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Evaluation of Four Techniques for the Preservation of the Porcine Stomach

Evaluación de Cuatro Técnicas para la Preservación del Estómago Porcino

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SUMMARY: In the study of the anatomy of domestic animals, it is challenging to have specimens of all species for dissection. Therefore, it is important to have preserved anatomical pieces of all species. However, most preservation techniques use toxic reagents, primarily formaldehyde, which is classified as carcinogenic. This study aimed to evaluate four preservation techniques for porcine stomachs: aqueous formalin, phenolated glycerine, alglifen, and alcohol-propylene glycol (developed at FES Cuautitlán UNAM). The size, weight, color, and texture of the preserved organs were assessed. The study found no statistically significant differences in the size and weight of organs preserved before and after the techniques. However, the aqueous formalin technique produces organs that emit irritating vapors due to the use of aqueous formalin. There were no significant changes in color that affected the anatomical study of the obtained pieces. When evaluated with a colorimeter, it was found that the alcohol-propylene glycol and alglifen techniques produced pieces with a predominance of yellow, the glycerin-phenol technique resulted in pieces with a predominance of red, and the aqueous formalin technique produced more whitish pieces. Evaluation of the texture revealed that the alcohol-propylene glycol technique resulted in softer and more flexible pieces, while with the other three techniques, the organs appeared more rigid. In conclusion, all four techniques preserved the organs adequately, showing no signs of decomposition. The alcohol-propylene glycol technique, which produces soft parts suitable for teaching, uses reagents with no toxicity, unlike the other techniques that use formalin and/or phenol.

KEY WORDS: Veterinary anatomy; Preservation techniques; Pig stomach.

INTRODUCTION

Veterinary anatomy involves studying the shape and structure of body parts in domestic animals, horses, ruminants, pigs, carnivores, and birds through cuts and dissections (Getty, 1975). However, it is often impractical to perform dissections on these species in laboratory settings due to high costs and management issues. Therefore, preserved anatomical pieces are necessary (García Tovar *et al*., 2020). Over the years, various techniques for preserving cadavers and anatomical pieces have been explored (Muñetón & Ortiz, 2013; Ortega, 2014; Pineda *et al*., 2024). Since the discovery of formaldehyde by William Hoffman in 1859 (cited by Muñetón & Ortiz, 2013), most preservation techniques have utilized this reagent due to its effective tissue fixation, cost-effectiveness, and ease of use in aqueous solutions (aqueous formalin). However, it is important to note that formaldehyde poses significant risks due to its toxicity and is classified as carcinogenic in humans and animals by the International Agency for Cancer Research (IACR) and the US National Toxicology Program (NTP).

Currently, there are techniques that use reagents with low or no toxicity; however, many of them still employ formaldehyde, even if at low concentrations (Muñetón *et al*., 2021). The Chilean preservative fixing solution uses 2 % aqueous formalin (Ortega, 2014; Villarroel & Troncoso, 2017; Muñetón *et al*., 2021), the DPineda solution contains 6 % aqueous formalin (Pineda *et al*., 2024), and the Walter Thiel solution contains 2 % aqueous formalin (Bertone *et al*., 2011; Ottone *et al*., 2016). Plastination poses no toxicity risks for the end user; however, it uses aqueous formalin in its development, affecting those who perform the technique (von Hagens, 1979; Sánchez *et al*., 2012; Riederer, 2014;

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Toaquiza *et al*., 2023). Formaldehyde free solutions have also been reported. Muñetón & Ortiz (2011), reported two solutions: one using white vinegar, glycerin, ethanol, sodium citrate, and malachite green, and another based on ethyl alcohol and glycerin. Ethanol-glycerin solutions have also been reported for fixation, followed by preservation with thymol (Niels *et al*., 2012); ethanol, glycerin, and benzalkonium (Tamayo-Arango & Garzón-Alzate, 2018); and fixation with alcohol followed by preservation in a hypersaturated sodium chloride solution (Guaraná *et al*., 2021). In a previous study, we successfully preserved sheep hearts using a formaldehyde-free solution consisting of ethanol, sodium chloride, propylene glycol, and white vinegar (Pichardo-Molinero *et al*., 2024).

This study aimed to compare four different techniques for preserving hollow organs by evaluating the size, weight, color, and texture of porcine stomachs.

MATERIAL AND METHOD

Biological material. Twenty porcine stomachs obtained from animals slaughtered for consumption were used following the guidelines outlined in the Mexican Official Standard NOM-194-SSA1-2004: Sanitary specifications for products and services in establishments dedicated to the slaughter and working of animals for feeding, storage, transport, and distribution. The stomachs were divided into 4 groups, each containing 5 stomachs, to apply the reported techniques.

Techniques

Preservation in aqueous formalin solution. The technique consisted of immersing the stomachs in a solution of water formalin 7 % which can remain in this solution for an indefinite time, as the organs are already fixed, they can be changed to a 3 or 5 % aqueous formalin solution to preserve them.

Preservation by phenolated glycerine technique. The porcine stomachs were fixed in a solution of aqueous formalin 7 % for 72 h. After fixing, the stomachs were removed from the solution and washed with running water for 24 h. Subsequently, they were impregnated with a 10 % phenol solution in glycerine (phenolated glycerine). During the processing time, the pieces were covered with cotton cloths saturated with phenolated glycerine solution for 21 days and massaged daily for 20 min during the days the process lasted. At the end of processing time, each piece was extracted, dried with cotton canvas, and stored covered with clean and dry cotton cloth in plastic bags closed at room temperature (Nieto *et al*., 2014)

Preservation with the Alglifen technique. It was carried out by successive immersion in 3 solutions at different concentrations of alcohol, glycerine and phenol, the concentration used will depend on the stage to which the stomach is exposed to preserve and each of these solutions remained submerged for a month, covered with cotton canvas; they were given a gentle massage twice a week at each step. Step 1: 3 parts alcohol, 1 part glycerine and 50 g phenol/5 L; step 2: 1 part alcohol, 1 part glycerine and 30 g phenol/5 L; step 3: 1 part alcohol, 3 parts glycerine and 10 g phenol/5 L (García *et al*., 2022).

Preservation with the alcohol-propylene glycol technique. Porcine stomachs were fixed in a solution of 50 % alcohol, 10 % sodium chloride and 10 % commercial vinegar for 10 days at room temperature. The excess solution was then removed and soaked in 90 % propylene glycol and 10 % commercial vinegar for 10 days at room temperature for impregnation. Eventually, the organs were removed, drained and dried with cotton cloths and then stored in plastic bags (Pichardo-Molinero *et al*., 2024).

Evaluation of the organs processed by the techniques. To compare the characteristics of the pieces processed by the techniques used in this study, we evaluated the following aspects: size, weight, color, texture. We measured the height (using a vertical line drawn at the level of the *Incisura angularis*) and length (using a horizontal line running from the right end to the left end of the stomach). Weight was measured using a balance. The color was determined by evaluating the L, a, and b color space on the CIELAB scale using a Minolta CR-400 colorimeter at three points in the stomach (*Fundus ventriculi*, *Corpus ventriculi* and *pars pylorica*). The texture was evaluated using a Shore A Durometer, 0-100HD (Jectse brand), with 5 measurements taken at three points of the stomach (fundus ventriculi, corpus ventriculi and pars pylorica), and the average of the measurements from 5 preserved stomachs with each technique was obtained.

Statistical analysis. Data were analyzed using the statistical software Prism 10. A T-test was used for the individual comparison of each technique (2 groups: before and after), and a 2-way ANOVA was used for the analysis between the different methods. The data shows the averages of the groups together with the standard error of the average (SEM).

RESULTS

Satisfactory results were obtained with all techniques. Figure 1 shows porcine stomachs preserved with each of the techniques, aqueous formalin (Fig. 1A), phenolated

glycerine (Fig. 1B), alglifen (Fig. 1C) and alcohol-propylene glycol (Fig. 1D). Measurements of height and length were kept constant before and after processing, indicating that the techniques did not cause tissue contraction, which is appropriate for preserving the anatomical size of the organs. As regards weight, the techniques resulted in a slight reduction of the weight of the organs. This can be attributed to the loss of water during processing, which is favorable as it reduces free water which promotes tissue decomposition. The statistical analysis did not reveal significant differences in the stomachs before and after processing by the different techniques (Table I).

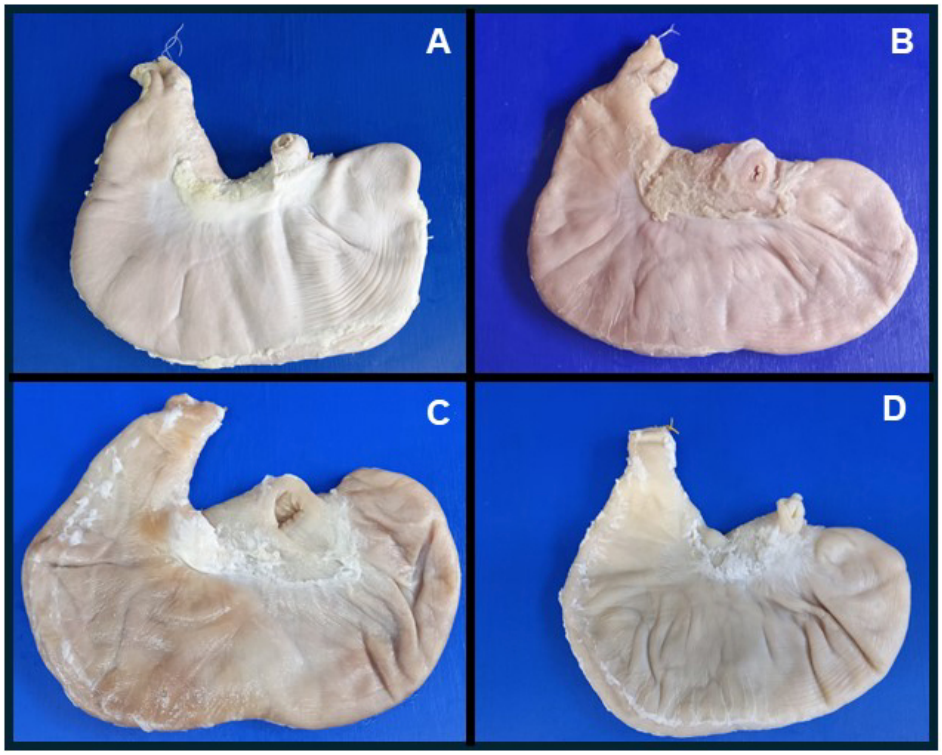

Fig. 1. Porcine stomach, parietal surface. A. Formalin aqueous technique, B. Phenolated glycerine technique, C. Alglifen technique, D. Alcohol-propylene glycol technique

Table I. Measurements of height, length and weight of the pig stomach before and after applying the four preservation techniques.

Technique	Height (cm)		Lenght (cm)		Weight (g)	
	Before	After	Before	After	Before	After
Aqueous formalin	12.6±0.42	13.7±0.97	24.8±1.60	23.8±2.02	579.8±60.45	499.8±72.73
Phenolated glycerin	14.6±1.98	14.2±1.10	26.1±2.07	24.7±1.52	540.8± 109.8	512.4± 104.61
Alglifen	13.5±1.06	14±0.79	27±1.27	27±0.71	595.6±100.09	502.2±59.25
Alcohol-propylene glycol	12.7±1.82	12.9±1.52	24.9±2.38	23.5±1.50	501±59.15	425.8±69.52

The color space was determined in preservation techniques and on the fresh organ (control) using the ICD scale L*a*b* (Luminosity value (L*) and chromatic coordinates (a* and b*) of the International Commission for Composition (ICD). The analysis determined that the stomachs processed by phenolated glycerine and alglifen techniques are less luminous than those of formalin and propylene glycol, with the latter technique providing parts of greater luminosity (Table II). The propylene glycol and alglifen technique generate predominantly yellow pieces dL* high, Table II), while glycerine pieces have a predominance of red (da*low, Table II). Organs preserved with water-based formalin were whitish.

As for the texture, during manual revision greater rigidity was noted in organs preserved with the aqueous formalin, phenolated glycerine and alglifen, techniques, unlike organs preserved with the alcohol-propylene glycol technique were found to be softer and more flexible. The data obtained with the durometer indicate a similarity between the organs preserved with aqueous formalin, phenolated glycerin and alglifen (22.75, 22.88 and 21.76 shore A, respectively) unlike the case of the alcohol-propylglycol technique which presented lower stiffness (16.38 shore A).

The external and internal anatomy was adequately

preserved. The mucous tunic of the stomachs preserved with the four techniques was adequately preserved without showing anatomical alterations due to the technique, or possible changes due to entering into a state of decomposition (Fig. 2). Figure 3 shows a preserved stomach with the alcohol-propylene glycol technique where the parts that make up the external anatomy (Fig. 3A) can be seen, as well as the internal anatomy (Fig. 3B). All terms used are in accordance with the Nomina Anatomica Veterinaria (International Committee on Veterinary Gross Anatomic Nomenclature, 2017).

Table II. Evaluation of the color of preserved organs with the four techniques.

Technique	δL	δa*	δb*
Aqueous formalin	9,45	-8.76	8.47
Phenolated glycerin	-6.15	-4.41	13.85
Alglifen	-7.99	-10.17	18.51
Alcohol-Propylene glycol	17.15	-14.53	38.13

The table shows the values of dL* = difference in light and dark, da* = difference in red and green, db* = difference in yellow and blue from stomachs processed with different techniques compared to the values of stomach control (mean L* value a* and b* of the fresh stomach).

There were no changes in color or smell that could indicate that the pieces were entering a state of decomposition.

DISCUSSION

Preservation techniques involve obtaining specimens with natural colors, tissue flexibility, absence of unpleasant odors and vapors, and safety against toxicity for students and staff while maintaining anatomical characteristics. In this study, four preservation techniques for porcine stomachs were tested as a model for hollow organs. The traditional method of preserving organs with aqueous formalin and three other techniques using different reagents. The phenolated glycerine technique involved using aqueous formalin as a fixative, along with glycerine and phenol. The other two techniques were formaldehyde-free. Alglifen technique used alcohol, glycerine, and phenol, while the other, alcohol-propylene glycol technique, used alcohol, sodium chloride, propylene glycol, and white vinegar. Although alglifen does not contain aqueous formalin, it still uses phenol, which is considered toxic. Therefore, the only solution free of toxic reagents is alcohol-propylene glycol.

The two formaldehyde-free techniques (alglifen and alcohol-propylene glycol) not only offer the advantage of being non-toxic but also

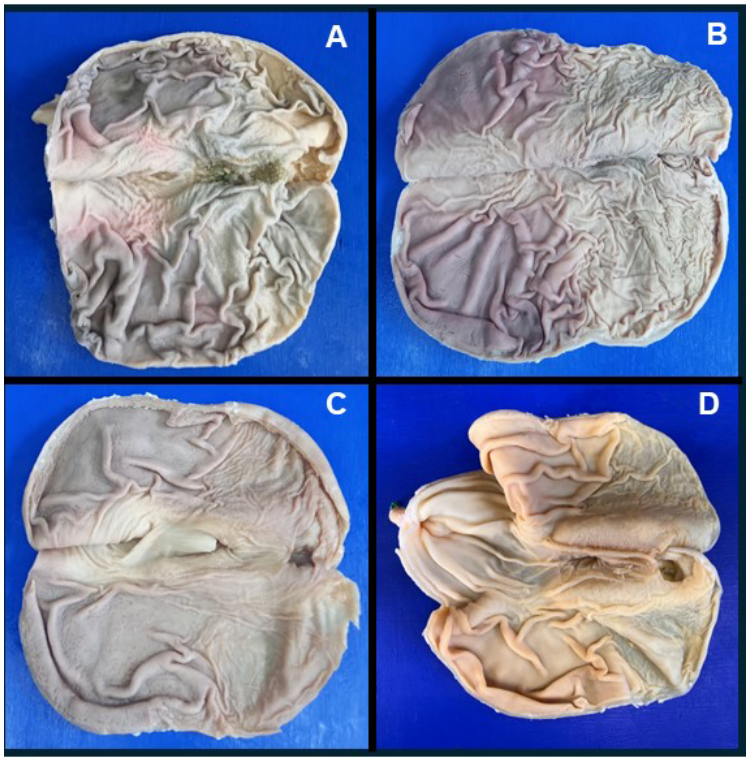

Fig. 2 Porcine stomach, *tunica mucosa*. A. Formalin aqueous technique, B. Phenolated glycerine technique, C. Alglifen technique, D. Alcohol-propylene glycol technique.

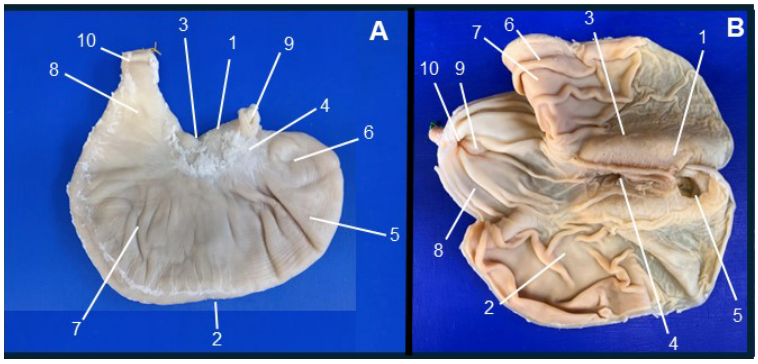

Fig. 3. Porcine stomach preserved by the alcohol-propylene glycol technique. A. *Facies parietalis*: 1. *Curvatura ventriculi minor*, 2. *Curvature ventriculi major*, 3. *Incisura angularis*, 4. *Pars cardiaca*, 5. *Fundus ventriculi*, 6. *Diverticulum ventriculi*, 7. *Corpus ventriculi*, 8. *Pars pylorica*, 9. *Esophagus*, 10. *Duodenum*. B. *Tunica mucosa*: 1. *Pars nonglandularis*, 2. *Pars glandularis*, 3. *Margo plicatus*, 4. *Ostium cardiacum*, 5. *Diverticulum ventriculi*, 6. *Plicae gastricae*, 7. *Sulci gastrici*, 8. *Antrum pyloricum*, 9. *Torus pyloricus*, 10. *Ostium pyloricum*.

do not require special facilities and are not complex. The formaldehyde-free techniques reported by Muñetón & Ortiz (2011), Niels *et al*. (2012), and Tamayo-Arango & Garzón-Alzate (2018) for the preservation of cadavers (human or animal) require submerging the cadavers in tanks, making these techniques complex and posing the challenge of needing facilities with tanks. Guaraná *et al*. (2021), working with porcine urinary bladder and intestine, used alcohol fixation followed by preservation in a hypersaturated sodium chloride solution, achieving satisfactory results, except that the viscera turned whitish. Regarding plastination, as mentioned in the introduction, it has the drawback of using formalin in its process, except for the technique reported by Baygeldi *et al*. (2022), who described a formaldehyde-free plastination technique. Plastination produces high-quality specimens, but they lose their original color, undergo a certain degree of shrinkage, and the resulting specimens are rigid, making them less suitable for educational purposes, as they cannot be easily manipulated (von Hagens, 1979; Sánchez *et al*., 2012; Riederer, 2014; Toaquiza *et al*., 2023). The specimens we obtained, particularly with the alcohol-propylene glycol technique, are soft and flexible, allowing for manipulation and dissection once the procedure is completed.

Comparing the four techniques evaluated in this study, preservation with aqueous formalin resulted in specimens that were difficult to handle during the duration of a class due to the irritating fumes, as well as their rigidity. In our experience, we also observed that when kept in aqueous formalin, this solution oxidizes, causing the specimens to darken. The phenolated glycerine technique also uses aqueous formalin in its process, with the advantage that the finished pieces do not emit irritating vapors; however, over time it also presents stiffness and darkening of the pieces. The alglifen technique gives satisfactory results, although it does not use aqueous formalin in its process it uses phenol which is also reported as a toxic reagent, although this could be avoided by replacing this reagent with commercial vinegar; the pieces also presented rigidity, with no change in color over time or unpleasant odors. Finally, the alcohol-propylene glycol technique was the only one that did not use toxic reagents throughout its development resulting in safe anatomical and flexible parts, without changes in color or unpleasant odors during its lifetime. In all techniques, the anatomical architecture was maintained to allow its use in the teaching-learning process of veterinary anatomy.

Preserved anatomical pieces are a great alternative to using corpses for teaching veterinary anatomy. They are especially useful for species that are difficult to obtain due to their price or size, and they also prevent the use of fresh material that decomposes quickly. By working with these anatomical pieces, students can visualize and understand the shape and structure of body parts of species that they may not have the opportunity to dissect. This allows them to combine theoretical knowledge with practical experience, enabling them to accurately identify the body parts of any domestic species and understandthe anatomy of each structure (Rodríguez, 2015; García Tovar *et al*., 2020).

The organs preserved using the four techniques have been kept for 3 months as of the time of this publication. However, it is worth noting that anatomical pieces preserved using alglifen and alcohol-propylene glycol have been maintained in good condition for over 3 years. This was achieved by wrapping them in flannel and storing them inside plastic bags.

CONCLUSIONS

The preservation of 20 porcine stomachs using four different techniques: aqueous formalin, phenolated glycerine, alglifen, and alcohol-propylene glycol was successful. No significant differences in size and weight were observed before and after the preservation techniques. There were no changes in color or smell indicating decomposition. However, the organs preserved with aqueous formalin showed a lighter color compared to the other three techniques, where the color was very similar to that of fresh organs. Additionally, pieces preserved with aqueous formalin, phenolated glycerine, and alglifen showed greater rigidity compared to those preserved with alcohol-propylene glycol, which exhibited greater flexibility. The external and internal anatomical structure was adequately maintained with all four techniques. Based on quality, flexibility, color, odor, and the use of non-toxic reagents, alcohol-propylene glycol was determined to be the best preservation technique.

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RESUMEN: En el estudio de la anatomía de los animales domésticos, es un desafío contar con especímenes de todas las especies para su disección. Por lo tanto, es importante contar con piezas anatómicas preservadas de todas las especies. Sin embargo, la mayoría de las técnicas de preservación utilizan reactivos tóxicos, principalmente formaldehído, el cual está clasificado como

cancerígeno. Este estudio tuvo como objetivo evaluar cuatro técnicas de preservación de estómagos porcinos: formalina acuosa, glicerina fenolada, alglifen y alcohol-propilenglicol (desarrollado en la FES Cuautitlán UNAM). Se evaluó el tamaño, peso, color y textura de los órganos preservados. En el estudio no se encontraron diferencias estadísticamente significativas en el tamaño y peso de los órganos preservados antes y después de las técnicas. Sin embargo, la técnica de formalina acuosa produce órganos que emiten vapores irritantes debido al uso de formalina acuosa. No hubo cambios significativos en el color que afectaran el estudio anatómico de las piezas obtenidas. Al evaluar con un colorímetro, se determinó que las técnicas de alcohol-propilenglicol y alglifen produjeron piezas con predominio de amarillo, la técnica de glicerina-fenol resultó en piezas con predominio de rojo y la técnica de formalina acuosa produjo piezas más blanquecinas. La evaluación de la textura reveló que la técnica de alcohol-propilenglicol resultó en piezas más suaves y flexibles, mientras que con las otras tres técnicas, los órganos aparecieron más rígidos. En conclusión, las cuatro técnicas preservaron los órganos adecuadamente, sin mostrar signos de descomposición. La técnica de alcohol-propilenglicol, que produce piezas blandas aptas para la enseñanza, utiliza reactivos sin toxicidad, a diferencia de las otras técnicas que utilizan formalina y/o fenol.

PALABRAS CLAVE: Anatomía veterinaria; Técnicas de preservación; Estómago de cerdo.

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