Quantitative Evaluation of Canine Stomach and Porcine Kidney Preserved with Ethanol-Glycerin Solution and Bleached with Different Concentrations of Hydrogen Peroxide

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ABSTRACT

The present study was to determine effects of the glycerin-ethanol based method and two different bleaching processes on quality of long term-formalin fixed organ specimens based on their coloration and volume changes. The study used 14 canine stomach samples which were obtained from formaldehyde embalmed cadavers and six porcine kidney samples which were preserved in 10 % formaldehyde solution. The results revealed that the color parameters including brightness, reddishness and bluishness were altered in all organ samples after the process of dehydration followed by bleaching but restored by impregnation with ethanol-glycerin mixture. One month storage of the specimens had different effects on coloration on canine and porcine specimens. All specimens' volume was retained at the end of the processing. The comparison of the effects of the two different concentrations of hydrogen peroxide solution on specimen quality indicated no significant differences in the color parameter values and volumes between groups. This technique was able to preserve anatomical structures of both canine stomach and porcine kidney. In conclusion, quality of organ samples was influenced by ethanol-glycerin method whose effects were varied between organs.

Key words: ethanol-glycerin solution, anatomy, dry preservation, canine, porcine

INTRODUCTION

The study of anatomy is not only build up ability to recognize body compositions and understand spatial relationships between body structures (Reid *et al*., 2018). Although, teaching aids such as video, models, interactive digital models are increasingly employed in veterinary anatomy teaching, conventional methods such as cadaver and organ specimens are still used as main tools for prosections and dissections (Varner *et al.,* 2021). Studying from cadavers and organs in veterinary anatomy laboratory also allows learners to comprehend individual anatomical variations which can be implemented for surgical practices.

Glycerin based techniques or glycerinization have been successfully employed for dry preservation of cadavers and organs. The products do not only have natural appearance, dry surface, flexibility and odorless but also show the suitability for learning of veterinary anatomy and clinical training (Carvaho *et al*., 2011, Cury *et al*., 2013, Elnady *et al*., 2015, Elnady, 2016). These techniques involves the four processes; fixation with formalin solution, water and fat elimination, replacement body fluid with durable chemicals (impregnation) and chemical stabilization of the impregnated materials (Karam *et al*., 2016)

Quality of anatomical specimens is based on its anatomical accuracy and appearance. Colorimetric and volumetric analyses of specimens can be performed in order to evaluate effects of the preservation process on specimen's quality (Akgün *et al*., 2017, Bakici *et al*., 2017). This study aimed to quantitatively analyze the effects of each stage of glycerin-ethanol based preservation on the quality of anatomical specimens consisting of the canine stomach representing a hollow organ and porcine kidney specimens representing a solid organ. The information gained from this study should improve the preparation methods for anatomical samples.

MATERIALS AND METHODS

Animals and specimen collection

Two organs including canine and porcine stomachs were used in this study. Canine stomach specimens (n=14) were obtained from donated cadavers which had been embalmed with embalming fluid containing 3.2% formalin, 20.5 % ethanol, 10.8 % glycerin and 5.4% phenol (V/V) in water and maintained in 10% formaldehyde solution for 1.5 years. Porcine kidneys (n=6) were purchased from fresh market vendors and fixed in 10% neutral buffer formalin for one year. The use of organ specimens in this study was approved by Kasetsart University's Institutional Animal Care and Use Committee, ID number: ACKU-VET032.

Glycerin-based preservation procedure

All formalin-fixed specimens were washed in tap water overnight and subsequently processed with the method reported by Carvalho and colleagues (2011) with some modifications. All organ samples were dehydrated in 70% ethanol solution for one week as the fresh solution was replaced every three days. Then, the canine stomachs and porcine kidneys were divided into two equal groups for bleaching for one week in one of two different solutions: 3% or 5% (V/V) hydrogen peroxide solution. The final stage of the process was to impregnate the specimens with a mixture of ethanol-glycerin at a 2:1 ratio (V/V) for two weeks. Finally, the specimens were air-dried overnight, stored and kept in containers sealed against light and air for one month. The volumes of all solutions used were at least twice specimen volumes. All stages of the preservation were conducted at ambient temperature.

Colorimetric evaluation

Color measurement of the organs was performed using a Miniscan EZ4500L colorimetric device (Hunter Lab, USA) with the CEI colorimetric system. Three colorimetric parameters were measured L* for the brightness of the organ, a* for the green-to-red color changes and b* for the blue-to-yellow color changes. From each sample, three readings of each parameter were taken at three different locations and the values were then averaged. The measurements were carried out at four different stages of the preservation: formalin fixation, dehydration and bleaching, after impregnation and after one-month storage.

Volumetric evaluation

The volumes of each fully expanded dog stomach inflated with fluid (water at the formalin-fixation stage and 70% ethanol at the end of impregnation stage) and porcine kidneys were measured according to Archimedes principle. The percentage of volume changes was obtained according to the following equation:

$$V\Delta = [(V2 - V1) \div V1] \times 100$$

where $V\Delta$ is the percentage of volume change, $V1$ is the volume measured pre-processing and $V2$ is the volume measured post processing.

Statistical analysis

The values of color parameters of all samples in each group were presented as means ± standard error of mean (mean ± S.E.). Two-way repeated measure analysis of variance was used to test for the effects of two variables (stages of the process and groups). Post hoc testing was performed using simple effect analysis with the Bonferroni correction if the effects of the variables were significant.

The volumes of the organs before and after the process were presented as means ± standard deviation. The normality of the data distribution and homogeneity of the variance were verified using the Shapiro-Wilk test and the Levene's test, respectively. The organ volumes of pre and post processing were compared using an independent T-test. Significance was $P<0.05$. The statistical analysis was performed using the IBM SPSS Statistics Version 27.

RESULTS

The ethanol-glycerin-based process was able to maintain the anatomy of both the canine stomach and porcine kidney samples. The anatomy correctness of the canine stomach samples was consistent throughout the process, with the texture of the organs being rubbery and flexible. All stomach samples had a dry and slightly wrinkled surface and the gastric folds were well-preserved (Figure 1). The porcine kidney samples maintained their anatomy in all stages of the process but the final products contained small crevices on the outer surface (for 2 of 6 kidneys). The appearance of the wrinkled surface from fixation on one kidney could not be eliminated by the process of glycerin-based preservation. The internal structures of the porcine kidneys were distinguishable (Figure 2).

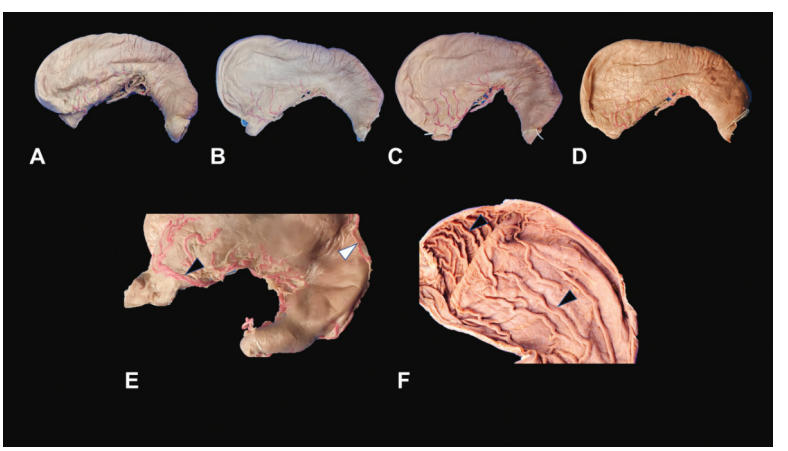

Figure 1 Canine stomach samples at each stage of glycerin-based process; (A) formalin fixation; (B) dehydration-bleaching; (C) ethanol-glycerin impregnation; (D) sample after storage. for one month; (E) external appearance of final product with prominent blood vessels, where white arrow head indicates branches of right gastric artery and, white arrow head indicates branches of left gastroepiploic artery; (F) internal appearance of final product; where black arrow heads indicate gastric folds.

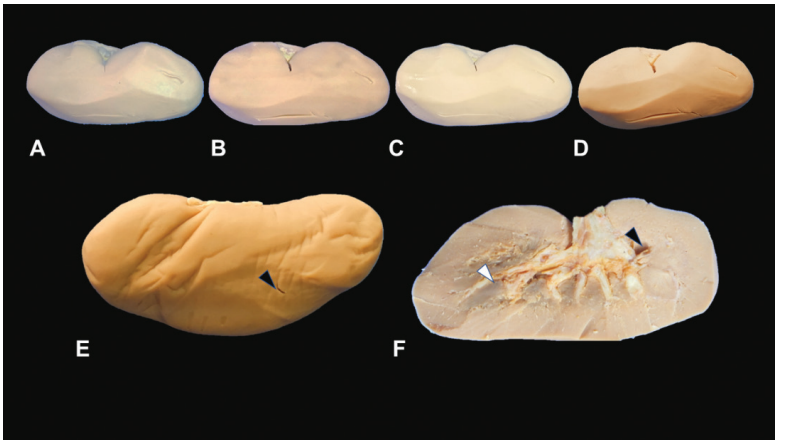

Figure 2 Porcine kidney samples at each stage of glycerin-based process; (A) formalin fixation; (B) dehydration-bleaching; (C) ethanol-glycerin impregnation; (D) sample after storage for one month; (E) external appearance of final product, where black arrow head indicates a small crevice; (F) internal structures of final product, where black arrow head indicates renal calyx and white arrow head indicates renal pyramid.

The brightness values (L*) of the canine specimens in both groups was significantly reduced by dehydration and bleaching but returned to similar levels of the pre-processing levels in the organs in after impregnation. The brightness of all specimen was maintained after one-month of storage (Table 1). Despite the different

Table 1 Colorimetric evaluation (mean ± S.E.) of canine stomach

Group	N	Parameter	Stage of process			
			Fixation	Dehydration and bleaching	Impregnation and air-drying	After storage for one month
3% H_2O_2	7	L*	46.32±2.11^{a,A}	39.20±2.24^{b,A}	44.59±1.98^{a,A}	47.61±1.65^{a,A}
5% H_2O_2	7		45.01±1.09^{a,A}	42.92±0.97^{b,A}	48.03±2.46^{a,A}	51.16±1.99^{a,A}
3% H_2O_2	7	a*	8.81±0.59^{a,A}	5.39±0.20^{b,A}	9.63±0.14^{a,A}	8.82±0.22^{a,A}
5% H_2O_2	7		7.86±0.67^{a,A}	5.43±0.27^{b,A}	9.97±0.24^{a,A}	8.83±0.33^{a,A}
3% H_2O_2	7	b*	9.95±0.53^{a,A}	5.85±0.38^{b,A}	10.11±0.82^{a,A}	9.99±0.41^{a,A}
5% H_2O_2	7		9.51±0.31^{a,A}	6.50±0.58^{b,A}	12.59±1.33^{a,A}	11.66±1.04^{a,A}

a, b: Different lowercase superscripts in the same row indicate significant ($P<0.05$) differences.

A, B: Different uppercase superscripts in the same column indicate significant ($P<0.05$) differences.

concentrations of hydrogen peroxide that the specimens were exposed to, the canine specimens of both groups had similar effects of dehydration-bleaching on brightness (Table 1). The stage of dehydration-bleaching had similar effects on the brightness of porcine kidney samples. The brightness levels of porcine kidneys in both groups increased after the impregnation but never reached to the higher levels of the pre-processing specimens. The storage had negative effects on the brightness of the porcine kidney of both groups not for the canine samples (Tables 1 and 2).

The dehydration-bleaching stage significantly decreased the values of the green-to-red (a*) and the blue-to-yellow (b*) changes in the canine and porcine organs of both groups (Tables1 and 2) compared to those measured in the pre-processing specimens of both species ($P<0.05$). The values of these two parameters in the canine and porcine organs of both groups significantly increased after the impregnation. The levels of reddishness and bluishness for the ethanol-glycerin-impregnated stomachs returned to the levels for formalin-fixed samples whereas those levels for porcine kidney samples were significantly higher ($P<0.05$).

Shrinkage occurred in both the canine and porcine specimens but volume differences were not significant (Tables 3 and 4). In addition, the two methods of bleaching had similar effects on the percentages of volume change for both the canine and porcine organ samples with some reduction (Tables 3 and 4).

Table 2 Colorimetric evaluation (mean ± S.E.) of porcine kidney

Group	N	Parameter	Stage of process			
			Fixation	Dehydration	Impregnation and air-drying	After storage for one month
3% H_2O_2	3	L*	64.52±0.87^{a,A}	46.51±0.38^{b,,A}	54.60±0.46^{c,A}	52.74±0.21^{d,A}
5% H_2O_2	3		64.29±0.13^{a,A}	44.56±0.54^{b,A}	54.34±0.22^{c,A}	50.53±0.64^{d,A}
3% H_2O_2	3	a*	6.03±0.08^{a,A}	3.71±0.06^{b,A}	7.15±0.29^{c,A}	7.01±0.17^{c,A}
5% H_2O_2	3		5.83±0.12^{a,A}	3.55±0.09^{b,A}	7.63±0.06^{c,A}	7.76±0.03^{c,A}
3% H_2O_2	3	b*	18.10±0.76^{a,A}	11.26±0.39^{b,A}	18.78±0.29^{a,A}	19.52±0.51^{a,A}
5% H_2O_2	3		18.54±0.52^{a,A}	11.23±0.45^{b,A}	19.53±0.16^{a,A}	19.90±0.25^{a,A}

a, b: Different lowercase superscripts in the same row indicate significant ($P<0.05$) differences.

A, B: Different uppercase superscripts in the same column indicate significant ($P<0.05$) differences.

Table 3 Volumetric evaluation of canine stomach (mean ± S.D.)

Group	N	Stage of process		Volume change (%)
		Fixation	Impregnation and air-drying	
3% H_2O_2	6	318.17 ± 135.56 ml. ^{a,A}	265.83 ± 108.31 ml. ^{a,A}	-15.86 ± 5.17
5% H_2O_2	6	346.64 ± 177.63 ml. ^{a,A}	290.28 ± 120.52 ml ^{a,A}	-13.46 ± 10.51

a, b: Different lowercase superscripts in the same row indicate significant (P<0.05) differences.

A, B: Different uppercase superscripts in the same column indicate significant (P<0.05) differences.

Table 4 Volumetric evaluation of porcine kidney (mean ± S.D.)

Group	N	Stage of process		Volume change (%)
		Fixation	Impregnation and air-drying	
3% H_2O_2	3	136.67 ± 14.70 ml. ^{a,A}	137.33 ± 12.68 ml. ^{a,A}	-1.44 ± 13.11
5% H_2O_2	3	120.73 ± 25.37 ml. ^{a,A}	114.00 ± 33.15 ml ^{a,A}	3.64 ± 11.92

a, b: Different lowercase superscripts in the in the same row indicate significant (P<0.05) differences.

A, B: Different uppercase superscripts in the same column indicate significant (P<0.05) differences.

DISCUSSION

Methods of preservation have been shown to influence the quality of anatomical specimens based on the evaluation of color alteration as well as retention of the specimen's volume and anatomy (Argűn *et al*., 2017, Bakici, *et al*., 2017). Glycerin-based preservation have been successful for retaining their natural appearance (Cury *et al*., 2013, Karam *et al*., 2016) as well as maintaining specimen dimensions (Carvalho *et al.,* 2011). This study provided information regarding of the colorimetric alterations caused by each stage of the processing method and the volumetric changes between pre and post processing.

Colorimetric and morphometric changes for specimens are common consequences of chemical exposure during anatomical preparation procedures (Turan *et al*., 2017). Myoglobin has been known as a main component that gives a reddish hue to organs (Oto *et al*., 2020) and is mostly denaturized by formaldehyde and ethanol (Brenner, 2014) during the fixation and dehydration stages. The reduction of reddishness in both specimens seemed to parallel the decrease in the brightness value and the blue-to-yellow changes at the stage of dehydration followed by bleaching. However, such effects of this stage of the processing on both canine and porcine samples were similar and not permanent.

Generally bleaching with hydrogen peroxide solution is used to improve the external appearance of specimens. The effect of this process depends on the concentration of the hydrogen peroxide, temperatures and the duration and amount of sunlight (Chen and Sui, 2018), suggesting that either the duration or concentration of hydrogen peroxide in this procedure must be optimized for effective bleaching of long-term formalin-fixed organs. An ethanol-glycerin mixture has been used successfully for soft embalming (Hammer *et al*., 2012) that results in a natural appearance and softness. This mixture seemed to exert a similar effect when used for impregnation of the canine stomach samples although the color parameters of the organ samples were not significantly improved. There was no perceived benefit for the porcine kidneys from using the ethanol-glycerin solution since there were crevices on the surface due to dryness. The duration of impregnation as well as the ratio of the ethanol-glycerin solution may need to be adjusted for solid organs such as kidneys to achieve similar effects to those observed for the canine stomachs.

In anatomical preparation, shrinkage of the preserved products is related to the process of dehydration (Ottone *et al*., 2019) with the magnitude of the effect varying with type of organs, chemical agent and period of dehydration (Brown et al., 2002, Holladay and Hudson, 1989). There was no significant difference in either the canine and porcine kidney sample volumes between pre and post processing, suggesting the ethanol-glycerin preservation can effectively retain the volume of both hollow and solid organs. This was contrary to a report that suggested tissue porosity was a factor that determines the degrees of organ shrinkages (Brown et al., 2002). It was likely that the organ samples in this study had been fixed with formaldehyde solution for a long period and so there was already a reduction in the volumes compared to their fresh state.

In conclusion, the quality of both canine stomach and porcine kidney samples was altered especially following the stage of dehydration-bleaching, with the color parameters being the most affected by this stage. The external appearance of the canine stomachs did not alter unlike for the porcine kidneys after processing. Shrinkage in both organs was not significant both following the processing and after storage.

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