Lysosomal Pellet Resuspension:** Carefully remove the supernatant. A small amount of distilled water was added to each centrifuge tube to resuspend the lysosomal pellet. If necessary, a dilute base solution was used to gently adjust the pH of the resuspended solution back to neutral (pH ~7.0) to stabilize the isolated components.
- **Clarification (Optional):** If the resulting lysosomal solution appeared cloudy due to residual particulate matter, it was filtered through filter paper or cheesecloth for clarification.

The relative quantity of lysosomal fluid was assessed visually and comparatively across the three egg types.

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## 3. Results

The comparative analysis of lysosomal fluid yield from eggs of different backgrounds is summarized in Table 1. Observable differences in the volume and apparent concentration of the isolated lysosomal fluid were noted.

**Table 1** Relative Lysosomal Fluid Quantity Comparison Across Egg Backgrounds

Egg Background	Relative Lysosomal Fluid Quantity
Organic Eggs	Highest amount of lysosomal fluid
Cage-Free Eggs	Middle amount of lysosomal fluid
Store-Bought Eggs	Lowest amount of lysosomal fluid

As presented in Table 1, organic eggs consistently yielded the highest perceived amount of lysosomal fluid following the extraction protocol. Cage-free eggs produced an intermediate quantity, while conventionally farmed (store-bought) eggs exhibited the lowest relative yield of lysosomal fluid.

#### 4. Discussion

The experimental procedure employed in this study, while rudimentary, effectively utilized fundamental biochemical principles to isolate and concentrate lysosomal components from egg white. The initial salting out with table salt (Step 2) serves to precipitate non-lysosomal proteins, a common technique for protein fractionation. The subsequent centrifugation steps, particularly the second run at higher centrifugal force (10000 x *g*), are crucial for concentrating the smaller, more soluble lysosomal components, including enzymes like lysozyme, into a pellet. While the isolated product is not entirely pure, it represents a relevant and comparable fraction of lysosomal material, allowing for a qualitative assessment of differences between egg types.

The primary finding of this study is the observed disparity in lysosomal fluid quantity among the different egg backgrounds, with organic eggs yielding the highest amount. This observation, while qualitative, provides preliminary support for the hypothesis that the environmental and management conditions associated with different egg production systems may influence the biochemical composition of eggs, specifically their lysosomal content.

From a biological perspective, lysosomes play a direct role in the egg's innate defense system. Their hydrolytic enzymes, particularly lysozyme, are potent antibacterial agents. They maintain an acidic environment crucial for inhibiting bacterial growth. Given that eggs, post-laying, are largely closed biological systems encased by a semi-permeable calcium carbonate shell (Magar, 2021), the internal biochemical environment is critical for preventing microbial spoilage. A higher concentration of active lysosomal components would theoretically provide a more robust and prolonged antibacterial defense within the egg.

Therefore, the inference can be made that eggs with a higher lysosomal concentration are likely to possess an extended shelf life. This aligns with consumer perception and some studies that suggest differences in egg quality and potentially shelf life based on production systems (Alig et al., 2024; Tumova et al., 2024). The proposed mechanism is that a greater presence of lysosomes contributes to a more effective and sustained antibacterial and acidic internal environment, thereby delaying the onset of internal decomposition caused by microbial activity.

It is important to acknowledge the limitations of this study. The quantification of lysosomal content was based on visual assessment of "lysosomal fluid" rather than precise biochemical assays for specific lysosomal enzymes or protein content. This qualitative approach, while indicative, requires validation through more rigorous quantitative methods (e.g., spectrophotometric assays for lysozyme activity, protein concentration measurements). Furthermore, the study did not directly measure shelf life; the correlation drawn is based on the known antibacterial function of lysosomes. Future research should involve controlled storage experiments to directly assess the shelf life of eggs from different backgrounds in conjunction with precise lysosomal enzyme quantification. Additionally, a deeper investigation into the specific rearing conditions (e.g., diet composition, stress levels, genetics) of hens from each background would be beneficial to elucidate the causal factors behind the observed differences in lysosomal content.

#### 5. Conclusion

This comparative study provides preliminary evidence suggesting a variability in lysosomal concentrations in eggs based on their production background, with organic eggs appearing to contain a higher relative amount of lysosomal fluid compared to cage-free and conventionally farmed eggs. Given the established role of lysosomes in the intrinsic antibacterial properties of eggs, these findings infer a potential link between egg background and their natural preservative capabilities, likely influencing shelf life. While further quantitative and direct shelf-life studies are

necessary to conclusively validate these assertions, the observed disparity underscores the importance of the hen's environment in shaping the biochemical composition of eggs and highlights a promising avenue for understanding and potentially enhancing egg quality through scientific inquiry.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict-of-interest to be disclosed.

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