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(Short Communication)

Comparative Evaluation of Acetone and 95% Ethyl Alcohol Dehydration of Canine Head, Heart, Lungs, and Thoracic Limb Specimens Using the Elnady Plastination Technique

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Abstract

Background: Plastination produces durable, odorless, and realistic teaching specimens, and the Elnady technique provides a low-cost, room-temperature preservation method. However, the effects of acetone versus alcohol dehydration on organ-specific shrinkage, odor, texture, and realism in canine specimens using this technique remain inadequately characterized. **Methods:** Six canine cadavers were used to collect the head, heart, lungs and thoracic limb after fixation in 10% formalin solution. Specimens were dehydrated using either 100% acetone (T1) or 95% ethyl alcohol (T2). All organs underwent glycerin impregnation and cornstarch curing following the Elnady plastination protocol. Pre- and post-plastination measurements were recorded to assess shrinkage. Odor, texture, color, and realism were evaluated by 80 veterinary students using a Likert type scale, and data were analyzed using nonparametric tests and effect size calculations. **Results:** Both dehydration protocols produced dry, odorless to mildly pleasant specimens suitable for veterinary anatomical instruction. No statistically significant differences in shrinkage were observed between groups, although effect sizes indicated biologically relevant variation. Greater shrinkage was observed in the head, heart, and thoracic limb with acetone, while lungs showed greater shrinkage with alcohol. Realism scores were comparable for the head and heart, but differed significantly in the lungs and thoracic limb. Acetone-treated thoracic limb demonstrated higher realism, while lung realism remained similar between treatments despite shrinkage differences. **Conclusions:** This preliminary study suggests that both acetone and 95% ethyl alcohol are suitable dehydration agents for plastination using the Elnady technique. Organ-specific differences were observed, particularly greater shrinkage of lungs dehydrated with alcohol and higher realism scores for acetone-dehydrated thoracic limbs. Because of the limited sample size, further studies with larger numbers of specimens are needed to confirm these findings.

Keywords

Plastination; Elnady technique; Canine anatomy; Dehydration methods

1. Introduction

The Elnady technique is a room-temperature, low-cost alternative plastination method that produces dry, flexible, soft and odorless organs that can be easily stored and handled. Its practical process and anatomical fidelity of specimens produced have been repeatedly and successfully implemented in a variety of species and organs [1,2].

During the plastination process, dehydration solutions significantly influence tissue shrinkage, color, and processing duration. The standard dehydration protocol is usually conducted using cold acetone to minimize shrinkage and rapid tissue penetration. Graded and absolute alcohol dehydration can also be conducted but it prolongs processing, increases potential shrinkage, and produces marked change in natural color of organs when compared to acetone dehydration [3]. However, alcohol produced a drier texture, which was easier to manage. These differences were particularly noted in heart and lung specimens, where alterations in morphological architecture may compromise their use in anatomical studies [4].

Recent studies have also been conducted to determine the optimal organ - solution ratio during dehydration. An organ-to-acetone ratio of at least 1:65 at −20 to −25 °C was reported to reduce dehydration time to approximately four days in small samples [5]. Despite this rapid dehydration process, another study suggests that water retention still persists even after dehydration with acetone. Residual water can exceed the traditional <1% retention assumption even after specimens are dehydrated in 99% acetone [6].

Although acetone and alcohol dehydration have been compared in conventional plastination protocols, limited information is available regarding their use in room-temperature plastination using the Elnady technique, particularly in canine head, heart, lungs, and thoracic limb specimens. Furthermore, organ-specific responses to different dehydration agents remain poorly characterized. Therefore, this study was conducted to compare acetone and 95% ethyl alcohol dehydration in terms of shrinkage, texture, color, realism, and overall specimen quality following Elnady plastination.

2. Methodology

Experimental Specimen and Fixation

Six dogs from the city dog pound were used in the study. Animals were euthanized using propofol (3mg/kg, IV), and potassium chloride (75mg/kg, IV) was used to stop the heart beating. Once the heart was confirmed that it had stopped beating using a stethoscope, the common carotid artery on the neck region was located, and the external jugular vein was incised open. The cannula was inserted caudally and cranially to one of the common carotid arteries for the perfusion of physiologic saline solution to wash off excess blood until the fluid comes out from the external jugular vein. After which, the animals were fixed with 10% formalin solution and were soaked for one week. Canine heads were separated using a saw by a transverse cut in the neck, 3 cm caudal from the base of head. The heart and lungs were collected and separated by dissection. The trachea was transected at the level caudal to the larynx. The heart was collected including its parietal pericardium and was incised open. The right and left thoracic limbs were also separated from the thorax. The head, heart, lungs, and thoracic limbs were collected and were distributed into two treatment groups based on predefined allocation protocol to ensure representation of all organ types in both groups.

Plastination Procedure

The plastination method employed was an adapted version of the Elnady technique [1]. Steps such as dye injection, muscle dissection, and bone drilling were omitted. Organs were washed in running water overnight and further soaked in clean water for 72 hours to wash off excess formalin solution and then allowed to drain prior to weighing. After washing, organs were subjected to a series of dehydration. Both dehydration and impregnation were performed passively at room temperature (approximately 25–28°C).

Dehydration

The canine head, thoracic limb, heart and lungs in Treatment 1 were subjected to 100% acetone for three weeks, while organs in Treatment 2 were subjected to 95% ethyl alcohol dehydration for three weeks in a stainless-steel airtight containers. The concentration of acetone and alcohol were monitored using a hydrometer (HG-100, Yiwu Weike Instrument Co., Ltd., China). After the dehydration process, the organs were drained and then subjected to impregnation.

Impregnation

The canine head, thoracic limb, heart and lungs in Treatments 1 and 2 were immersed in glycerin for three weeks in a stainless-steel

container to allow passive impregnation of the specimen. The glycerin volume was maintained at approximately five times the specimen volume [1]. After three weeks, the specimens were removed from the glycerin bath and drained in the same container and were left for one week to remove the excess fluid.

Curing with Corn Starch

After the removal of excess fluid, the organs were wiped off of excess glycerin with absorbent paper and were placed in a cloth bag. The bags were tightly tied up, then covered with cornstarch from outside of the bag for a week. When cornstarch powder became saturated with glycerin/alkyd resin by the presence of clumps, these were replaced with new powder. Using a soft brush, the residues of cornstarch were brushed off.

Data Collection and Analysis

The weight and surface area of organs were obtained after washing as pre-plastination weight and after curing as post-plastination weight to determine the extent of possible shrinkage of the specimen using a digital weighing scale (ACS-30, Accura Precision Scales Trading, Philippines), and a vernier caliper (0–150 mm Electronic Digital Caliper, INGCO Tools Co., Ltd., China) and tape measure, respectively. The length of the canine head was measured using a vernier caliper from the tip of the nose to base of the skull. The circumference of canine head was obtained using tape measure at the level of the top of the canine head, cranial to the ears and base of the mandible. The vernier caliper was used to measure the height from the tip of the cranial lobe to the tip of the caudal lobe of both left and right lung lobes, width was measured at the broadest transverse part of the lungs and thickness was measured at the thickest area on the dorsoventral region of the lung lobes. For the heart, the length was measured from base to apex of the heart, width and thickness was obtained from the broadest transverse region of the heart. The length of the canine thoracic limb was measured from the most proximal part of the scapular region and the most distal part of the manus in extended position. The circumference of the thoracic limb was measured using tape measure in three regions (the mid-scapular, mid-brachial, and mid-antebrachial).

Due to the limited sample size, organ weight and surface area data were analyzed using within-specimen percent change. Data were summarized using both mean ± standard deviation and median (interquartile range). Comparison between treatment groups was performed using Mann–Whitney *U* tests. Effect sizes were calculated to assess the magnitude of observed differences. Percentage reduction was calculated using the following equation:

Percentage Reduction (%) = [(Pre-plastination weight – Post-plastination weight) ÷ Pre-plastination weight] x 100

Plastinated organs were evaluated for odor, texture, color, durability, and realism after complete drying by eighty (80) veterinary medicine students who were enrolled in anatomy courses and had prior experience dissecting dog specimens. Randomization and blinding were applied to minimize bias, with only the researcher aware of treatment assignments. Evaluations were conducted using a Likert-type scale. Odor was evaluated as: 1 (Unpleasant): Strong, offensive, or irritating smell; 2 (Tolerable): Noticeable odor but not overwhelming; 3 (Neutral): Faint or no detectable smell, neither pleasant nor unpleasant; 4 (Pleasant): Mild, inoffensive, or slightly agreeable scent and 5 (Very Pleasant): Odorless or has a subtle, refreshing aroma. The organs were evaluated at a distance of 30 cm (approximately one foot) from the evaluators during odor assessment. Texture was rated as 1 (soft): easy to press and smooth; 2 (firm): when pressure is applied, it leaves an impression, but the impression isn't permanent; the impression rebounds to the original shape or 3 (hard): when pressure is applied, it leaves no impression. Color was rated as 1 = Black; 2 = Dark brown; 3 = Brown; or 4 = Light brown. Durability was evaluated by observing whether the specimens showed signs of decomposition and was rated as follows: 1 (Very Poor Durability): Easily damaged, not suitable for handling; 2 (Poor Durability): Fragile, shows wear quickly with minimal use; 3 (Moderate Durability): Holds shape under gentle use, may degrade over time; 4 (Good Durability): Resilient, withstands repeated handling with minor wear; 5 (Excellent Durability): Highly durable, maintains integrity despite frequent or rough use by observing the specimens if it showed signs of decomposition. For realism criteria, plastinates were rated as: 1 (Not Realistic): Appears synthetic, exhibits inaccurate

coloration and lacks intricate details; 2 (Somewhat Realistic): The basic shape is accurate, however, it lacks detailed texture and precise color variation; 3 (Realistic): Distinctive details and coloration, exhibits a mildly rigid or smooth surface; 4 (Highly Realistic): Exhibits intricate detail, precise textures, and accurate coloration, possesses a realistic appearance with subtle imperfections; 5 (Extremely Realistic): Virtually indistinguishable from a genuine organ in appearance, texture, and flexibility; devoid of any visible artificial elements. For color, odor, texture, and realism, the Wilcoxon signed-rank test was used to analyze differences between treatment groups for each category. Statistical analyses were performed using jamovi version 2.7.17 (The jamovi Project, Sydney, Australia).

Table 1 shows the percentage reduction in weight and surface area of plastinated organs. Head, heart, and thoracic limb specimens in Treatment 1 (acetone dehydration) exhibited greater shrinkage in terms of weight and surface area reduction when compared to Treatment 2, while lungs dehydrated with 95% ethyl alcohol (T2) exhibited greater weight reduction. Despite the substantial weight loss and surface area observed, there were no significant differences between treatment groups of the different organs evaluated (Table 1). Although no statistically significant differences were detected, large effect sizes ($|r| \approx 0.78–1.0$) suggested potentially meaningful biological differences between dehydration methods. Because of the small sample size, effect sizes were used to supplement hypothesis-testing results and to identify treatment-related trends that may warrant further investigation.

Table 1. Mean (IQR) percentage reduction in weight and surface area of the canine head, heart, lungs and thoracic limb post-plastination.

Parameter	Organ	Treatment 1	Treatment 2	p value	Effect size (r)
Weight	Head	30.9% (1.58)	21.1% (0.48)	0.1	-1.00
	Heart	38.2% (4.22)	36.3% (4.89)	1.0	-0.11
	Lungs	46.9% (4.06)	52.9% (2.40)	0.2	0.78
	Limb	39.1% (4.11)	34.1% (3.74)	0.2	-0.78
Surface Area	Head	12.9% (2.18)	5.50% (2.50)	0.1	-1.00
	Heart	26.4% (6.06)	25.2% (9.19)	1.0	-0.11
	Lungs	34.6% (9.85)	62.6% (8.75)	0.1	1.00
	Limb	32.8% (7.97)	30.1% (3.34)	1.0	-0.11

Institutional Animal Care and Use Committee Permit

Six (6) dogs from the city dog pound were used in the study. The procedures conducted in the handling and use of the dogs in the study were approved by the Institutional Animal Care and Use Committee of the Cagayan State University with protocol number: 2025-007, approval date: July 23, 2025.

3. Results and Discussion

A comparative evaluation of acetone and 95% ethyl alcohol dehydration for room-temperature plastination of canine head, heart, lungs, and thoracic limb specimens was conducted. Changes in specimen weight and surface area were used as indicators of tissue shrinkage following plastination. Organoleptic properties, including odor, texture, color, durability, and realism, were also evaluated.

The lungs showed greater shrinkage of 62.6% in Treatment 2 (95% alcohol) versus 34.6% in Treatment 1 (acetone). Lung tissue is porous and delicate because alveolar spaces can collapse, which may contribute to greater tissue shrinkage. In contrast, the head, heart, and limb muscle architecture having extensive connective tissue scaffolding [3,7] yielded minimal differences between treatment groups.

A previous study showed that shrinkage depends on the type of dehydration solution and temperature, where cold acetone dehydration produced less shrinkage when compared to room-temperature acetone or alcohol dehydration processes [3]. In the present study, the larger tissue shrinkage in terms of weight and surface area may be associated with the process of room temperature plastination and the use of glycerin impregnation which could potentially influence

the shrinkage when compared to cold silicone plastination [1,7]. In various plastination studies, acetone is preferred as a dehydration solution because it reduces shrinkage during cold-temperature processing while alcohol dehydration increases shrinkage [7]. Similarly, acetone can cause additional loss in organ mass in room temperature dehydration because of its degreasing effect to organs which may contribute to substantial weight reduction [8]. Furthermore, room-temperature plastination approaches are feasible but emphasize that solvent choice, temperature, and exchange schedules affect specimen stability and quality, consistent with organ-specific shrinkage differences between acetone and alcohol dehydration [1,9].

Post-plastination evaluation by 80 veterinary medicine students was conducted to compare the odor, texture, color and realism of the plastinated organs as shown in Table 2. Results of odor evaluation, on both treatment groups, show a median score of 3 (neutral) for lungs and thoracic limb and 4 (pleasant) for head and heart. Statistical analysis shows no significant differences between treatment groups on all organs evaluated. Both treatment groups produced odorless to mildly pleasant specimens, which is consistent with the result of using Elnady technique eliminating unpleasant odor of formalin from organs [1,2,5,25].

In terms of texture, the head evaluation showed firm (2) and hard (3) texture in Treatment 1 and 2, respectively. For heart, lungs and thoracic limb, a median texture score of 2 (firm) was observed in both treatment groups. Significant differences were observed between treatment groups of head ($p = 0.008$, $r = -0.41$), heart ($p < 0.001$, $r = -0.88$) and thoracic limb ($p < 0.001$, $r = -0.61$) while lungs texture did not differ significantly ($p = 0.39$, $r = -0.16$). Perceptible differences, as shown by large effect sizes, were also noted in the heart and thoracic limb.

The harder texture observed in the head with alcohol dehydration, despite both treatments undergoing glycerin impregnation and cornstarch curing, may be attributed to the initial intensity of protein coagulation during dehydration [10]. Alcohol causes a stronger and more rapid denaturation and precipitation of proteins, leading to tightly packed, rigid protein networks and greater tissue shrinkage [11]. In dense anatomical regions like the head, which contain abundant connective tissues and complex structures, this effect becomes more pronounced and may not be fully reversed by subsequent processing steps.

Although both methods include glycerin impregnation—which helps restore some flexibility—and cornstarch curing, these steps primarily act as post-dehydration modifiers. In the case of alcohol-treated tissues, the extent of prior protein coagulation is often too great, so the softening effect of glycerin is limited. By contrast, acetone dehydration tends to produce less severe protein rigidity, allowing the same glycerin and

Table 2. Median (Range) result of student evaluation (n=80) post-plastination of organs for odor, texture, color and realism.

Organ	Criteria	Treatment 1	Treatment 2	p value	Effect size (r)
Head	Odor	4 (1-5)	4 (1-5)	0.673	0.08
	Texture	2 (1-3)	3 (1-3)	0.008	-0.41
	Color	4 (2-4)	4 (2-4)	0.008	-0.67
	Realism	4 (2-5)	4 (2-5)	0.667	0.12
Heart	Odor	4 (2-5)	4 (2-5)	0.249	-0.22
	Texture	2 (1-3)	2 (1-3)	<.001	-0.88
	Color	4 (2-4)	4 (2-4)	0.076	-0.47
	Realism	4 (3-5)	4 (3-5)	0.859	-0.05
Lungs	Odor	3 (2-5)	3 (2-5)	1.000	0.00
	Texture	2 (1-3)	2 (1-3)	0.390	-0.16
	Color	1 (1-3)	1 (1-3)	1.000	-0.01
	Realism	3 (1-5)	3 (2-5)	<.001	-0.68
Limb	Odor	3 (1-5)	3 (1-3)	0.053	-0.37
	Texture	2 (1-3)	2 (1-3)	<.001	-0.61
	Color	4 (2-4)	4 (3-4)	0.035	-0.50
	Realism	5 (3-5)	4 (3-5)	0.001	0.79

cornstarch steps to more effectively maintain softness and flexibility [1,2,5]. Consequently, even with identical downstream treatments, the head remains harder in alcohol-dehydrated specimens due to the stronger initial structural changes induced by alcohol.

Color evaluation results showed differences among organs. Head, heart, and thoracic limb color resulted in median score of 4 (light brown) on both treatment groups. While lungs showed a median score of 1 (black) on both treatment groups as shown in Figure 1. Statistical results showed a significant difference between treatment groups of the head (p = .008, r = −0.67) and thoracic limb (p = .035, r = −0.50), while no significant difference was noted in heart (p = .076, r = −0.47) and lungs (p = 1.00, r = −0.01). Significant differences may occur despite similar median values, as nonparametric tests account for differences in distribution, variability, and ranking of observations rather than central tendency alone.

Post-plastination color is organ dependent and is influenced by the fixation process, dehydration reactions and impregnation. In this

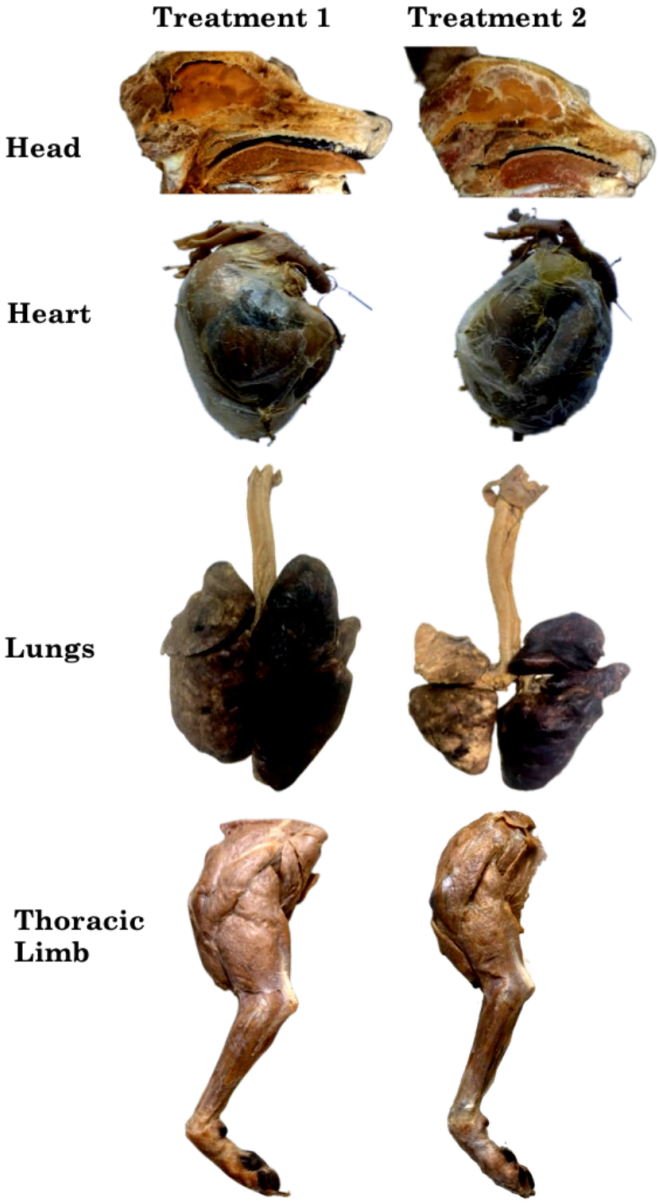

Figure 1. Representative coloration of canine head, heart, lungs, and thoracic limb specimens following plastination using acetone and 95% ethyl alcohol dehydration.

study, both dehydration methods produced acceptable coloration. This coincides with reports that Elnady technique (acetone-based preservation) can produce realistic specimens while color remains sensitive to processing conditions [1,12].

Results of realism evaluation show a range of 3 – 5. Head and heart median scores in both treatment groups showed a score of 4 (highly realistic) which exhibits intricate detail, precise textures, and accurate coloration; possesses a realistic appearance with subtle imperfections. The lungs showed a median score of 3 (Realistic) with distinctive details and coloration; exhibits a mildly rigid or smooth surface in both treatment groups. The median realism score for the thoracic limb was 5 (Extremely Realistic) in Treatment 1 and 4 (highly realistic score) in Treatment 2. Statistical analysis result showed that lungs ($p = <0.001$; $r = -0.68$) and thoracic limb ($p = 0.001$; $r = 0.79$) exhibited significant differences between treatment groups. The realism scores of the head and heart did not differ significantly between treatment groups (head: $p = 0.667$; heart: $p = 0.859$).

Realism in plastinated specimens is a multidimensional attribute that integrates accurate shape, natural texture, appropriate coloration, and functional flexibility [13]. In this study, the significant differences observed in the lungs and thoracic limb may be attributable to dehydration-induced tissue shrinkage and surface alterations. Alcohol-dehydrated specimens exhibited greater shrinkage and discoloration, reducing perceived realism, whereas acetone dehydration more effectively preserved morphology and flexibility, particularly in musculoskeletal tissues [3,4]. Plastination yields dry, durable, and anatomically precise specimens that maintain structural integrity and are suitable for educational and pathological applications [7]. Consistent with prior educational research, higher realism enhances learner acceptance and perceived authenticity of specimens [14]. Additionally, studies employing locally available materials at ambient conditions report that plastinated specimens remain odorless, dry, flexible, and durable, with preserved color and texture, allowing long-term handling and storage without decomposition and making them well suited for gross anatomical instruction [1,2,5,15-25].

4. Conclusions

This preliminary study shows that acetone and 95% alcohol dehydration using Elnady technique resulted in neutral to pleasant odor and dry specimens that are suitable for anatomical teaching; furthermore, the choice of dehydration solution depends on organ type, with lungs showing a tendency toward greater shrinkage, while thoracic limbs subjected to acetone dehydration demonstrated higher perceived realism. Furthermore, regardless of the dehydration solution used, head and heart plastinates provided acceptable and realistic specimens. These results support an organ-specific approach to organ plastinate preparation considering the limited accessibility of acetone as a dehydration solution. This preliminary study demonstrates that both dehydration methods are effective; however, limitations in sample size necessitate further investigation using a larger number of specimens to validate these observations and determine their broader applicability.

Author Contributions

Conceptualization, J.D.C., G.Z.D.C., J.P.B., J.P.C., and K.J.P.O; Methodology, J.D.C., G.Z.D.C., J.P.B., J.P.C., and K.J.P.O; Investigation, J.D.C., G.Z.D.C., J.P.B., J.P.C., and K.J.P.O; Writing – Original Draft, J.D.C., G.Z.D.C., J.P.B., J.P.C., and K.J.P.O; Writing – Review & Editing, J.D.C. G.Z.D.C., J.P.B., J.P.C., K.J.P.O., M.F.C., R.T.C., S.L.A., L.V.A.P., and M.A.M.S.; Funding Acquisition, J.D.C., M.F.C.,R .T.C., S.L.A., L.V.A.P., and M.A.M.S.; Resources, J.D.C., G.Z.D.C., J.P.B., J.P.C., and K.J.P.O; Supervision, J.D.C., M.F.C., R.T.C., S.L.A., L.V.A.P., M.A.M.S., and R.T.C.

Ethics Approval and Consent to Participate

The Institutional Animal Care and Use Committee permit was secured prior to the conduct of the study with Protocol Number: CSU-IACUC-2025-0007.

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Conflict of Interest

The authors declare no conflict of interest.

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