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Room Temperature Plastination of Three Species of Fish (Tilapia, African Catfish and African Bonytongue) in Ghana

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Abstract - **This study assessed the suitability of room temperature plastination with locally available silicone paste and hardener in Ghana for producing plastinates of Tilapia, Catfish and Bonytongue for use as teaching aids. Fresh fish samples were fixed in 10% neutral buffered formalin solution at room temperature, dehydrated in acetone at room temperature, impregnated with a silicone polymer solution at room temperature, and then hardened by curing in acidic fumes under UV light in a dehumidified environment at room temperature. The weights, morphometric measurements of Girth-Behind-Operculum (GBO), Girth Medially (GM), Head Length (HL), Total Length (TL) and StandardLength (SL), and anatomical proportions of TL/GBO, HL/SL, TL/GM, HL/TL, GBO/GM, and SL/TL were determined for each fresh sample and at the end of each plastination stage. Differences in weights and anatomical dimensions of fresh and plastinated samples of the three fish species were not significant except for the Catfish GM and Tilapias weights. Differences in the anatomical proportions of fresh and plastinated samples were not significant (P>0.05) with the exception of the Catfish TL/GBO and TL/GM. Mean weight losses at the end of plastination were 35.2%, 34.5% and 28.2% for Tilapia, Catfish and Bonytongue respectively. The plastinates were well preserved and dry, hard, and odorless, and could be handled with bare hands. Since the anatomical dimensions and proportions of the plastinates were not significantly different from those of the fresh samples it shows that room temperature plastination is suitable for preserving Tilapia, Catfish and Bonytongue.**

1. INTRODUCTION

Body fluids are liquids that originate from within the body of a living organism. The fluid may be expelled by excretion or secretions from the body or may be retained within the body. Body fluids consists mainly of body water and the remainder as lipids. Body water occurs as intracellular fluid (35% – 40%) and extracellular fluid (60% – 65%). The extracellular fluid consists of interstitial fluid bathing the cells, lymph, blood, and transcellular fluid within epithelial lined spaces.

In a living organism, the body fluids play a very important role in maintaining the shape and weight of the organism. However, immediately the organism dies, decomposition begins. The process of decomposition is aided by the body fluids which provide a good medium for the body's own chemicals and enzymes to autolyse the body tissues, and for bacteria to breakdown the tissue's by putrefaction.

The long term preservation of organisms could be enhanced by the:

1. Destruction of microorganisms and autolytic enzymes.
2. Removal of water and fats from the tissues of the body to halt the processes of autolysis and putrefaction.
3. Replacement of the water and fats lost from the tissues with another medium in order to prevent shrinkage to maintain the structural integrity of the organism
4. Simultaneous movement of body fluids out of the body tissues and diffusion of the replacement medium into the body tissues so that the shapes of the tissues, organs and organism are maintained throughout the process.
5. Prevention of loss of the replacement fluid from the tissues of the organism to the atmosphere by diffusion and evaporation.
6. Ensuring that the replacement medium does not contain any water or lipid as this would only serve to facilitate enzyme autolysis and microbial putrefaction of the organism, should resists decolourisation, and be compatible with biological tissue (Weiglein, 2005).

The process of plastination invented in 1878 by von Hagens (Grondin, 1998) ensures the long term preservation of organisms by satisfying the six criteria listed above.

During plastination, the decomposition of the organism is halted through fixation. The water and fats in the tissues are then totally replaced by dehydration with acetone. The acetone is completely evaporated from the tissues and replaced with a low viscosity and reactive polymer such as silicon or polyester through forced vacuum impregnation (Suganthy, 2012). The polymer is then hardened by curing with light, heat or certain gases (Weiglein, 2005). The preserved specimens are known as Plastinates (Priya *et al*, 2007). The type of polymer used determines the look and feel of the plastinates (Weiglein, 2005). Plastinates are of superior quality than specimen preserved by other methods (Lee *et al*, 2005). Plastinates are used as teaching aids in the life sciences (Roach, 2000; Cook, 1997) and they have advantages over models preserved by the traditional method of formaldehyde preservation. Formaldehyde is toxic, has an unpleasant smell and irritates the skin. The specimen deteriorate quickly when they are taken out of the formaldehyde (Roach, 2000). Plastinates look natural and they are odorless and very durable (Grondin, 1998). They can be handled with bare hands because they are dry and nontoxic (Lee *et al*, 2005).

The standard method of plastination called the S10 method therefore has the following procedures: fixation, dehydration, impregnation and curing (Grondin, 1998).

Zheng *et al.* (2000) showed that the standard S10 plastination method could be adapted by using locally available materials as substitutes for the standard materials to produce plastinates at a lower cost.

In a previous study, (Ameko *et al.*, 2013a) locally available silicone paste and hardener in Ghana were used as substitutes for the standard materials to plastinate three types of fish (Tilapia, Bonytongue and Catfish). Fixation, pre-impregnation, intermittent forced impregnation, and curing stages were done at room temperature, and dehydration and post-impregnation at 4^{o}C. The plastinates retained their general shapes and their anatomical structures were preserved. The anatomical dimensions and proportions of the plastinates were not significantly different ($p>0.05$) from that of the fresh samples.

In another study (Ameko *et al.*, 2013b)the use of low temperature was eliminated during the plastination of whole guinea pigs and dissected guinea pigs. Fixation, dehydration, pre-impregnation, intermittent forced impregnation, post-impregnation and curing were all done at room temperature. The shapes and sizes of the plastinates did not differ significantly ($p>0.05$) from those of the live samples. The results showed that room temperature plastination with locally available materials would be cheaper to produce because of reduced energy cost in production.

This current study assessed the suitability of room temperature plastination with locally available silicone paste and hardener in Ghana for producing plastinates of Tilapia, Catfish and Bonytongue. The hypothesis (H_0) was that the anatomical dimensions and proportions of the plastinates would be significantly different ($p>0.05$) from those of the fresh samples.

2. MATERIALS AND METHODS

2.1. Specimen collection

Live Tilapias, African Catfish and African Bonytongue fish were procured from ponds on the Volta Lake in Ghana. The fish were immediately fixed by immersion in 10% neutral buffered formalin solution in plastic containers until they died. The specimens were tagged with different colored rubber bands for easy identification. The color and the texture of the fish were also noted. The specimens were transported to the laboratory.

2.2. Procedure for Room Temperature Plastination

2.2.1. Fixation

The fish were kept in neutral buffered formalin solution at room temperature for 10 days. Some of the fixative was injected into the body cavities of the fish through their orifices (Figure 2).

Figure 1. Injecting fish through the orificewith silicone polymersolution

2.2.2. Dehydration

Samples were removed from the fixative, drained and then immersed in 30% acetone at room temperature for 3 days and then finished off in 80% acetone for a further three days at room temperature.

2.2.3. Pre- impregnation

A Silicone Polymer Solution was prepared by dissolving 635g of Silicone Paste (Asmaco Antifungal 100% Sealant 2540^{TM}) and 60ml of Silicone Hardener (Sikkens 123^{TM}) in 6 liters of Xylene. The samples were removed from the 80%acetone solution, drained of excess acetone, and then immersed for 7 days in the polymer solution in a desiccator at room temperature.

2.2.4. Intermittent forced impregnation

The specimens were immersed in the polymer solution in a sealed desiccator at room temperature and low pressure from a vacuum pump applied intermittently for 12 hours and then off for 12 hours for 3 days. The vacuum caused the acetone to vaporize from the tissues and allowed the polymer to diffuse into the tissues.

2.2.5. Post-Impregnation

The vacuum pump was switched off and the pump disconnected and the specimens left immersed in the silicon solution at room temperature for 4 days.

2.2.6. Curing and hardening

The specimens were removed from the silicon solution and excess polymer wiped off. The external surfaces of the specimens were coated with the Hardener solution. The specimens were arranged on a clean absorbent cloth on a shelf (Figure 2) and then exposed to acidic fumes from a dilute acid solution obtained by diluting concentrated H_2SO_4 with an equal volume of distilled water. The specimens and flasks containing the acid solution were placed in a sealed chamber under UV light together with petri dishes of calcium chloride desiccant to keep the environment dehumidified for 14 days.

Figure 2. Coating fish with hardener then curing fish on a shelf

2.3. Morphometric Measurement on Fish

Morphometric measurements of each sample were taken to the nearest centimetre using a string and ruler. This was done on each sample when fresh and also at the end of each stage of plastination. The Girth-Behind-Operculum (GBO) and Girth Medially (GM) were measured by the method of (Stergiou *et al*. 2003), and Head Length (HL), Total Length (TL) and Standard Length (SL) by the method of (Herrel, *et al*. 2005).

2.3.1. Head Length (HL)

A rope was stretched from the tip of the mouth to the root of the first dorsal spine and the length recorded (Figure 3).

Figure 3. Head Length and Total Length

2.3.2. Total Length (TL)

The total length is the maximum length of the fish with the mouth closed and the tail fin pinched together. A rope was stretched from the tip of the mouth through the ventral side to the pinched end of the tail (Figure 3).

2.3.3. Standard Length (SL)

A rope was stretched from the tip of the mouth through the ventral side to the end to the base of the caudal fin.

2.3.4. Girth-Behind-Operculum (GBO)

Girth-Behind-Operculum was measured by stretching a piece of rope around the head to pass behind the gill-cover and directly behind the right pectoral fin, and left pectoral fin.

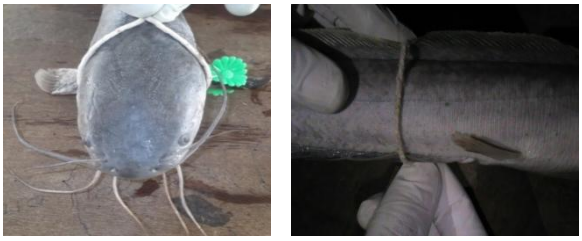

Figure 4. Girth-Behind-Operculum and Girth-Medially

2.3.5. Girth Medially (GM)

This was measured for the Tilapia by stretching a rope around the medial girth by passing it over the first dorsal fin, and infront of the tips of the right pectoral fin, pelvic fins, and left pectoral fin.

For the Catfish, a piece of rope was stretched around the medial girth by passing it over the first dorsal fin, and behind the right and left pelvic fins.

The girth was measured medially for Bonytongues' by stretching a piece of rope around the medial girth, passing it behind the right and left pelvic fins.

2.3.6. Anatomical Proportions

The following anatomical proportions for fresh and plastinated samples were determined: TL/GBO, HL/SL, TL/GM, HL/TL, GBO/GM, and SL/TL. A two-tail T-test was used to determine for significant differences ($\alpha = 0.05$) between the means of the fresh and plastinated samples.

3. Results

Table 1. Single factor Anova for differences in anatomical dimensions among fresh samples of three fish species ($\alpha = 0.05$)

	F	*F crit*	*Sig*
MW	4.48	3.68	SD
SL	10.8	3.68	SD
GM	4.39	3.68	SD
HL	2.72	3.68	NSD
GBO	0.82	3.68	NSD
TL	9.6	3.68	SD

SD – Significant difference
NSD – No Significant difference

Table 2. Single factor Anova for differences between anatomical dimensions of fresh and plastinated samples ($\alpha = 0.05$)

	Tilapia	Catfish	Bonytongue
MW	SD	NSD	NSD
SL	NSD	NSD	NSD
GM	NSD	SD	NSD
HL	NSD	NSD	NSD
GBO	NSD	NSD	NSD
TL	NSD	NSD	NSD

Table 3. Mean values of anatomical proportions of fresh and plastinated samples.

	Tilapia	Catfish	Bonytongue
TL/GBO	1.34(1.38)	2.20(2.48)	1.93(1.99)
HL/SL	0.37(0.34)	0.27(0.28)	0.21(0.22)
TL/GM	1.33(1.37)	2.63(3.22)	2.05(2.10)
HL/TL	0.31(0.27)	0.24(0.24)	0.19(0.20)
GBO/GM	0.99(0.99)	1.20(1.31)	1.06(1.06)
SL/TL	0.84(0.81)	0.87(0.87)	0.93(0.90)

Values for plastinated samples are in brackets.

Table 4. Single factor Anova of the anatomical proportions of fresh and plastinated samples (α = 0.05)

	Tilapia	Catfish	Bonytongue
TL/GBO	NSD	NSD	SD
HL/SL	NSD	NSD	NSD
TL/GM	NSD	NSD	SD
HL/TL	NSD	NSD	NSD
GBO/GM	NSD	NSD	NSD
SL/TL	NSD	NSD	NSD

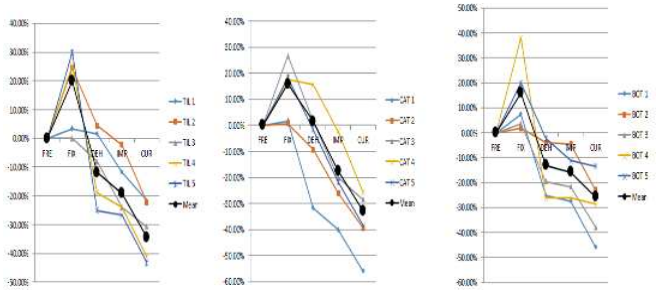

Figure 4. Percent Changes in Mean Weight of Tilapia, Catfish and Bonytongue

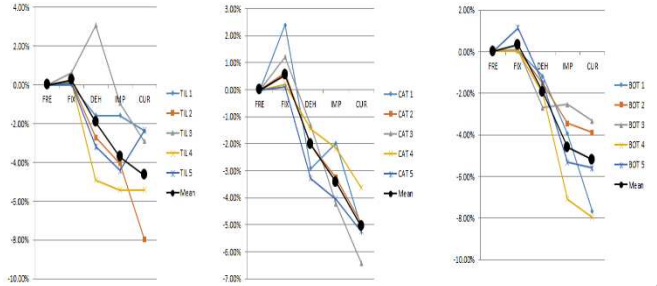

Figure 5.Percent Changes in Standard Length for Tilapia, Catfish and Bonytongue

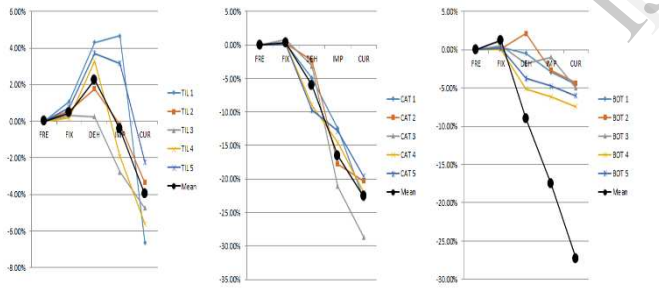

Figure 6.Percent Changes in Girth Medially for Tilapia, Catfish and Bonytongue

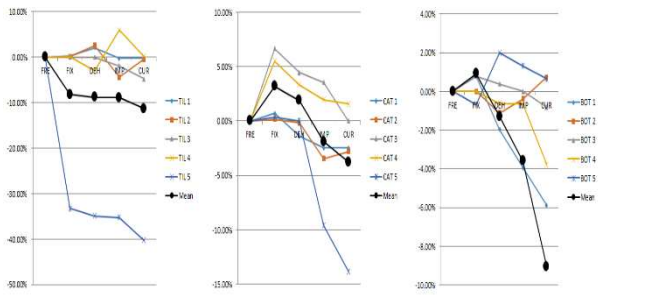

Figure 7.Percent Changes in Head Length for Tilapia, Catfish and Bonytongue

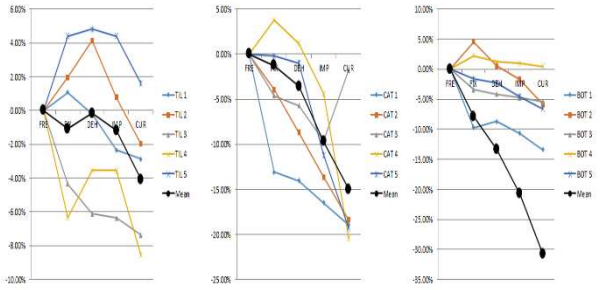

Figure 8.Percent Changes in Girth-Behind-Operculum for Tilapia, Catfish and Bonytongue

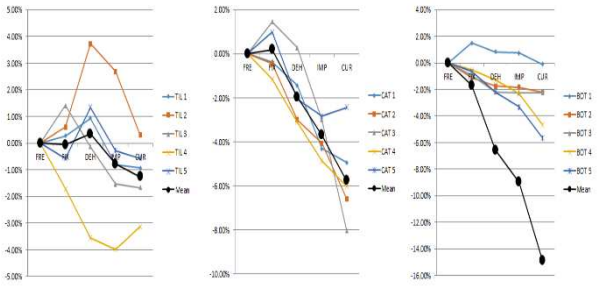

Figure 9.Percent Changes in Total Length for Tilapia, Catfish and Bonytongue

Figure 10. Tilapia, Catfish and Bonytongue samples at the end of curing

4. DISCUSSIONS

The individual fresh samples varied significantly (P≤0.05) in weights and sizes. Changes in the sizes of the individual samples occurred at various stages of plastination.

The MW, SL, GM, and TL of fresh fish samples varied significantly (p≤0.05) among the three fish species (Table 1). However, the HL and GBO of the fresh samples were not significantly different (p>0.05) among the three fish species.

Statistical analysis showed that differences in the weights and anatomical dimensions of fresh and plastinated samples of the three fish species were not significant (P>0.05) with the exception of the Catfish where the GM of fresh and plastinated samples were significantly different (P≤0.05) and the Tilapias where the weights of fresh and plastinated samples were significantly different (P≤0.05) (Table 2).

In a previous work (Ameko *et al.*, 2013a) with both dehydration and post impregnation at 4°C, the longitudinal and head region dimensions of Tilapia samples did not change significantly (P>0.05) during plastination but changed significantly (P≤0.05) in the mid-sections of the samples. However, in this study the longitudinal

dimensions of all three fish species changed significantly during plastination (P≤0.05) but that of the head region remained significantly (P>0.05) the same. In the previous work (Ameko *et al*., 2013a) plastinated Catfish samples maintained their overall shapes, i.e. longitudinally, in the mid-section and in the head region, while plastinated Bonytongues' maintained their shapes longitudinally but shrunk significantly in their head regions and in their mid-sections. In another previous work (Ameko *et al*., 2013b) the size and shapes of white Agouti Guineapigs' plastinated at room temperature did not differ significantly(P > 0.05) from that of the fresh euthanised samples. In this study, the mean weights of the samples increased at fixation, followed by progressive decrease from the stage of room temperature dehydration through room temperature post impregnation to curing (Figure 4).

Mean weight losses at the end of plastination were -35.2%, -34.5% and -28.2% for Tilapia, Catfish and Bonytongue respectively. This agrees with the results of a previous study (Ameko *et al*., 2013b), of room temperature plastination of Whole Guineapigs' where the weight of the Guineapigs' increased by 35.8% at fixation but reduced progressively from dehydration onwards, and with a mean weight loss of 58.2% after curing.

The variations in SL (Figure 5), GM (Figure 6), HL (Figure 7), GBO (Figure 8), and TL (Figure 9), all followed the same trend as that of the mean weight.

The anatomical proportions of fresh Catfish and Bonytongue samples were similar (Table 3) indicating that they had similar shapes which were quite different from the shapes of the Tilapia samples. Similar results were obtained by Ameko et al. (2013a).

Statistical analysis showed that differences in the anatomical proportions of fresh and plastinated samples were not significant (P>0.05) with the exception of the TL/GBO and TL/GM of Catfish where the differences were significant (P≤0.05) (Table 4).

Conclusions

Since generally the anatomical dimensions and proportions of the plastinates were not significantly different from those of the fresh samples it shows that room temperature plastination is suitable for preserving Tilapia, Catfish and Bonytongue. This procedure could be used to preserve these samples for use as teaching aids.

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