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VOLUMETRIC AND COLORIMETRIC EVALUATION OF FORMALIN AND KAISERLING FIXATION METHODS IN DOMESTIC AVIAN SPECIMENS

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Summary

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Otolithic activity is very rapid in the avian body, due to the catabolic enzymes in cells and tissues after death. Otolysis is an undesirable condition for scientists focused on the preservation of tissues. In the post mortem period, tissue and organs should be preserved as soon as possible by various fixation methods. Among these methods, formalin is the most frequently used one due to its low cost. However, the formaldehyde, the main ingredient of the formalin solution, is exceedingly detrimental to human health. Moreover, formalin causes undesirable changes in the natural structure of the tissues. At this stage, Kaiserling solutions come to the forefront because of their colour preservation ability. The aim of this study is to evaluate the colour and volume alterations on the heart, spleen and liver of domestic avian species fixated with formalin and Kaiserling solutions. It was determined that the Kaiserling solution played an active role in terms of effective colour protection and minimum volumetric shrinkage.

Key words: avian, colorimeter, fixation, formalin, Kaiserling, volume

INTRODUCTION

Following the end of the vital activity, the process of fixing the tissues and organs in order to preserve in the closest form to the living organism is called the process of fixation. Fixation stops the autolysis and stabilises the organic components (Ulmer, 1994; Ravi & Bhat, 2011).

Today, various solutions are being used for the fixation processes. Among these solutions, formalin (water – formaldehyde) is the common used one due to its low cost (Hayashi *et al*., 2016). But formaldehyde, main component of the formalin solution, is extremely harmful to human health. Formaldehyde can cause eye irritation, nasal obstruction, burning in the throat, headache, dizziness, contact dermatitis, asthma crisis, but most important of ever, cancer (Brenner, 2014). The most significant statement of researchers is not only the harmful effects formalin solution to human health, but also the undesired

changes in the natural form of the tissues. One of the disadvantages is the remarkable alterations in the natural colour of the tissues (von Hagens *et al*., 1987; Sandhyamani *et al*., 2005; Brenner, 2014). However, the demonstration of the specimens in the closest form to the natural color and shape after fixation process is very important for the observers and students as well (Coon, 1949).

At this point, Kaiserling solutions (1897) have an unquestionable place to provide tissue or organs a natural outlook (Coon, 1949; Pulvertaft, 1950; Boushey & Stultz, 1983; Sandhyamani *et al*., 2005; Natarajan *et al*., 2012; Patil *et al*., 2013; Brenner, 2014; Prasad *et al*., 2015; Kamath *et al*., 2016). Three types of solutions, Kaiserling fluid no. I-II-III, are mainly used in the Kaiserling fixation method. Kaiserling fluid no. I containing potassium acetate, potassium nitrate and formalin (low concentration, 3–4%) is used basically for the fixation. Kaiserling fluid no. II containing ethyl alcohol is used mainly for the colour restoration. Kaiserling fluid no. III containing potassium acetate, glycerin and distilled water is used fundamentally for the mounting (Pulvertaft, 1950; Patil *et al*., 2013).

The aim of this study is to calculate the colour and volume alterations on the formalin and Kaiserling fixated heart, spleen and liver of domestic poultry species and to indicate the convenient solution in terms of these parameters.

MATERIAL AND METHODS

Sixteen broiler chickens were used for statistical interpretation in the present study. The heart, spleen and livers of chickens used in the study were obtained from a licensed slaughterhouse. The study was approved by Ankara University Animal Experiments Local Ethics Committee (Decision no.: 2017-13-108).

Organs were dissected out within 15 min after the animals were slaughtered. The natural colour of the organs was measured using the CR-400 Minolta portable colorimeter (The Konica Minolta Chroma Meter CR-400, Tokyo, Japan) (Fig. 1A). Colour alterations were quantitatively evaluated using the colour data software (SpectraMagic NX, Tokyo, Japan). Volume calculations were performed by Archimedes Method (Fig. 1B). After the first colour and volume measurement procedure was finished, the or-

Fig. 1. The natural colour measurement phase of the organ with the colour analyzer (A); Volume measurement of organs according to Archimedes principle (B).

Table 1. Statistical evaluation of volumetric shrinkage rates of organs (mean ± SEM)

Organ	n	Kaiserling solution	Formalin solution (10%)
Spleen	8	20.96 ± 5.34	28.54 ± 3.63
Heart	8	11.63 ± 0.89	18.99 ± 1.12
Liver	8	6.66 ± 0.43	11.09 ± 0.79
Est. Marginal Means*	24	13.082 ± 1.573 [b]	19.542 ± 1.573 [a]

[a,b,c]: Different letters in the same line demonstrate statistically significant difference (P<0.05).

gans were divided into two groups. Eight sets of heart, spleen and liver; 10% formalin (Sigma, Steinheim, Germany) (37% formaldehyde was accepted as 100%) and other 8 sets were fixed with Kaiserling solutions (Kaiserling fluid no. I, no. II, no. III, respectively). The organs were stored for 10 days in Kaiserling fluid no. I including 85 g potassium acetate (Merck, Darmstadt, Germany), 45 g potassium nitrate (Merck, Darmstadt, Germany), 4800 mL 4% formalin solution, in Kaiserling fluid no. II including ethyl alcohol 80% (Delta, Konya, Turkey) for half an hour and in Kaiserling fluid no. III containing 200 g potassium acetate, 300 ml glycerin (Emir Kimya, Ankara, Turkey), 900 ml distilled water for 10 days. The formalin fixation process of the other group was maintained for 20 days in order to equalise the period with the formalin fixation process. At the end of the process, colour and volume analyses of the organs in each group were performed again. The data obtained after fixation were compared statistically with the results of natural color and volume measurements. Data were expressed as mean±SEM. Data were subjected to two-way ANOVA (analysis of variance) using General Linear Model procedure. *Post hoc* testing was only carried out for significant interactions and was performed using simple effect analysis with Bonferroni adjustment. A probability value of less than 0.05 was considered significant, unless otherwise noted. SPSS 14.01 was used for statistical analysis.

RESULTS

The volumetric shrinkage of the Kaiserling and 10% formalin solution after fixation on the organs was different as expected. The organs in the 10% formalin solution shrank more than the Kaiserling solution in terms of volume for all organs (P<0.05) (Table 1). Volume shrinkage and colour changes among the solutions were statistically evaluated.

The brightness (dL) values of Kaiserling and 10% formalin solutions showed a variety. When compared to Kaiserling solution, brightness of all organs fixated with formalin was increased (P<0.05) (Table 2). The difference between the change in brightness caused by the Kaiserling solution on the heart and liver was not significant (P>0.05) (Table 2). The effects of Kaiserling and 10% formalin solution on the change between the blue and yellow color (da) of all organs were statistically significant (P<0.05) (Table 2). Formalin changed the natural colour of all organs to blue when compared with Kaiserling solution. The colour alteration between the blue and yellow on the heart and spleen fixated with Kaiserling solution was not statistically significant (P>0.05) (Table 2). Moreover, there was no significant distinction in blue and yel-

Table 2. Statistical evaluation of colour change in organs (mean ± SEM)

	Organ	n	Kaiserling solution	Formalin solution (10%)
dL	Spleen	8	4.87 ± 0.43 $^{b. A}$	13.43 ± 0.75 $^{a. A}$
	Heart	8	2.24 ± 0.34 $^{b. B}$	11.08 ± 0.56 $^{a. B}$
	Liver	8	0.87 ± 0.62 $^{b. B}$	5.83 ± 0.32 $^{a. C}$
da	Spleen	8	−6.65 ± 0.61 $^{a. B}$	−12.87 ± 0.71 $^{b. B}$
	Heart	8	−5.58 ± 0.52 $^{a. B}$	−10.04 ± 0.53 $^{b. A}$
	Liver	8	−0.54 ± 0.41 $^{a. A}$	−12.41 ± 0.44 $^{b. B}$
db	Spleen	8	−3.16 ± 0.49 ^{B}	−3.51 ± 0.57 ^{B}
	Heart	8	−2.49 ± 0.15 $^{b. B}$	0.10 ± 0.19 $^{a. A}$
	Liver	8	−0.33 ± 0.49 ^{A}	−0.45 ± 0.49 ^{A}
dEab	Spleen	8	9.52 ± 0.44 $^{b. A}$	19.70 ± 0.38 $^{a. A}$
	Heart	8	6.61 ± 0.40 $^{b. B}$	14.67 ± 0.23 $^{a. B}$
	Liver	8	2.81 ± 0.50 $^{b. C}$	13.67 ± 0.37 $^{a. B}$

^{a,b,c}: Different letters in the same line demonstrate statistically significant difference ($P<0.05$), ^{A,B,C}: Different letters in the same column demonstrate statistically significant difference ($P<0.05$).

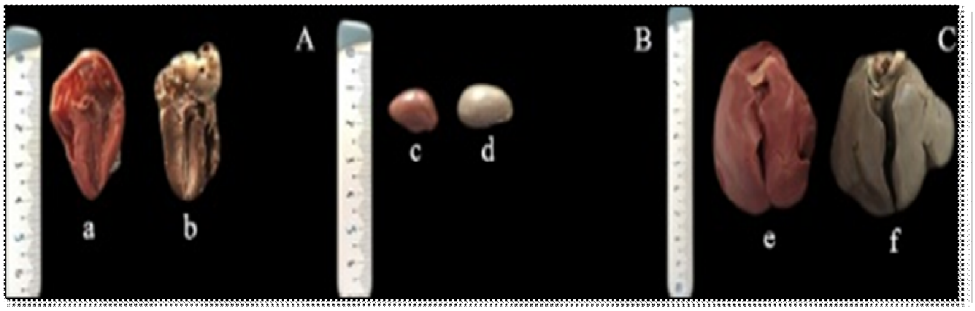

Fig. 2. Coloration of hearts (A), spleens (B) and livers (C) after Kaiserling solution (a, c, e) and 10% formalin solution fixation (b, d, f).

low colour change between spleen and liver when formalin was used. The change between green and red color (db) on organs, which were fixed by different solutions, was significant only in heart ($P<0.05$) (Table 2).

The natural colour of the heart turned red when the formalin solution was used and it turned green in the Kaiserling solution. All the organs were turned green with the use of both solutions, except effect of the 10% formalin solution on heart. The visually perceptible natural colour change (dEab) in all organs after fixation was significant in between solutions ($P<0.05$) (Table 2). It was found that the Kaiserling solution preserved the natural colour of the organs much more than the 10% formalin solution (Fig. 2). Among these organs, Kaiserling solution especially protected the liver's natural colour.

DISCUSSION

Many scientists had pointed out that Kaiserling solution plays an effective role in protecting the natural colour of the organs (Sandhyamani *et al*., 2005; Natarajan *et al*., 2012; Patil *et al*., 2013; Brenner, 2014; Prasad *et al*., 2015; Kamath *et al*., 2016). However, it has not been mentioned how effective this protection is. In

this study, the colour protection effect of the Kaiserling solution was evaluated quantitatively. The colour changes that can be noticed on the organs were interpreted statistically. The frequency of usage due to the cheapness of formalin solution was stated in the literature (Hayashi *et al*., 2016). It has been however noted that frequent use of formaldehyde is detrimental to human health (Brenner, 2014). The Kaiserling solution used in the study is more expensive than the 10% formalin solution. However, the low percentage of formaldehyde content in Kaiserling solution is quite remarkable in terms of human health. The presence of glycerin in fixative solutions seemed to have a reducing effect of tissue shrinkage (Sandhyamani *et al*., 2005). Glycerin in the Kaiserling solution had a positive effect on the tissue shrinkage in this study.

CONCLUSIONS

In this research it was determined that Kaiserling solution protected the natural colour of the organs more than the 10% formalin solution. Moreover, the visible colour change among the organs using Kaiserling solution was at least in the liver. At the same time, the volumetric shrinkage after the fixation in the organs used Kaiserling solution is lower than that of 10% formalin. It is also thought that the natural colour of the organs will be preserved by using Kaiserling solution in fixation, which is an important step of plastination.

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