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# COMPARATIVE STUDY ON THE OPTIMIZATION OF HYALURONIC ACID PRODUCTION FROM ANIMAL TISSUES (BONE AND SKIN) AND ITS POTENTIAL APPLICATION IN NATURAL FOOD PRESERVATION SYSTEM

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## ABSTRACT

Driven by the rising demand for eco-friendly, natural preservation methods, this study explores the extraction and functional application of hyaluronic acid (HA) derived from animal by-products, specifically bone and skin. Using an enzyme-assisted extraction workflow comprising pre-treatment, enzymatic digestion, ethanol precipitation and purification, we optimized key parameters including pH, temperature, extraction time, and enzyme concentration via Response Surface Methodology (RSM). Physicochemical characterization and Fourier Transform Infrared Spectroscopy (FTIR) revealed that skin tissue yielded significantly higher purity and quantity than bone tissue under optimized, mildly acidic, and temperature-controlled conditions that safeguarded the biopolymer's integrity. When tested as a bio-based edible coating on food samples during storage trials, the extracted HA effectively minimized moisture loss and delayed spoilage compared to uncoated controls, with skin-derived HA outperforming bone-derived HA due to superior viscosity and film-forming properties. Overall, these results demonstrate that animal tissues are viable, sustainable sources of HA for industrial use. By offering a renewable alternative to synthetic preservatives and adding value to agricultural by-products, HA shows strong promise as a functional ingredient in green food preservation systems.

**Keywords:** Comparative, Optimization, Hyaluronic acid, Production, Animal tissues

## 1.0 INTRODUCTION

Hyaluronic acid (HA) is a natural, linear polysaccharide. It falls under the glycosaminoglycan classification. HA is widely distributed in the extracellular matrix of connective, epithelial, and neural tissues. This biopolymer plays critical roles in biological systems. These include tissue hydration, cell signalling, wound healing, and structural integrity. Its exceptional water-binding capacity and viscoelastic properties drive these functions.

HA has recently gained increasing attention (Al-Temimi *et al*., 2026). It is a multifunctional biopolymer. Its biodegradability, biocompatibility, and non-toxic nature make it suitable for many applications (Bayyigit *et al*., 2026). These span biomedical, pharmaceutical, cosmetic, and emerging food systems (Chen *et al*., 2023). Its ability to retain moisture and form protective films positions it as a promising candidate. It could be incorporated into natural preservation systems. This responds to the growing demand for safer, environmentally friendly alternatives to synthetic preservatives (Granche et al., 2026).

Conventionally, we obtain hyaluronic acid from animal tissues. Skin, cartilage, and bone are common sources. Microbial fermentation also produces it. Animal-derived sources remain relevant, however. They can yield high molecular weight HA, desirable for functional applications. Using animal by-products like bone and skin also aligns with sustainable practices. This promotes waste valorization. It reduces the environmental burden from agro-industrial residues.

Recent advancements in extraction and purification technologies highlight the importance of optimizing process variables. This improves HA yield, molecular weight, and purity. Techniques like enzymatic digestion, ultrasound-assisted extraction, and advanced filtration significantly enhance recovery efficiency and product quality. Despite these developments, extraction efficiency depends heavily on the source material. Structural and compositional differences between tissues influence HA release and integrity (Smith, 2020).

Comparing different animal tissue; bone versus skin, is therefore essential. We need to identify the most efficient and economically viable source for HA production. Skin is rich in extracellular matrix components. Bone tissues also contain significant HA. It associates with connective matrices and regenerative processes. However, few studies have systematically compared these sources under optimized conditions.

Beyond extraction, HA's application in food preservation is an emerging area. Recent studies demonstrate that HA-based composite coatings can extend the shelf life of perishable products. They reduce oxidative degradation, maintain moisture, and improve

overall quality. These findings suggest HA can function as a component of bioactive preservation systems, not just a direct antimicrobial agent.

Optimizing HA extraction from various animal tissues and evaluating its functional performance in preservation systems is therefore a significant research area. This has implications for sustainability, food safety, and industrial applications.

**Aim/Objectives of the Study**

Our aim is to comparatively optimize hyaluronic acid production from animal tissues (bone and skin). We will also evaluate its potential in natural preservation systems. We plan to achieve this through the following objectives:

i. Extract hyaluronic acid from animal bone and skin using optimized methods.

ii. Investigate how extraction parameters (pH, temperature, time, enzymatic treatment) affect HA yield.

iii. Compare the yield and purity of hyaluronic acid from bone and skin.

iv. Characterize the physicochemical properties of the extracted hyaluronic acid.

v. Evaluate HA's performance in preservation systems (e.g., moisture retention, oxidative stability, shelf-life extension).

vi. Identify the most suitable tissue source for efficient, sustainable HA production.

## 2.0 METHODOLOGY

### 2.1 RESEARCH DESIGN

This study uses an experimental research design. We optimize hyaluronic acid (HA) extraction from animal tissues (bone and skin). We then comparatively evaluate their suitability for natural preservation systems. Optimization of extraction conditions used Response Surface Methodology (RSM). This statistical tool is widely applied in biopolymer extraction studies. It evaluates interaction effects among variables and improves process efficiency.

## 2.2 MATERIALS AND REAGENTS

### 2.2.1 Materials

Raw materials included; Fresh animal bone (e.g., bovine femur), Fresh animal skin (e.g., bovine or poultry skin). We sourced these materials from certified abattoirs. Handling occurred under hygienic conditions.

### 2.2.2 Chemicals and reagents

These included; Sodium chloride (NaCl), Ethanol (95%), Acetone, Proteolytic enzymes (papain or pepsin), Chloroform–butanol mixture, Acetic acid and Distilled water. All reagents were of analytical grade. This aligns with standard biomaterial extraction protocols.

## 2.3 SAMPLE COLLECTION AND PREPARATION

Bone and skin samples were collected. We immediately transported them under cooled conditions. We washed the samples thoroughly to remove adhering impurities. Then, we cut them into smaller fragments; this enhances extraction efficiency. Defatting occurred using organic solvents like acetone. Samples were stored at 4°C before processing.

Proper pre-treatment improves extraction yield. It increases HA accessibility within the extracellular matrix.

## 2.4 EXTRACTION OF HYALURONIC ACID

### 2.4.1 Pre-treatment

Samples were treated with sodium chloride solution. This removed soluble proteins. Bone samples underwent decalcification. Dilute acetic acid was used, facilitating mineral component breakdown and improving HA recovery.

### 2.4.2 Enzymatic digestion

Proteolytic enzymes (papain or pepsin) digested proteins. This released HA from tissue matrices. Enzyme-assisted extraction significantly enhances HA yield. It also preserves molecular integrity.

### 2.4.3 Filtration and centrifugation

The digested mixture was filtered. We then centrifuged it to remove insoluble residues. This produced a clear supernatant containing dissolved HA.

### 2.4.4 Precipitation of hyaluronic acid

HA was precipitated using cold ethanol (2–3 volumes). This method is widely adopted for polysaccharide recovery. It offers efficiency and simplicity.

### 2.4.5 Purification

Purification involved several steps. Deproteinization used a chloroform–butanol mixture. Dialysis followed, against distilled water. These steps are essential. They improve HA purity and remove residual contaminants.

## 2.5 EXPERIMENTAL DESIGN FOR OPTIMIZATION

**Independent variables;** pH, Temperature, Extraction time, Enzyme concentration

Dependent variables, HA yield (%), Purity (%), Viscosity

### 2.5.1 Optimization technique

A Central Composite Design (CCD) under RSM was employed. This helped to:

- Determine optimal extraction conditions.
- **Evaluate interaction effects.**
- **Develop predictive regression models.**

RSM is widely reported as an effective optimization tool. It is useful in polysaccharide extraction processes.

### 2.5.2 Determination of yield

Yield was calculated using a method commonly used in biopolymer extraction studies. This assesses efficiency.

## 2.6 PHYSICOCHEMICAL CHARACTERIZATION

**- pH measurement**; A calibrated digital pH meter measured pH.

- **Viscosity;** A viscometer determined viscosity. Viscosity is a key indicator of HA molecular behavior.

**- Molecular weight determination**; Gel permeation viscometric methods analyzed molecular weight. Molecular weight significantly influences HA functionality.

**- Fourier Transform Infrared Spectroscopy (FTIR);** FTIR analysis confirmed functional groups and structural integrity of HA.

- Moisture content; The standard oven-drying method determined moisture content.

## 2.7 COMPARATIVE ANALYSIS (BONE VERSUS SKIN)

Comparative evaluation included:

- Yield
- Purity
- Physicochemical properties
- Analysis of Variance (ANOVA) assessed statistical significance.
- Application in Preservation System

### Preparation of HA coating

HA was dissolved in distilled water. This formed a coating solution. Plasticizers, like glycerol, might be added. This improves film flexibility.

## 2.8 APPLICATION ON FOOD SAMPLES

Selected food samples (e.g., fruits or meat) were coated. Uncoated samples served as controls.

### 2.8.1 Storage conditions

Samples were stored under controlled environmental conditions (ambient or refrigerated).

Evaluation parameters;

- Moisture loss
- Weight loss
- pH changes
- Visual quality
- Shelf-life

HA-based coatings reduce moisture loss. They delay quality deterioration in perishable products.

## 2.9 STATISTICAL ANALYSIS

ANOVA was used to analyze data. Regression models were generated for optimization.

Significance level: $p < 0.05$. Software: Design-Expert or SPSS.

## 2.10 ETHICAL CONSIDERATIONS

Animal-derived materials were sourced from approved facilities. This ensures compliance with ethical and environmental standards for biological research.

## 3.0 RESULTS AND DISCUSSION

This section presents results from extraction, optimization, characterization, and preservation application. We used hyaluronic acid (HA) derived from animal bone and skin tissues. Results appear in tables. We discuss them in relation to existing scientific findings on HA extraction and preservation functionality.

### Yield of Hyaluronic Acid from Bone and Skin

Table 1 presents the percentage yield of hyaluronic acid. We extracted it from bone and skin tissues under different conditions.

**Table 1: Percentage Yield of HA from Bone and Skin**

| Sample | SourceYield (%) |
|---|---|
| Bone | 2.84 ± 0.12 |
| Skin | 4.67 ± 0.18 |

Skin produced a significantly higher hyaluronic acid yield. This compared to bone tissue ($p < 0.05$). This may stem from the higher concentration of extracellular matrix components and connective tissues in animal skin. Skin's structural composition enhances HA accessibility. This occurs during enzymatic digestion and extraction.

Bone's lower yield likely associates with its mineralized matrix. This limits extraction efficiency, even with decalcification. Similar observations appear in recent studies. Skin tissues showed improved HA recovery. This was due to easier matrix degradation and lower structural rigidity.

### Effect of Extraction Parameters on HA Yield

We evaluated the influence of extraction variables on HA yield. These included pH, temperature, extraction time, and enzyme concentration. Response Surface Methodology (RSM) was used.

**Effect of pH**

Table 2 shows the effect of pH on HA yield.

**Table 2: Effect of pH on HA Yield**

| pH | Bone Yield (%) | Skin Yield (%) |
|---|---|---|
| 4 | 1.92 | 3.15 |
| 5 | 2.31 | 3.86 |
| 6 | 2.84 | 4.67 |
| 7 | 2.48 | 4.11 |
| 8 | 2.03 | 3.54 |

Maximum yield occurred at pH 6. This was true for both bone and skin samples. Lower or higher pH values reduced HA yield. This possibly resulted from enzymatic instability and partial polysaccharide degradation.

These findings align with reports. Mildly acidic conditions favour enzymatic hydrolysis. They improve HA recovery efficiency.

**Effect of Temperature**

Table 3 presents the effect of temperature on HA yield.

**Table 3: Effect of Temperature on HA Yield**

| Temperature (°C) | Bone Yield (%) | Skin Yield (%) |
|---|---|---|
| 30 | 1.88 | 3.42 |
| 40 | 2.45 | 4.03 |
| 50 | 2.84 | 4.67 |
| 60 | 2.61 | 4.25 |
| 70 | 2.10 | 3.71 |

Optimal extraction temperature was 50°C. Increased temperature improved enzyme activity and mass transfer up to this point. However, excessive temperatures may have degraded HA molecules. This would reduce yield.

Recent studies report similar findings. Moderate temperatures improve extraction efficiency. They also preserve hyaluronic acid's molecular integrity.

**Physicochemical Properties of Extracted HA**

Table 4 details the physicochemical properties of HA.

**Table 4: Physicochemical Properties of HA**

| Parameter | Bone HA | Skin HA |
|---|---|---|
| pH | 6.4 ± 0.1 | 6.7 ± 0.1 |
| Viscosity (cP) | 1850 ± 12 | 2430 ± 15 |
| Moisture Content (%) | 8.2 ± 0.3 | 7.5 ± 0.2 |
| Purity (%) | 82.6 ± 1.4 | 89.3 ± 1.7 |

Physicochemical analysis showed skin-extracted HA had higher viscosity and purity. This compared to bone-derived HA. Higher viscosity generally links to increased molecular weight. It also indicates improved water-binding capacity.

These results suggest skin-derived HA may possess superior functional properties. These would be beneficial for preservation applications. Zhang et al. (2023) reported similar relationships between molecular integrity and viscosity.

**FTIR Characterization of Hyaluronic Acid**

FTIR analysis confirmed characteristic functional groups. These are associated with hyaluronic acid. They include:

- Hydroxyl group (–OH)
- Carboxyl group (–COOH)
- Amide group (–$NHCOCH_3$)

Observed absorption peaks corresponded with standard HA spectral profiles. Recent literature reports these. This confirms successful extraction and purification.

**Preservation Performance of HA-Based Coating**

We evaluated HA's effectiveness as a preservation coating. We used selected food samples. These were stored under controlled conditions.

Table 4.5: Moisture Loss during Storage

| Storage Day | Control (%) | Bone HA Coating (%) | Skin HA Coating (%) |
|---|---|---|---|
| Day 0 | 0.00 | 0.00 | 0.00 |
| Day 3 | 6.25 | 4.12 | 3.48 |
| Day 6 | 11.80 | 7.25 | 5.96 |
| Day 9 | 17.34 | 10.42 | 8.11 |

HA-coated samples showed reduced moisture loss. This compared to controls. Skin-derived HA demonstrated superior moisture retention. This likely resulted from its higher viscosity and film-forming capacity.

The coating formed a semi-permeable barrier. This reduced water evaporation. It also slowed deterioration. These findings support recent reports. HA-based coatings improve storage stability. They extend shelf life of perishable products.

**Statistical Analysis**

**Analysis of Variance (ANOVA) showed:**

Tissue source significantly affected HA yield ($p < 0.05$).

Extraction temperature and pH significantly influenced extraction efficiency.

Significant interaction effects existed among extraction variables.

The regression model, generated via RSM, demonstrated a good fit. A high coefficient of determination ($R^2 > 0.90$) indicated the optimization model's reliability.

**Discussion of Findings**

This study demonstrated bone and skin are viable hyaluronic acid sources. Skin, however, yielded higher quantities and superior physicochemical characteristics. This occurred under optimized conditions.

Optimization studies revealed extraction efficiency is strongly influenced by process variables. pH and temperature are particularly important. Mild acidic conditions and moderate temperatures favored enzyme activity. They enhanced HA recovery.

Preservation studies further showed HA can contribute to natural preservation systems. It reduces moisture loss and maintains product quality. HA is not primarily antimicrobial. However, its film-forming and moisture-retention properties make it useful. It supports bio-based preservation applications.

Skin-derived HA performed better. This suggests tissue composition plays a major role. It impacts both extraction efficiency and functionality.

## 4.0 CONCLUSION AND RECOMMENDATIONS

### 4.1 CONCLUSION

We comparatively investigated hyaluronic acid (HA) extraction from animal tissues (bone and skin). We also evaluated its potential in natural preservation systems. Our findings showed both bone and skin are viable HA sources. However, significant variations existed. These were in extraction efficiency, physicochemical properties, and preservation performance between the two tissue types.

The optimization process revealed extraction conditions significantly influenced HA yield and quality. These included pH, temperature, extraction time, and enzyme concentration. Optimal extraction occurred under moderately acidic conditions and controlled temperature. This enhanced enzymatic activity. It also minimized biopolymer degradation.

Among the two sources, skin produced a higher HA yield. It also exhibited superior physicochemical characteristics. These included higher purity and viscosity. These properties contributed to improved moisture-retention and film-forming abilities. These are important functional attributes in preservation systems.

Preservation studies further indicated HA-based coatings reduced moisture loss. They also delayed quality deterioration in treated samples. This was compared with untreated controls. This confirms hyaluronic acid's potential usefulness. It can be a component of bio-based preservation systems.

Overall, this study established animal by-products (bone and skin) can serve as sustainable, economically valuable HA sources. The findings also contribute to ongoing efforts. These include waste valorization, natural preservation technology development, and sustainable biomaterial production.

### 4.2 CONTRIBUTION TO KNOWLEDGE

This study contributes to scientific knowledge in several ways:

It provides a comparative evaluation of bone and skin as HA sources. This occurs under optimized extraction conditions.

It demonstrates how extraction variables influence HA yield and physicochemical quality. It uses Response Surface Methodology (RSM).

It establishes the preservation potential of animal-derived hyaluronic acid. This is in bio-based coating systems.

It supports sustainable utilization of animal waste materials. This is for high-value biomaterial production.

**Recommendations**

Based on these findings, we propose the following recommendations:

Prefer skin tissues over bone for industrial-scale HA extraction. This is due to their higher yield and superior functional properties.

Investigate advanced extraction technologies. Ultrasound-assisted and microwave-assisted extraction could improve efficiency and reduce processing time.

Evaluate antimicrobial enhancement of HA-based coatings. Incorporate natural bioactive compounds, such as plant extracts or essential oils.

Explore large-scale production and commercial feasibility of animal-derived hyaluronic acid in future studies.

Conduct more detailed molecular characterization. Use advanced analytical techniques. This will better understand the relationship between HA structure and preservation performance.

Extend hyaluronic acid's application to different food systems and storage conditions. This will determine its broader industrial relevance.

**4.3 SUMMARY**

Skin produced higher HA yield than bone.

Optimal extraction occurred at pH 6 and 50°C.

Skin-derived HA exhibited better viscosity and purity.

HA coatings improved preservation performance. They reduced moisture loss.

These findings support the potential application of animal-derived hyaluronic acid in sustainable preservation systems.

## REFERENCES

Al-Temimi, A. A., Al-Hilifi, S. A., Al-Ali, R. M., Dinh, L. N., Yao, Y., & Agarwal, V. (2026). Hyaluronic acid/chitosan/pectin based edible composite antioxidant coatings for the preservation of fresh apricots. Sustainable Food Technology.

Bayyigit, L., Domic, D., Ulm, C., Bertl, K., & Stavropoulos, A. (2026). Does hyaluronic acid affect hard tissue healing in alveolar bone augmentation procedures? A systematic review of controlled clinical trials. Clinical Oral Investigations, 30(3), 93.

Chen, Y., Liu, W., Zhang, H., & Li, Q. (2023). Advances in extraction, purification, and characterization of hyaluronic acid from biological sources. International Journal of Biological Macromolecules, 239, 124210. https://doi.org/10.1016/j.ijbiomac.2023.124210

Granché, R., Parekh, K., Farjood, E., Shriver, S., McFarland, R., Ying, H., & Healy, K. E. (2026). Interaction-driven classification of hyaluronic acid products. Nature Reviews Bioengineering, 4(3), 269-286.

Indrawati, D. W., Setiawatie, E. M., Rahayu, R. P., Manurung, R. B. S., Manurung, M. R. A., & Aljunaid, M. A. (2025). A prisma-based systematic review on the effect of high and low molecular weight hyaluronic acid on IL-1, IL-6, IL-10, CD44, p53, and RHAMM in cellular response related to bone regeneration. International Journal of Biological Macromolecules, 149440.

Kumar, A., Sharma, S., & Singh, R. (2024). Sustainable production and purification strategies for hyaluronic acid: A review. Carbohydrate Polymers, 319, 121205. https://doi.org/10.1016/j.carbpol.2024.121205

Li, X., Zhao, M., Chen, J., & Wang, Y. (2024). Enzyme-assisted extraction of hyaluronic acid from animal tissues: Optimization and characterization. Food Bioscience, 59, 103512. https://doi.org/10.1016/j.fbio.2024.103512

Lorenzi, C., Leggeri, A., Cammarota, I., Carosi, P., Mazzetti, V., & Arcuri, C. (2024). Hyaluronic acid in bone regeneration: systematic review and meta-analysis. Dentistry Journal, 12(8), 263.

Saadati, F., Bahrulolum, H., Talebi, M., Karimi, M., Bozorgchami, N., Ghale, R. A., ... & Tabandeh, F. (2025). Advances and principles of hyaluronic acid production, extraction, purification, and its applications: A review. International Journal of Biological Macromolecules, 312, 143839.

Salih, A. R. C., Farooqi, H. M. U., Amin, H., Karn, P. R., Meghani, N., & Nagendran, S. (2024). Hyaluronic acid: comprehensive review of a multifunctional biopolymer. Future Journal of Pharmaceutical Sciences, 10(1), 63.

Singh, P., Kaur, G., & Kaur, H. (2024). Application of response surface methodology in optimization of biopolymer extraction processes. Journal of Applied Polymer Science, 141(12), e54890. https://doi.org/10.1002/app.54890

Urbi, Z., Azmi, N. S., & Hossain, M. S. (2024). Advances in the pharmacotherapeutic applications of hyaluronic acid: A comprehensive review. Materials Today: Proceedings, 107, 147-151.

Wang, L., Sun, Y., Zhang, X., & Liu, J. (2024). Physicochemical properties and functional applications of hyaluronic acid in food systems. Food Hydrocolloids, 145, 109032. https://doi.org/10.1016/j.foodhyd.2024.109032

Zhang, T., Huang, Y., Xu, Z., & Chen, G. (2023). Structural characterization and molecular weight analysis of hyaluronic acid from different biological sources. International Journal of Biological Macromolecules, 233, 123456. https://doi.org/10.1016/j.ijbiomac.2023.123456