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Methodological improvement of the plastination technique in birds' plumage

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Methodological improvement of the plastination technique in birds' plumage

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Abstract: Plastination is a technique that preserves biological tissues in a natural and non-toxic manner, ensuring long-term stability and ease of handling. However, reports on avian plastination remain scarce due to the structural complexity of feathers. Because birds constitute the second most diverse class of vertebrates, documenting and preserving their diversity through modern preservation methods is essential. This study aimed to adapt the plastination process for avian plumage while maintaining its morphological integrity and natural appearance. Thirty-two carcasses of domestic pigeons (*Columba livia*) were plastinated using two protocols: a room-temperature protocol (≈25°C) and a cold-temperature protocol (–25°C). After impregnation, four post-treatment procedures were tested on specimens from each group: cornstarch adsorption, paper absorption, heating (60°C), and emulsification. Macroscopic and microscopic analyses were performed to evaluate structural alterations caused by silicone impregnation and to assess the effectiveness of each treatment. The absorption and adsorption treatments yielded the most significant aesthetic improvements, effectively removing excess silicone from feather surfaces. While cornstarch removed considerable amounts of silicone, it left minute residues adhering to the feathers; in contrast, heating and emulsification resulted in a thin silicone layer covering the barbs. These findings provide key insights into how plastination affects feather structures and demonstrate how the technique can be optimized for avian specimens. The results expand the potential applications of plastinated birds in teaching collections, museum exhibitions, and research. Furthermore, absorption and adsorption treatments offer cost-effective and environmentally friendly solutions, achieving higher quality than conventional methods. Overall, this study addresses a challenge persisting since the introduction of plastination in 1978—preserving avian specimens while maintaining feather integrity—representing a significant advancement for this diverse vertebrate group.

Keywords: Plastination; birds; methodology; protocol; feathers.

Introduction

Biological tissues naturally undergo decomposition through autolysis and microbial activity (Saeed, Rufai & Elsayed, 2001; Monteiro, 2022). Preserving specimens in a state that closely resembles their natural condition

has therefore long challenged collectors and anatomists. The development of plastination by Gunther von Hagens in 1977 offered an effective and durable solution to this problem. The technique replaces bodily fluids with a polymer that is subsequently cured, resulting in dry, odorless, and long-lasting specimens (Latorre et al., 2016; Monteiro et al., 2022). The standard plastination process involves four main stages: fixation with formalin, dehydration in acetone, forced impregnation, and curing.

Although plastination is well-documented in the literature, few studies have addressed how feathers and fur behave during the process or how they interact with polymers such as silicone. When applied to birds, plastination often produces unsatisfactory outcomes, including a wet or matted appearance and a loss of feather volume, which leads to substantial morphological alterations. Starchick and Henry (2019) highlighted the potential advantages of room-temperature impregnation for preserving feathers and fur, yet detailed accounts of how these delicate structures respond to plastination are still lacking. Understanding the microscopic interactions between silicone and avian plumage is therefore essential to overcome the limitations of traditional plastination in birds.

Birds play diverse and crucial ecological roles as pollinators, seed dispersers, pest controllers, and participants in multiple trophic levels. They also possess commercial relevance and can act as potential reservoirs or transmitters of zoonoses (Whelan et al., 2008). Consequently, the proper documentation and long-term preservation of avian specimens are fundamental to supporting research in zoology, taxonomy, morphology, ecology, and evolutionary biology. Furthermore, these collections serve as repositories of genetic and morphological information accessible to the global scientific community (Percequillo et al., 2022).

Implementing new preservation techniques that require less maintenance and enable broader analytical applications is therefore essential for the sustainability of biological collections. In this context, this study proposes and validates an optimized protocol capable of producing avian specimens of high aesthetic and scientific quality. This approach seeks to enhance the role of plastinated birds in research, education, and public exhibition, contributing to the advancement and dissemination of anatomical and zoological knowledge.

Materials and methods

A total of thirty-two rock pigeon (Columba livia) carcasses, authorized for use by the Animal Ethics Committee of UFES (CEUA-UFES, protocol no. 31/2019), were obtained from a licensed company approved to manage and control harmful synanthropic fauna, in accordance with IBAMA Normative Instruction no. 141/06. The specimens were divided into two groups: a control group (eight specimens) and a treatment group (twenty-four specimens).

To evaluate the effectiveness of different post-impregnation treatments on feathers, two plastination protocols were tested: a room-temperature protocol (RT) and a cold-temperature protocol (CT). This design allowed for direct comparison of treatment performance under distinct plastination conditions. The CT group followed the standard method of von Hagens et al. (1987), conducted at −25 °C, whereas the RT group used the “Dow Corning™/Corcoran-Silicone” protocol proposed by Glover, Henry, and Wade (1998), conducted at +25 °C. Treatments were applied after mechanical removal of excess silicone following forced impregnation and prior to the curing stage.

Specimens were initially fixed in 10% formalin through subcutaneous infiltration, followed by immersion in 3% formalin for 30 days to minimize pigment loss (Oostrom, 1987; Ekim et al., 2017). Dehydration was

carried out in four successive acetone baths (95%, 95%, 100%, 100%), each lasting one week, over a total of 28 days. The final acetone concentration exceeded 99% purity (v/v).

During impregnation, specimens were immersed in silicone mixed with a DBTL catalyst (100:1) at −25 °C for CT or in silicone combined with a TEOS crosslinker (8:100) at room temperature for RT. The process was performed under progressive vacuum until bubble cessation, following the procedure described by DeJong and Henry (2007). Impregnation lasted an average of 20 ± 4.28 days for CT and 7 days for RT, under pressures below 5 mmHg.

Following impregnation, specimens were drained within the same chamber for 48 hours maintaining −25 °C for CT and room temperature for RT followed by an additional 24-hour drainage at 25 °C for both groups. Manual removal of surface silicone was then performed over five consecutive days, with three five-minute sessions per day. After this step, the following specific treatments were applied (Figure 1).

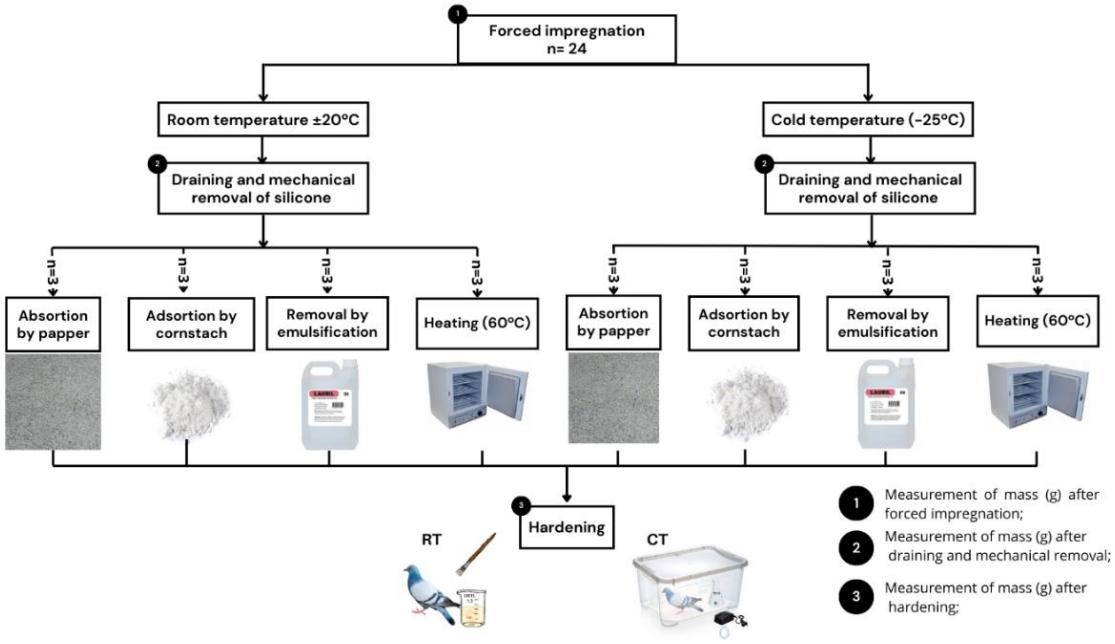


Figure 1. Flowchart of experimental treatment applied among the two different impregnation protocols (RT and CT). Copyright 2024, Authors.

I. Adsorption with shredded paper: Six specimens were completely embedded in recycled paper shredded into granules of approximately 3 mm, prepared using a blender. The specimens remained in the container for 21 days, and the paper was replaced whenever it became saturated with silicone, identified by increased saturation. After the treatment period, any paper adhering to the specimens was gently removed with a soft-bristle brush and compressed air (Figure 2).

II. Adsorption with cornstarch: Following the protocol proposed by Bernal et al. (2022) for preparing glycerinated specimens using the "Elnady" technique, six specimens were fully immersed in cornstarch for 21 days. Silicone and starch clumps were periodically removed, and additional starch was added as needed to keep the specimens completely covered. After the treatment period, residual starch was removed using a soft-bristle brush and compressed air (Figure 2).

III. Emulsification with detergent: Six specimens underwent two daily immersion baths, each lasting one hour, for five consecutive days. The detergent used was an alkaline surfactant containing Sodium Laureth Sulfate as the active ingredient, diluted at a 1:40 ratio (v/v) (Wong, 1997; Story & Simon, 2000). After each bath, the specimens were dried with a cloth and air blower, followed by mechanical removal of surface silicone using paper for three minutes (Figure 2).

IV. Heating treatment: Six specimens were placed in an oven at 60 °C for one hour, twice daily, over five consecutive days. After each heating cycle, the specimens were blotted with absorbent paper for three minutes to remove softened silicone residues (Figure 2).



Figure 2. Specimens subjected to different post-impregnation treatments for mechanical removal of excess silicone. (A) Immersion in shredded paper; (B) Immersion in cornstarch; (C) Immersion in alkaline detergent solution; (D) Heating treatment at 60 °C in a drying oven. Copyright 2024, Publisher.

For the hardening stage, specimens impregnated under the CT protocol were cured through TES vaporization in 15-minute cycles, repeated every 2 hours over a period of three days (Hagens, Tiedemann &

Kriz, 1987). In the RT protocol, hardening was achieved by spraying a DBTL–acetone solution (1:3), ensuring homogeneous curing. This process promoted silicone polymerization and yielded a dry, stable final surface.

Analysis

Five researchers jointly evaluated the specimens, assessing each treatment's effectiveness in restoring aesthetic appearance, preserving natural feather texture and coloration, minimizing silicone clumping, and maintaining flexibility. Photographs and descriptive notes were used as references, with control specimens serving as a baseline. A cross-rating scale was applied: 0 = ineffective, + = slightly effective, ++ = moderately effective, +++ = effective, and ++++ = highly effective.

Specimens were photographed throughout all processing stages. Feather samples were collected for optical and scanning electron microscopy (SEM) before processing, after impregnation, and after curing. Silicone reduction was determined by comparing specimen weights (g) before and after each treatment.

Optical microscopy slides were prepared following Lee et al. (2015) and examined under 4×, 10×, and 40× magnifications. For SEM, samples were mounted on carbon tape, gold-coated (Desk V, Denton Vacuum), and imaged using a JEOL® JSM-6610LV microscope at 20 kV under 20×, 100×, and 500× magnifications.

To assess the influence of impregnation temperature (RT vs. CT) on silicone drainage and compare treatment effectiveness, statistical analyses included Student's t-tests, one-way ANOVA, and two-way ANOVA (for interaction effects). All tests were conducted with a 95% confidence interval using GraphPad Prism 8, Microsoft Excel 2024, and Jamovi.

GenAI/Generative AI use

Generative artificial intelligence tools were used in this study exclusively for language-related support, including translation and grammatical revision of the manuscript. Specifically, ChatGPT and LanguageTool were employed for these purposes. These tools did not contribute to the study design, data analysis, or interpretation of results. All data analyses, as well as the creation of figures and images, were conducted solely by the authors. The authors take full responsibility for the content of this work.

3. Results

At the end of the process, the specimens exhibited the characteristic features of plastinated material: odorless, dry to touch, easily handled and stored, and slightly rigid. Despite reducing the formaldehyde concentration to 3%, pigment loss was observed in the scales, oral mucosa, and rhamphotheca of all specimens during fixation. In the control group, a persistent wet appearance remained after plastination, indicating that the standard protocol still has limitations. These include silicone accumulation beneath contour feathers and feather displacement on the back, chest, and neck, leading to reduced aesthetic quality and overall visual appeal (Figure 3).



Figure 3. Control group specimens impregnated using the cold-temperature protocol (−25 °C) proposed by Hagens, Tiedemann & Kriz (1987). (A) Ventral view; (B) Dorsal view. Scale = 10 cm. Copyright 2024, Publisher.

In general, the treatment groups produced distinct outcomes, and this section outlines their main results.

I. Adsorption with cornstarch

This adsorption-based treatment produced excellent results. Consistent with Elnady (2016), cornstarch effectively removed silicone from specimen surfaces, yielding superior aesthetic quality. The curing catalyst at room temperature often caused an oily, wet appearance and reduced feather volume, making a second cornstarch application necessary in some cases (Figure 4A).

Throughout the 21-day treatment, accumulated cornstarch was periodically brushed off and replaced to maintain contact with the specimen. Excess buildup occasionally occurred in areas such as the ventral wings, axillary folds, and thighs, leaving faint whitish residues that required gradual removal. After cleaning, treated specimens exhibited improved contrast, natural feather arrangement, and preserved flexibility with minimal silicone clumping (Figure 4A).

II. Adsorption with shredded paper

Shredded paper also produced satisfactory outcomes, effectively wicking excess silicone, and preventing residue accumulation between feather vanes, thereby preserving the microscopic barb structure (Figure 4B). A slight wet sheen persisted due to residual silicone, less pronounced than in other groups but more noticeable than in cornstarch-treated specimens.

Although paper was less effective in maintaining feather volume and flexibility, it offered practical advantages such as availability, eco-friendly disposal, and easy residue removal with minimal adhesion to feathers or body cavities (Figure 4B).

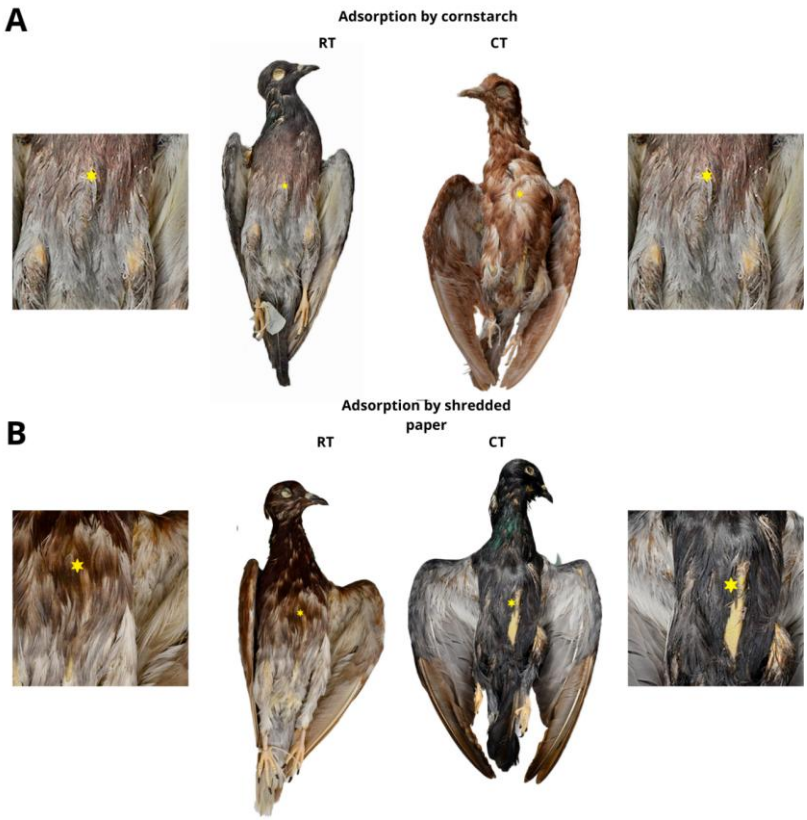


Figure 4. Plastinated specimens after their respective treatments under different impregnation protocols. A) Specimens treated by adsorption with cornstarch at room temperature (RT) and cold temperature (CT). B) Specimens treated by adsorption with shredded paper at cold temperature (CT) and room temperature (RT).

III. Emulsification treatment

Specimens exhibited wet appearance, feather misalignment, and volume reduction, resulting in poor overall quality (Figure 5A). Although some silicone drainage occurred, the material adhered to feathers during removal, forming clumps and deforming the vane structure, regardless of the impregnation protocol. Alkaline surfactant detergents containing sodium laureth sulfate were largely ineffective in removing silicone (PDMS), likely due to the hydrophobic nature of polydimethylsiloxane, which forms a water-repellent surface layer (Métivier et al., 2018).

IV. Heating treatment

Thermal treatment resulted in loss of plumage volume, feather disarray, and a persistent wet appearance, yielding low-quality plastinates. Microscopy revealed a thin silicone layer beneath the feathers, with accumulation especially in the abdominal region near evisceration incisions. Despite its limited efficiency in silicone removal, some feather iridescence was preserved (Figure 5B).

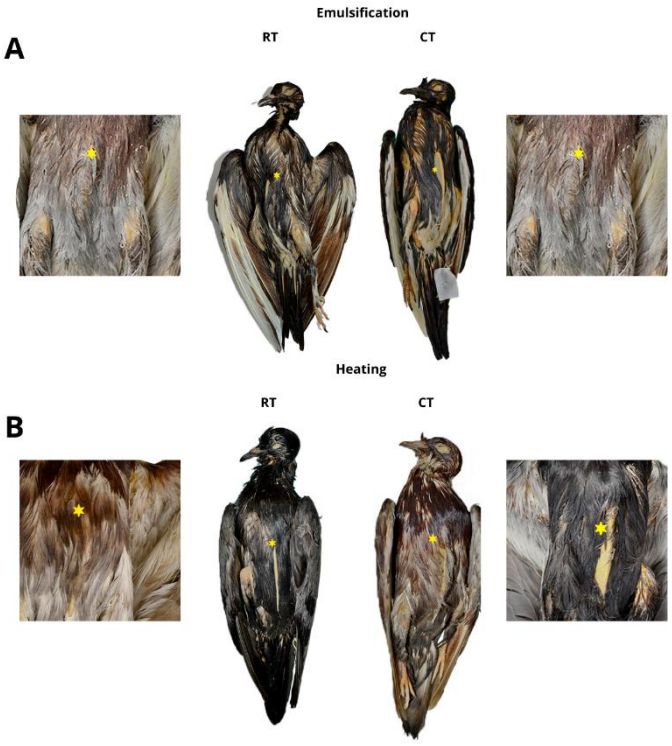


Figure 5. Plastinated specimens after their respective treatments in different impregnation protocols. A) Specimens treated by emulsification impregnated at room temperature (RT) and cold temperature (CT). B) Specimens treated by heating impregnated at cold temperature (CT) and at room temperature (RT).

As expected, some specimens showed silicone accumulation. However, some treatments performed well, especially those using cornstarch and paper — with cornstarch producing the best results. Specimens subjected to the emulsification process displayed the poorest aesthetic outcomes. Similarly, specimens heated at 60 °C produced unsatisfactory visual results.

The table below summarizes the qualitative evaluation of the different treatments applied to plastinated specimens, focusing on feather color preservation, silicone lump formation, and malleability. Each characteristic was analyzed relative to the control group, using an effectiveness scale described in the legend. The final evaluation integrates these criteria to provide an overall quality ranking for each treatment method (Table 1).

Table 1. Results of the visual assessment of specimen properties after their respective treatments, based on evaluations conducted by the LabPlast team.

Treatment	Group	Feather Color Preservation	Silicone Lump Formation	Feather Malleability	Final Evaluation
Adsorption with cornstarch	RT	++++	+++	++++	Excellent
	CT	++++	+++	++++	Excellent
Adsorption with shredded paper	RT	++++	++++	++++	Excellent
	CT	+++	++++	++++	Excellent
Detergent emulsification	RT	+	0	+	Very poor
	CT	+	0	+	Very poor
Heating	RT	++	++	++	Poor

	CT	++	++	++	Poor
Legend: 0 = *Ineffective;* + = *Slightly effective;* ++ = *Moderately effective;* +++ = *Effective;* ++++ = *Highly effective.*					

Mean mass reduction across treatments was 20.1 g (range: -10 to 105 g), with mass gain observed in some specimens treated by emulsification (Table 2).

Table 2. Efficacy of silicone removal (%, minimum, maximum, mean, and standard deviation) following different treatment applications under distinct impregnation protocols.

Plastination protocol	Treatment	Amount	Min	Max	Mean	Standard Deviation
CT	Cornstarch	3	2.01	11.7	5.89	5.11
	Paper	3	6.33	7.02	6.72	0.352
	Detergent emulsification	3	-4.10	2.35	-1.73	3.54
	Heating	3	8.48	10.5	9.21	1.14
	Total TB	12	-4.10	11.7	5.02	5.05
RT	Starch	3	13.3	39.9	23.72	14.2
	Paper	3	9.17	18.3	12.97	4.74
	Detergent emulsification	3	5.88	20.8	12.83	7.49
	Heating	3	5.12	5.53	5.32	0.26
	Total TA	12	5.12	39.9	13.7	9.9
Total CT and RT		24	-4.10	39.9	7.74	5.29

Statistical analysis revealed no significant relationship between the impregnation protocol (RT or CT) and overall silicone removal efficiency (Figure 7). The student's t-test indicated no significant difference in total mass reduction ($t(30) = 8.08$, $p = 0.951$, $d = -0.02$) (Figure 7C). However, during the draining phase, RT specimens showed greater silicone reduction within the first 24 hours ($t(30) = -9.99$, $p < 0.008$, $d = -1.03$) (Figure 7A), while in the manual removal phase, CT specimens exhibited higher mass reduction over five days ($t(30) = -9.99$, $p < 0.008$, $d = -1.03$) (Figure 7B). These variations reflect protocol-specific methodological differences influencing silicone removal dynamics.

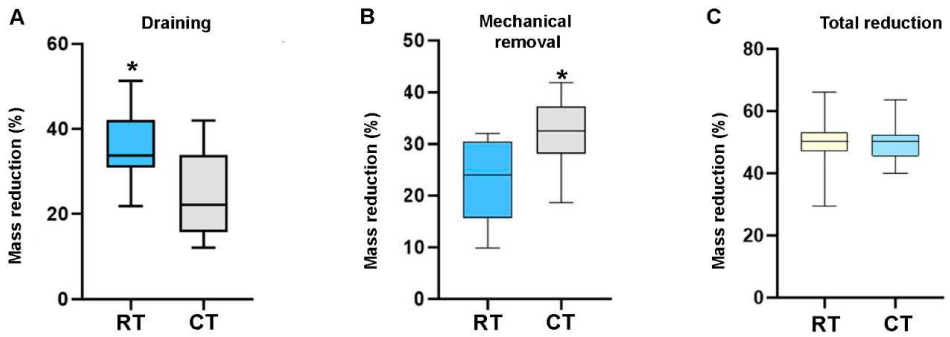


Figure 7. Average percentage reduction in the mass (g) of silicone removed from impregnated specimens, comparing the protocols conducted at room temperature (RT) and cold temperature (CT, −20 °C). (A) Differences between RT and CT groups during the draining phase post-impregnation; (B) Differences between RT and CT groups during the manual removal of silicone; (C) Total silicone reduction at the end of the

plastination process, showing that both protocols (CT and RT) resulted in similar final mass reductions. Copyright 2024, Publisher.

The effects of the different treatments on mass reduction were initially analyzed independently of the plastination protocol. One-way ANOVA showed no significant difference among treatments ($F = 1.01$, $df_1 = 3$, $df_2 = 9.60$, $p = 0.43$), considering only silicone removal (Figure 8A).

When the interaction between treatment and impregnation protocol was assessed (Figure 8B), differences emerged. Under the CT protocol, a significant difference was found between the Heating and Emulsification groups, with emulsification even increasing specimen mass ($F = 7.49$, $df_1 = 3$, $df_2 = 3.55$, $d = 0.714$, $p < 0.05$). Conversely, no significant differences were observed among treatments under the RT protocol ($F = 3.77$, $df_1 = 3$, $df_2 = 3.34$, $d = 0.479$, $p = 0.138$).

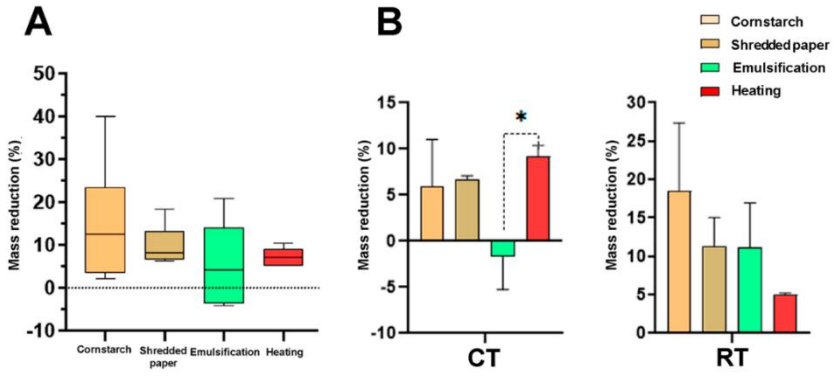


Figure 8. Effects of the different treatments on silicone removal. (A) Effectiveness of each treatment in reducing specimen mass. (B) Comparison of treatment effectiveness when considering the impregnation protocol used, showing that a significant difference in silicone removal occurred only when treatments were associated with the cold temperature plastination protocol (CT). Copyright 2024, Publisher.

In ANOVA analysis comparing treatments and impregnation protocols, the F and p values indicated a significant interaction between these two factors in relation to silicone reduction in the specimens ($F = 4.598$, $gl_1 = 3$, $gl_2 = 3.34$, $d = 0.479$, $p < 0.0167$). Specifically, the cornstarch and emulsification treatments exhibited significant differences in mass reduction between the RT and CT groups. For the remaining treatments, no statistical evidence supported the hypothesis that mass reduction during processing depended on the impregnation protocol used (Figure 9).

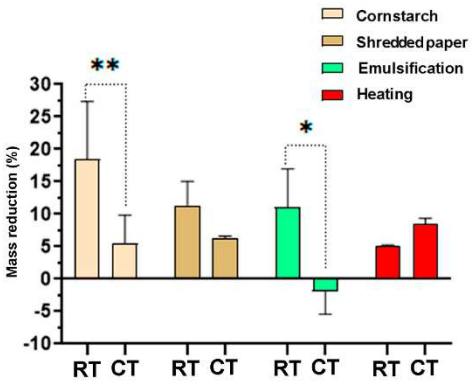


Figure 9. Average percentage reduction in silicone mass (g) per treatment across the different impregnation protocols (RT and CT), indicating the groups with statistical differences based on two-way ANOVA. Legend: *; ** = statistical significance. Copyright 2024, Publisher.

Microscopic analyses revealed that, following the impregnation process, the feather barbules became less transparent to light and exhibited poorly defined edges, which hindered detailed observation under optical microscopy. Nevertheless, the overall feather architecture remained discernible, albeit with reduced visual clarity compared to control specimens. Regarding silicone detection, most samples showed no severe clumping; however, small, localized accumulations were occasionally observed, particularly at 10× and 40× magnifications. Scanning electron microscopy (SEM) confirmed the formation of a thin silicone layer coating on feather surface, indicating that plastination partially obscures the visualization of the inter-barbule interfaces (Figure 10).

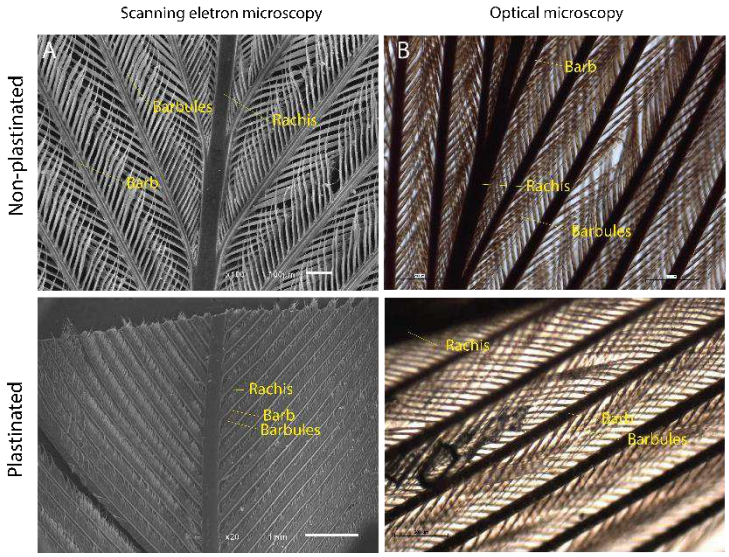


Figure 10. Images of feather surfaces under different microscopy methods before (control) and after plastination (plastinated). Scale = 20 µm. Copyright 2024, Publisher.

After plastination, optical microscopy remained feasible, though image quality decreased due to specimen opacity (Figure 11A).

Both starch and emulsification treatments caused notable structural alterations under SEM. Starch-treated samples showed small surface artifacts on barbules, barbs, and rachis, likely corresponding to starch granules, while emulsification led to barbule deformation and a continuous silicone layer that reduced the visible feather surface (Figure 11B).

SEM analysis also revealed that untreated plastinated feathers retained a thin silicone film, partially clumping barbules and reducing flexibility, giving a “wet-like” appearance (Figure 11B). Treatments based on adsorption and absorption were generally more effective in removing excess silicone, indicating mechanical trapping as a promising post-impregnation cleaning strategy (Figure 11C).

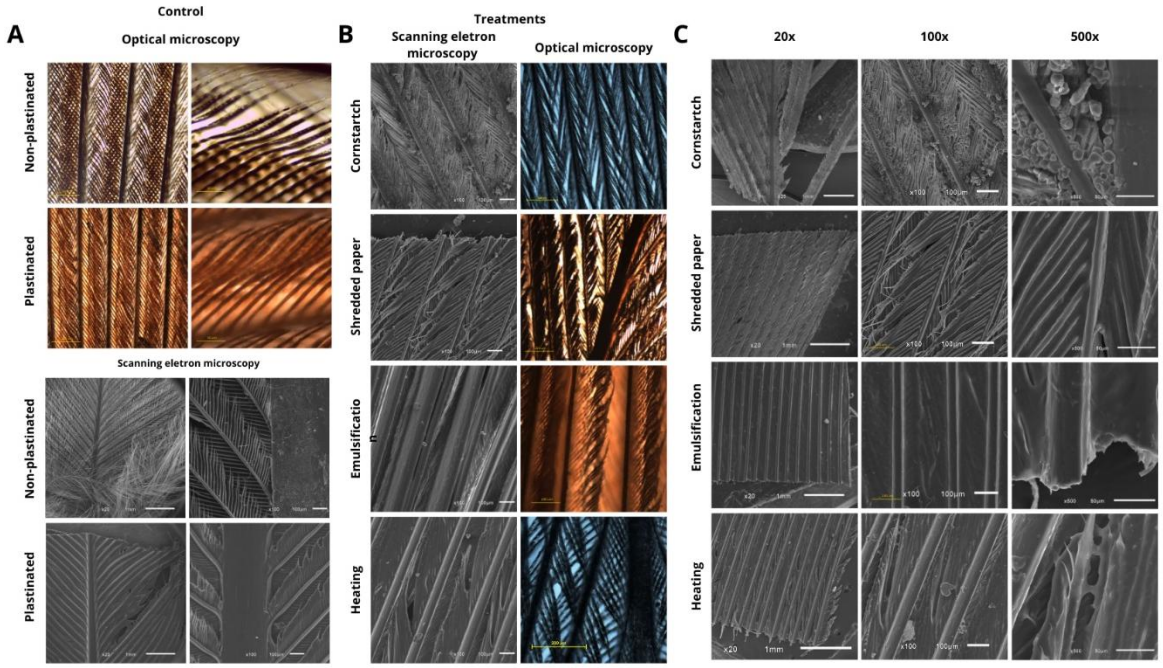


Figure 11. Microscopic comparison of non-plastinated and plastinated feathers, including post-treatment samples. A) Optical microscopy images (10× and 40× magnifications) and scanning electron microscopy (SEM) images (20× and 100× magnifications) of non-plastinated and plastinated feathers from the control group. B) Comparative assessment of treatment effectiveness (cornstarch, shredded paper, emulsification, and heating) under SEM (100×) and optical microscopy (20×), showing the feather surface after the plastination process. Scale = 1 µm. C) SEM images of feather surfaces after treatments at different magnifications (20×, 100×, and 500×). Scales = 20 µm, 100 µm, and 500 µm.

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4. Discussion

This study demonstrated that bird plastination can be successfully achieved while preserving plumage integrity and satisfactory external appearance. Feathers represent a particular challenge during plastination because residual silicone may alter texture, reduce volume, and interfere with the natural arrangement of barbs and barbules. Similar technical difficulties have been reported for feathered and furred specimens, in which excess polymer compromises final aesthetics (Starchik & Henry, 2015; Elnady, 2019).Among the treatments tested, cornstarch and shredded paper provided the best overall results, improving both silicone removal and specimen appearance. These findings reinforce previous observations that absorbent dry materials may be effective alternatives during finishing stages of plastination, especially when delicate external surfaces must be preserved (Elnady, 2019; Ottone, 2023).

Cornstarch showed high efficiency, particularly in room temperature specimens. This effect may be associated with lower silicone viscosity under warmer conditions, facilitating adhesion between polymer residues and starch particles. Cornstarch contains amylose and amylopectin, compounds capable of interacting with hydrophobic materials through weak intermolecular forces, favoring adsorption phenomena (Khoo et al., 2023). However, starch residues accumulated in feather folds and between barbs, requiring additional cleaning.

Similar residue-related limitations have been described for powder-based absorbents used in preservation procedures (Melendres et al., 2022).

Shredded paper also performed well and was operationally advantageous because it left minimal residues and simplified final cleaning. Its porous cellulose matrix likely enhanced silicone uptake through capillary absorption and surface retention, mechanisms widely recognized in fibrous absorbent materials (Zhao et al., 2022). Although a slight reduction in feather volume was observed, the overall external appearance remained natural.

The room-temperature and cold-temperature impregnation protocols showed similar final drainage efficiency, indicating that both methods are technically suitable for bird plastination. However, room-temperature processing promoted faster early-stage drainage because silicone viscosity decreases as temperature rises, facilitating polymer flow and recovery. This may reduce material costs through silicone reuse. In contrast, cold temperature plastination provides slower curing and greater procedural control, which may be advantageous for fragile specimens or complex anatomical preparations (Sora, 2015; Ottone, 2023).

Heating at 60 °C was less satisfactory than the absorbent treatments. Although elevated temperature can initially reduce silicone viscosity, prolonged exposure may accelerate catalyst-induced crosslinking, increase surface thickening, and reduce feather flexibility (Zhai, 2019). As a consequence, thermal treatment negatively affected specimen aesthetics and should be used cautiously when plumage preservation is required.

From an applied perspective, these findings are relevant for zoological museums, academic collections, and science outreach. Plastinated birds offer durable, dry, odorless, and low-maintenance specimens, representing an attractive alternative to traditional wet preservation and complementary use alongside taxidermy (Chytas et al., 2019; Sora et al., 2019; Silva et al., 2024).

This study has some limitations, including sample size and logistical constraints associated with specimen acquisition and processing time. Future studies should evaluate larger sample numbers, test combined finishing treatments, and compare responses among different feather categories and avian taxa, since feather microstructure varies substantially among species (Kardong, 2016; Foth & Rauhut, 2020).

Overall, the present work advances the refinement of avian plastination methods and demonstrates that appropriate post-impregnation treatments can substantially improve the final quality of plastinated plumage.

5. Conclusion

This study demonstrated that bird plastination is feasible while preserving plumage morphology and maintaining a natural external appearance.

The main findings of this study are summarized as follows:

1. Cornstarch and shredded paper were the most effective post-impregnation treatments for silicone removal, providing better aesthetic and structural results than the other tested methods.
2. Room-temperature and cold-temperature impregnation protocols showed similar overall drainage efficiency, although each presented distinct practical advantages during processing.
3. Heating at 60 °C was not recommended, as it negatively affected feather flexibility and specimen appearance.

These findings provide a practical basis for improving avian plastination protocols and supporting the use of plastinated birds in scientific, educational, and museum collections.

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