

Original Article:

Effects of Temperature on Colour of Selected Pig Organs in Silicone (S10) Plastination

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Abstract:

Background: Temperature is one of the most important factors that are responsible for plastination procedure. **Objective:** The present study was designed to determine a suitable method of plastination of skeletal muscle in a low-resource setting in Bangladesh. **Methods:** This observational study was carried out in the Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, between March 2015 and February 2016. Six whole pig kidneys (as firmer organ) and six whole pig lungs (as softer organs) were collected from a government authorized slaughterhouse in Dhaka city. Same numbers of organs were designated as 'Cold Temperature Group' and 'Room Temperature Group'. We observed the change in colour at cold and room temperatures after different stages of plastination with a colour chart. **Results:** After fixation, both the brownish kidneys and reddish pink lungs turned brownish and darker. After dehydration, both the kidneys and lungs got paler. After forced impregnation, the colour turned much darker in both groups. The colour change continued towards a darker tone with time. The specific colour changes quantified into frequencies were very variable in both temperature groups. **Conclusion:** In observed colour changes, the difference was indeterminate.

Keywords: Plastination, temperature, firmer organ, softer organ, colour change, silicone (S10) method.

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Introduction:

Human cadavers and gross specimens of body parts have been considered essential tools for the teaching of anatomy in medical education. Traditional methods of preservation include drying, immersion in chemical preservatives or perfusion of blood with chemical preservatives¹. Commonly, cadavers and specimens of human body are preserved in formalin. In the anatomy dissection rooms, cadavers and gross anatomical specimens are found soaked with formalin, discoloured and they spread unpleasant odour that cause tearing of eyes, burning sensation in nose and throat, tightening of the chest and palpitation

of the heart². Students lose their concentration while studying in the dissection hall. Therefore, there has always been a desire for specimens that would be dry, odourless, real, non-dangerous that do not require rigorous maintenance and do not deteriorate with time and which can be used in classrooms without gloves³. These expectations from anatomical learning tools have been fulfilled to a great extent by applying the modern method of body preservation named 'plastination'. Plastination is a novel method of preservation of biological specimens developed by Dr. Gunther von Hagens in 1977 at the Department of Anatomy of Heidelberg University in Germany, who patented it between 1977 and 1982.

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The plastination method consists of slowly replacing tissue fluids and a portion of the tissue lipids with a plastic polymer, under vacuum. The results are clean, dry, odourless, and durable real biological specimens that can be handled without gloves and do not require any special storage conditions or care⁴. These specimens prevent exposure of staff and students to the toxic substances (e.g., formaldehyde, phenol and alcohols) used in the classical preservation of biological tissues⁵. It even preserves cell identity at a microscopic level⁶.

Although plastination is a simple process, the results are often not as satisfactory as expected. Various factors have been found to contribute to the determination of the quality of plastinates. Among them temperature is one of the most important factors. Bangladesh is a tropical country and temperature ranges from 14°C to 30°C⁷. It is said that cold temperature (-18°C to -21°C) plastination method produces good quality specimens. However, it is also possible in room temperature (20°C to 28 °C). If a good quality specimen can be produced by room temperature method the expenditure will be reduced. Previous studies showed the effects of temperature on the procedural times and on the gross morphology of selected pig in silicone (S10) plastination. However, those studies could not conclude a consistent result regarding temperature difference in which the plastination process takes place. Therefore, it demands a series of research to acquire a consistent result and reach a conclusion.

To date, there are no known reports of the use of plastinates⁸ in Bangladesh as teaching aids in medical education. Recently, the first plastination laboratory of the country has been established in the Department of Anatomy of Bangabandhu Sheikh Mujib Medical University (BSMMU) in 2012, through the funding of a Subproject (CP-036) of the Higher Education Quality Enhancement Project (HEQEP) of the University Grants Commission of Bangladesh provided by the Academic Innovation Fund (AIF) of the World Bank⁹. The present study was designed to determine a suitable method of plastination of skeletal muscle in a low-resource setting in Bangladesh.

Methods:

This observational study was carried out in the Department of Anatomy, Bangabandhu Sheikh

Mujib Medical University (BSMMU), Dhaka, Bangladesh, between March 2015 and February 2016.

The specimens were collected from a government authorised slaughterhouse of Bangladesh (Shandon Pork Meat Shop, Farmgate, Dhaka). Six pig kidneys were taken as the firmer organs and six lungs were also taken from same animal as the softer organ. Six (6) pig kidneys and six (6) pig lungs were designated as the 'Cold Temperature Group' and the same numbers of organs as the 'Room Temperature Group'. The colour was observed in the fresh organ and after every stage of plastination done in cold temperature and in room temperature. The colour changes were determined visually and qualitatively by comparing with an authorised colour chart (Fig. 1A) of a standard paint manufacturer ('Colour Creations Palette' of Nippon Paint, Japan)¹⁰.

The colour of the ventral surface of the kidney was compared with the colours of the chart in daylight and the name of the colour that most closely matched that of the ventral surface was recorded from the chart (Fig. 1B). A point was identified by tying a thread on the visceral surface of the caudal lobe of the lung (Fig. 1C). A circular area about 2 centimetres in diameter was drawn centring the thread-marked point. This circular area was the selected particular area for determining the colour change. The deepest and the lightest colours of the selected area were compared with the colours of the chart at daylight and the names of the colours that most closely matched those of the lung surface were recorded from the chart (Fig. 1D). The organs were fixed with 10% formalin solution^{11,12}. Then rinsed with water^{11,13}, pre-cooling was done only in 'Cold Temperature Group'¹⁴. Afterwards, the specimens of Cold Temperature Group were dehydrated with cold temperature acetone at -19°C to -23°C in deep freezer¹⁵ and the specimens of Room Temperature group was dehydrated with room temperature acetone at 20°C to 28°C separately. Forced impregnations of the specimen of two groups were done with silicone S10 and S3 at both temperatures separately^{14,16}. Lastly, gas-curing of the specimens of two groups were done with S6 at room temperature¹⁴.

The specific colour changes quantified into frequencies were very variable in both temperature groups.

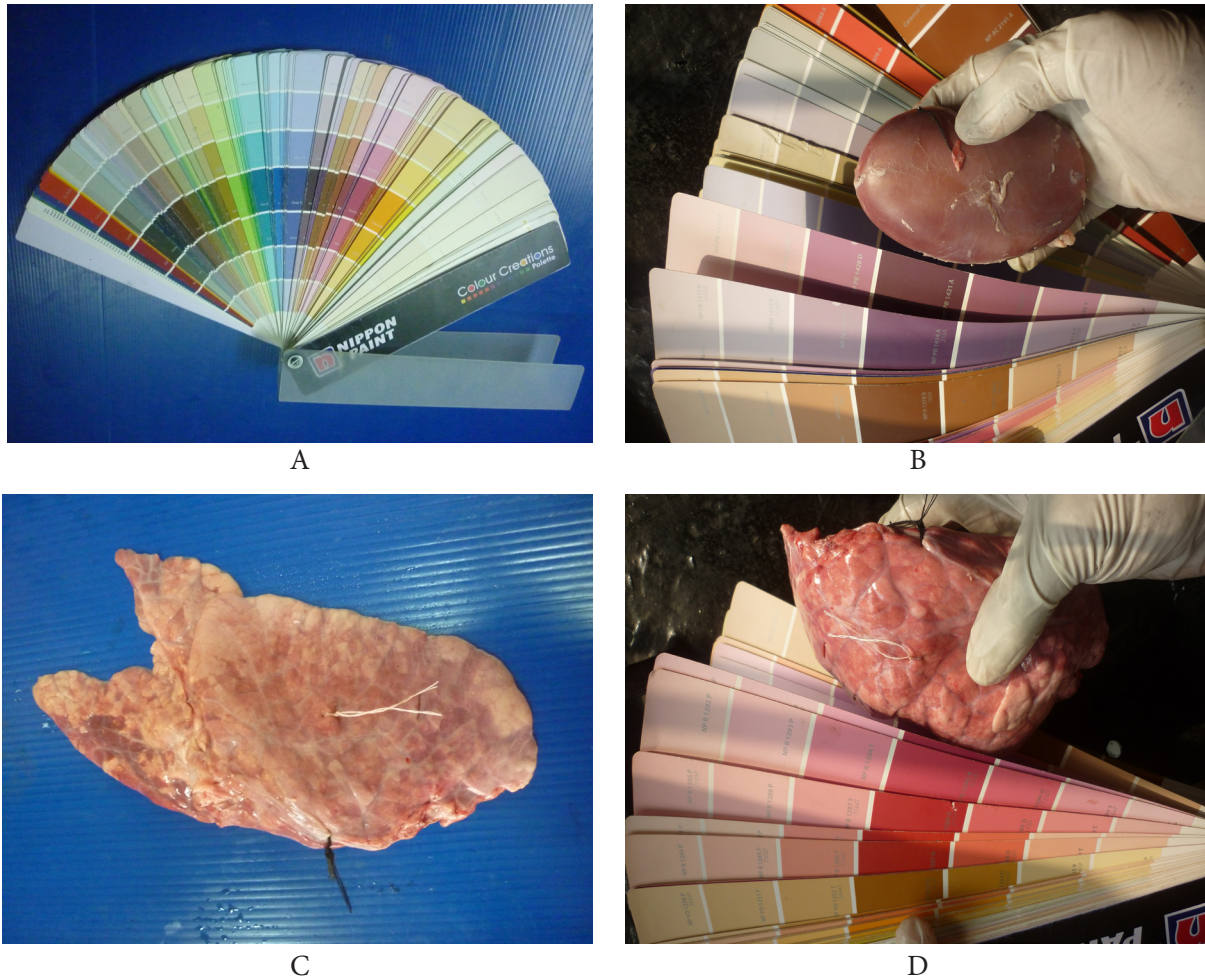


Figure 1: Procedure of observing the changes in colour: A) the colour chart used for determining the changes in colour; B) comparison of the colour of the ventral surface of the whole kidney with those of the colour chart; C) a white thread tied on the visceral surface of the caudal lobe of a lung to mark the area to be examined for colour change; D) comparison of the colours of the selected part of the lung with those of the colour chart.

Results:

The changes in colour were assessed by matching with a colour chart. The percentage frequencies of different colours were calculated for each stage in the two temperature groups. The observations made regarding colour changes in the firmer organ (kidney): In the fresh stage, the kidneys had shades of brownish pink colour. After fixation, the colour changed to a darker brownish one. This brownish tone was broadly maintained throughout plastination. However, broadly the kidneys became slightly paler on dehydration while they became gradually darker through the forced impregnation and gas-curing stages. The changes at room temperature and cold temperature are described below:

The observations of colour changes in the softer organ (lung): at fresh stage, the lungs were of

variable shades of reddish pink colour with an irregular distribution. After fixation, the colour changed to a brownish and darker one. This brownish tone was maintained throughout plastination. However, broadly, the lungs became slightly paler on dehydration, while they became gradually darker through forced impregnation and gas-curing stage. Fresh stage: in firmer organ, four colours were found in the Cold Temperature Group and two colours in the Room Temperature Group, but the colour 'Razzle Dazzle' was common to both groups. Largest difference in the frequencies (66.66% for cold temperature and 0% for room temperature) was found for the colour 'Ground Clove'. In case of the softer organ, three colours were found in the Cold Temperature Group and three colours were found in the Room Temperature Group. The colour 'Pioneer Red' was common in both groups. Largest differences in the frequencies

(45.45% for room temperature and 41.66% for cold temperature) were found for colours 'Rose Violet' and 'Sarah's Sash'. After-fixation percentage frequencies of different colours of the organs at room temperature and cold temperature showed on Figure 2. After dehydration: in case of the firmer organ, two colours appeared at Cold Temperature Group and two different colours appeared at Room Temperature Group. Largest difference in frequency were found for 'Stair Step' (66.66% for cold temperature and 0% for room temperature). 'Tavern Buff' and 'Old Barn' (0% for cold temperature and 50% for room temperature). In case of the softer organ, three colours appeared at cold temperature and different four colours appeared in Room Temperature Group. Largest difference in frequency was found for 'Hand Painted' (57.14% for room temperature and 0% for cold temperature). 'Curling Bark' had 50% frequency for cold temperature.

temperature and 62.50% for room temperature appeared the most. After-forced impregnation percentage frequencies of different colours of the organs at room temperature and cold temperature showed on Figure 3. After gas-curing: in firmer organ, two colours appeared at Cold Temperature Group and different two colours appeared at Room Temperature Group. Largest differences in frequency were found for 'Stair Step' (67% for cold temperature and 0% for room temperature) and 'Hand Painted' had 66.66% frequency for room temperature and 0% for cold temperature. In case of softer organ, four colours appeared in Cold Temperature Group and different three colours appeared in Room Temperature Group. Largest difference in frequency was found for 'Hand Painted' (57.14% for room temperature and 0% for cold temperature). 'Curling Bark' had 50% frequency for cold temperature.

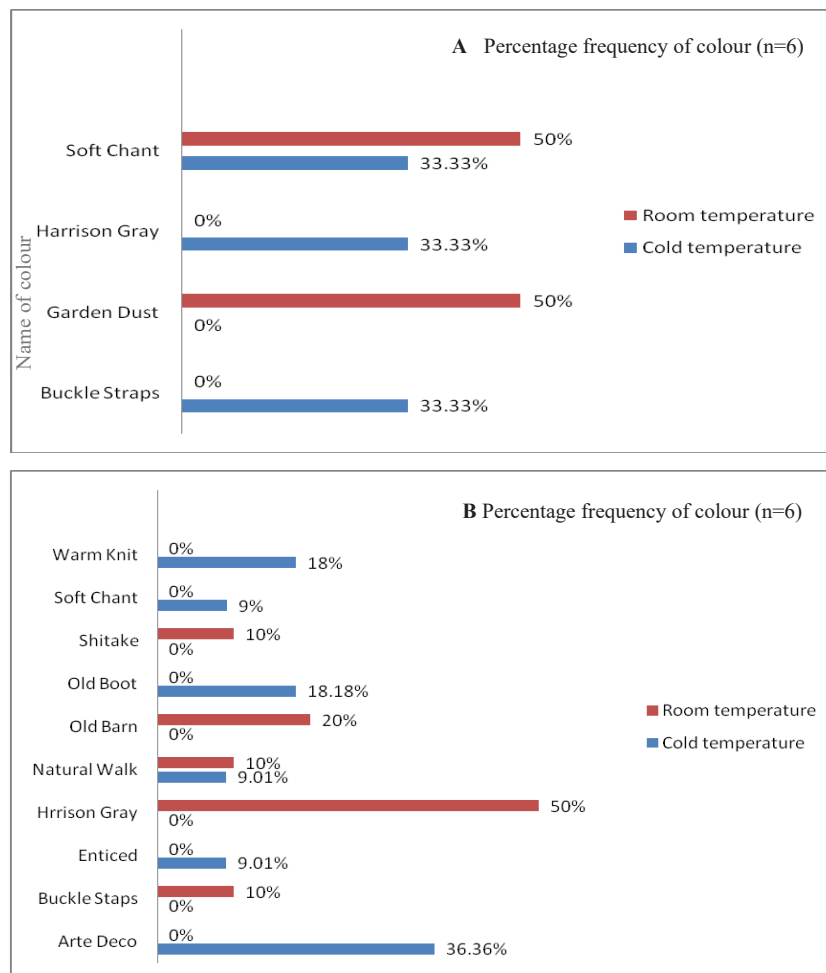


Figure 2: After-fixation percentage frequencies of different colours of the organs at room temperature and cold temperature: (A) For firmer organ; (B) For softer organ.

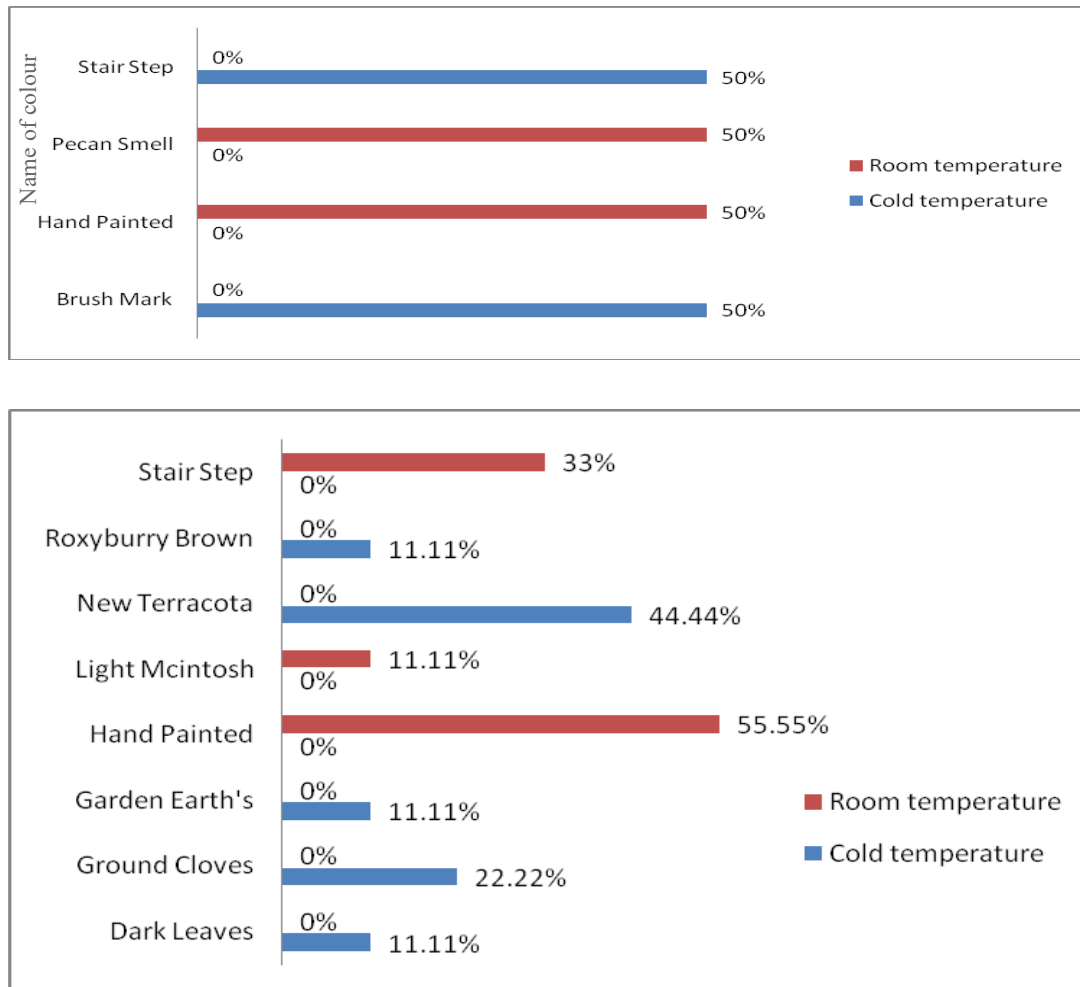


Figure 3: After-forced-impregnation percentage frequencies of different colours of the organs at room temperature and cold temperature: (A) For firmer organ; (B) For softer organ.

Discussion:

Broadly speaking, the colours of the fresh kidneys were brownish pink and that of the fresh lungs were reddish pink (with some paler areas irregularly found). The brownish pink kidneys turned more brownish and the reddish pink lungs turned brownish after fixation. Although this brownish tint in both the organs continued, the organs became paler after dehydration. Then this brownish colour gradually darkened through the next two stages (forced impregnation and gas-curing) of plastination. The specific colour changes quantified into frequencies showed much variability. Ameko et al.¹ found that cow kidney turned brown from pink after plastination. In another study, Ameko et al.¹⁷ observed that the colour of the guinea-pig's lungs changed from pink to dark brown. Results of the present study

were similar to those of both the above studies. On the other hand, Ottone et al.¹⁸ observed that the colour of the tissues in both pork and human heart after plastination stayed 'very similar' to the original, without undergoing any type of darkening or lightening that would cause poor visualisation of the anatomical structures. However, it must be noted that various tones of different colours, as represented by the names of the colours that matched the organs after different stages, are very difficult to quantitate. These names do not suggest any definitive directions of darkness/paleness or otherwise. One option was to use a digital technique that expresses different colours as relative percentages of cyan, magenta, yellow and black (CMYK)¹⁶. However, experience during the present study revealed that the colours in the photographs do not match properly with the originals. Therefore, direct comparison of the

organs with the colour chart (printed on papers) was preferred for the present study. Nevertheless, this method could not give clue whether there was any difference in the direction of the change in colour between the two temperature groups.

Conclusion:

Broadly speaking, cold temperature silicone (S10) plastination showed variable effects on pig organs regarding colour compared to room temperature S10 plastination. Further and more specified research with matched samples as far as feasible are recommended for arriving at definitive conclusions. It is evident from the above discussion that it is difficult to identify any specific pattern of differences in the changes in the two temperature groups. Therefore, a large pool of studies needs to

be available before deciding confidently on what and how to do for getting good quality palatinates.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval issue: The study was approved by the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

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Authors' contribution: Conception and design of the study: TA; Data collection and compilation: TA, TA, FA, SNA; Data analysis: TA, MRM, MA; Critical writing, revision and finalizing the manuscript: TA, MRM, MA, TA, FA, SNA.

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