Research Article

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DEVELOPMENT OF A TECHNIQUE AND COMPARISON OF TRADITIONAL PRESERVATION METHOD USING SAINDHAVA LAVANA AND SAMUDRA LAVANA WITH EXISTING FORMALDEHYDE ORGAN PRESERVATION TECHNIQUE

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ABSTRACT

Introduction: Formaldehyde, a common preservative for specimens, causes various side effects. Salt, traditionally used to preserve food like meat, fish, and dairy, is cost-effective, widely available, and poses no health risks. Our study aimed to develop an alternative organ preservation method using Saindhava Lavana (Rock salt) and Samudra Lavana (Sea salt) instead of formaldehyde. Methodology: Seven fresh Goat Stomach and Liver specimens were selected, otherwise discarded from a meat shop. The specimens were observed based on assessment criteria and preserved in formalin, Saindhava Lavana (Rock salt), and Samudra Lavana (Sea salt) for 40 days. Salt-preserved jars were opened every 10 days, with batch-wise observations recorded. Specimens were tested for Colony-Forming Units (CFU) to detect microbial growth. After 40 days, cedarwood oil and resin were applied to preserve the specimens further. Observation and Results: Most specimens were preserved successfully, with no microbial growth observed in any sample, including the controls. Conclusion: Salt preservation, while slightly more costly, proves highly effective for preserving organs, particularly hollow ones like the Stomach, and when resin-coated, is suitable for display purposes.

Keywords: Preservation**,** Formaldehyde, Formalin, Salt, Saindhava lavana, Samudra lavana.

INTRODUCTION

The Chinchorro culture in the Atacama Desert of Chile and Peru practised artificial mummification as early as 5000–6000 BCE. Ancient Egypt took mummification to its greatest extent, with specialized priests performing mummification from the First Dynasty (3200 BCE). They removed organs, dehydrated the body, and used natron for preservation[1]. Later, formaldehyde became a common method for preserving dead bodies. The introduction of formaldehyde as a preservative in 1893 was an important step in the history of preservation. Formaldehyde is used to embalm bodies because it changes the tissues on a cellular level so that bacteria cannot grow. Breathing in formaldehyde fumes can cause formaldehyde poisoning, with symptoms such as breathing difficulties, COPD, headaches, skin irritation, and oesophagus and stomach burning. It is linked to ALS and other nervous system disorders and is a known human carcinogen, particularly associated with leukaemia. Concentrations above 0.1 ppm can irritate eyes and mucous membranes, causing watery eyes and, at higher levels, severe damage, headaches, burning sensations, coughing, wheezing, nausea, skin irritation, and difficulty breathing.[2]

Salt was traditionally added to meals primarily for preservation purposes. In particular, meat, fish, dairy products, and other food products have been preserved with salt. Enzyme hindrance via decreased catalytic activities and altered cofactors, respiration inhibition, O-nitrophenyl-β-galactoside hydrolysis, depletion of cell energy source (ATP molecule), cellular plasmolysis, deterrence of substrate transport into the cells across cell membranes, and restricted oxygen solubility are some of the mechanisms that salt uses as a preservative to inhibit microbial growth.

In Egypt, during 2600 BC, salt was used to mummify dead bodies.[3]

Lavana (Salt) has been used as medicine as well as food since ancient times. Several types of lavana are described in every classic of Ayurveda; some are not available in the present day, but Panchalavana is mainly used for medicinal purposes.[4,5,6]

The group of pancha lavana viz. Saindhava Lavana (Rock salt), Samudra Lavana (Sea salt), Vida Lavana (Ammonium chloride), Sauvarchala Lavana (Black salt), Romaka Lavana (Sambhar salt/ Earthen salt). Among all of them, Saindhava Lavana (Rock salt)

is the best one. We have selected Saindhava lavana (Rock salt) and Samudra lavana (Sea salt) for the research.[5,7,8]

Saindhava Lavana (Rock salt) is considered Sodium chloride/Rock salt/Bay salt. Sodium chloride is the primary ingredient, making up 98% sodium bicarbonate ($NaHCo_3$) 0.07%. It contains many useful minerals and elements, such as minor quantities of magnesium chloride, calcium chloride, and calcium sulphate. It also contains iodine, lithium, magnesium, phosphorus, potassium, chlorium, manganese, iron, zinc, and strontium. Another chemical which is in minute quantity also acts as a preservative. Chemicals like sodium bicarbonate, sodium chloride, and sodium sulfate are similar to Natron salt, but the only difference is in their percentage.[9]

Samudra Lavana (Sea salt) is considered Sodium chloride / common salt /sea salt, the major ingredient, making up 91.3%, total sulphide (Na_2S) 0.121%, iron 0.0089%. It contains many valuable minerals and elements like minor quantities of $MgCl_2$, $MgSO_4$, $CaSO_4$, iodine, Mg, Ph, K, Cr, Fe, sulphide, etc. also act as a preservative. Chemical compositions like Sodium chloride and Sodium sulphide are similar to Natron salt, but the difference is in their percentage.[9]

MATERIALS AND METHODS

For this experimental study, we used 7 Goat Liver organ samples and 7 Goat Stomach samples, Glass jars, Surgical gloves, Face masks, Paper towels, Ethyl alcohol, Formaldehyde, Saindhava lavana (Rock salt) and Samudra lavana (Sea salt), Gauze, Cedarwood oil (essential oil), Synthetic resin, Paint and Paintbrush.

For our study, we have not sacrificed any animal. We have taken the fresh specimen, which was supposed to be discarded at the meat shop. So, Institutional CPCSEA ethical clearance for animal samples was not taken.

Phase 1: Preservation Method

1. Seven fresh samples of the Liver (solid organ) and seven fresh samples of the Stomach (soft organ) of the Goat were collected from the meat shop early in the morning within half an hour of removal.
2. One sample of each organ was used for preservation using the traditional formaldehyde method; three samples were used for preservation using Saindhava lavana, and three samples were used for preservation using Samudra lavana.
3. Wearing masks and hand gloves, organs were washed with water within half an hour of collection. All organs were dried using a paper towel, and observation was performed using assessment criteria. And organs were rubbed with ethyl alcohol both inside and outside (Stomach).
4. Two glass jars were taken, and a sample of Liver and Stomach were placed in each with a 1:9 combination of Formaldehyde and Water.
5. Three glass jars were taken, and Saindhava lavana (Rock salt) was spread inside. Livers were kept inside the jar. Jars were filled with Saindhava lavana (Rock salt) so that the lavana covered the whole organ, and jars were locked airtight.
6. Three glass jars were taken, and Saindhava lavana (Rock salt) was spread inside. Stomachs were kept inside the jar. Jars were filled with Saindhava lavana (Rock salt) so that the whole organ, inside out, was covered by the lavana and jars were locked airtight.
7. The same procedure was followed to preserve three Liver and three Stomach samples of goat using Samudra lavana (Sea salt).
8. The jars with Lavana were opened once in 10 days batch-wise and observed for the changes mentioned in the assessment criteria. Existing salts were taken, and each jar with the same lavana ensured that jars were locked airtight.
9. Step 8 was repeated on the 20th and 30th day as well.
10. On the 40th day, all organs were removed and cleaned to ensure no salt residues remained. As mentioned in the assessment criteria, they were observed, and swab samples were taken for the CFU test.
11. Cedarwood oil (essential oil) was applied to the organs placed with Lavana and covered with gauze for 10 days.
12. After 10 days, gauzes were removed, synthetic resin was applied using a brush over the organ, and it was kept safe.
13. The organs were observed for one year for their durability, smell, and decomposition.

Figure 1: Fresh Stomach samples 7 in number

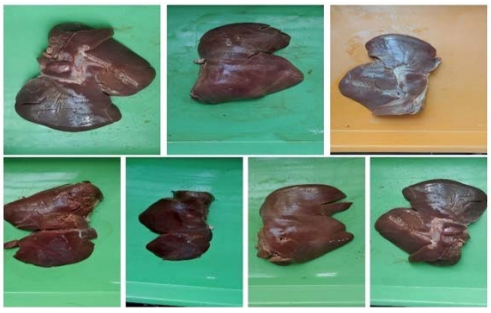

Figure 2: Fresh Liver samples 7 in number

Table 1: Division of sample for storage

7 Liver samples	7 Stomach samples
Sample 1 - Saindhava	Sample 1 - Samudra
Sample 2 - Saindhava	Sample 2 - Samudra
Sample 3 - Saindhava	Sample 3 - Saindhava
Sample 4 - Samudra	Sample 4 - Saindhava
Sample 5 - Samudra	Sample 5 - Saindhava
Sample 6 - Samudra	Sample 6 - Samudra
Sample 7 - Formalin	Sample 7 - Formalin

Table 2: Formation of batches

Batch Number	**Saindhava Lavana** (Rock salt)	**Samudra Lavana (Sea salt)**
1st Batch – opened every 10 days	Liver sample 2 Stomach sample 5	Liver sample 6 Stomach sample 6
2nd Batch – opened on 20th day then 10 days	Liver sample 3 Stomach sample 3	Liver sample 4 Stomach sample 2
3rd Batch – opened on 30th day then on 40th day	Liver sample 5 Stomach sample 4	Liver sample 1 Stomach sample 1
Formalin	Liver sample 7	Stomach sample 7

Figure 3: Method of preservation of Liver

Figure 4: Preservation of Liver and Stomach samples in Formalin

Figure 5: Method of preservation of Stomach

Phase 2: Detection was performed for its anti-microbial effect and signs of decomposition. After 40 days, all organ swabs are taken and sent for a CFU (colony forming unit) test. All samples were observed for signs of decomposition.

Microbial Analysis - Sample Preparation

Sample 10mg was dissolved in 1 mL of saline (1% w/v Sodium chloride), and 100 µL was used for analysis.

Analysis

Platting of samples for Microbial Analysis

Luria Bertani (LB) agar media (Tryptone 10 gm, sodium chloride 10 gm, yeast extract 6 gm, agar 15 gm, distil water 1000 mL) 200 mL was prepared and autoclaved at 121 °C for 15 minutes. 100 µL of the samples were poured into the sterilized Petri plate respectively, and in 1 plate, 100 µL Saline was poured as Control, and approximately 25 mL of the LB agar was poured into the Petri

plate, allowed for solidification (pour plate method) and incubated at 37 °C for 24 hours. After 24 hours, the plates were observed, and the colony-forming units (CFU) were recorded.

Assessment Criteria: In this experimental study, the following are considered assessment criteria at par with the control group - appearance: shape, size, texture, colour, smell/odour, weight, and transparency of media in which it is stored.

OBSERVATION

Table 3: Batch 1 - Liver sample 2 and Liver sample 6

Parameters	**1st day**		**10th Day**		**20th Day**		**30th Day**		**40th Day**	
Lavana	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra
Weight	229 gm	325 gm	135 gm	194 gm	130 gm	192 gm	126 gm	185 gm	124 gm	171 gm
Length	10 cm	11 cm	9 cm	9 cm	9 cm	9 cm	9 cm	9 cm	9 cm	9 cm
Breadth	19 cm	19 cm	12 cm	14 cm	12 cm	13 cm	12 cm	13 cm	12 cm	13 cm

Table 4: Batch 1 - Stomach sample 5 and Stomach sample 6

Parameters	**1st day**		**10th Day**		**20th Day**		**30th Day**		**40th Day**	
Lavana	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra
Weight	230 gm	320 gm	116 gm	202 gm	90 gm	123 gm	69 gm	102 gm	67 gm	84 gm
Length	33 cm	31 cm	27 cm	28 cm	25 cm	27 cm	25 cm	27 cm	23 cm	25 cm
Upper width	17 cm	19 cm	15 cm	18 cm	15 cm	16 cm	15 cm	16 cm	14 cn	16 cm
Middle width	8 cm	10 cm	10 cm	12 cm	10 cm	11 cm	10 cm	11 cm	10 cm	11 cm
Lower width	7 cm	6 cm	8 cm	9 cm	8 cm	8.5 cm	8 cm	8.5 cm	8 cm	8.5 cm

Table 5: Batch 2 - Liver sample 3 and Liver sample 4

Parameters	**1st day**		**20th Day**		**30th Day**		**40th Day**	
Lavana	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra
Weight	385 gm	319 gm	251 gm	230 gm	240 gm	225 gm	234 gm	221 gm
Length	13 cm	23 cm	10 cm	16 cm	10 cm	15 cm	10 cm	15 cm
Breadth	19 cm	13 cm	15 cm	11.5 cm	15 cm	11.5 cm	15 cm	11 cm

Table 6:I Batch 2 - Stomach sample 3 and Stomach sample 2

Parameters	**1st day**		**20th Day**		**30th Day**		**40th Day**	
Lavana	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra
Weight	270 gm	316 gm	114 gm	210 gm	86 gm	129 gm	72 gm	100 gm
Length	35 cm	38 cm	31 cm	37 cm	30 cm	33 cm	30 cm	32 cm
Upper width	19 cm	19 cm	19 cm	17 cm	19 cm	17 cm	19 cm	17 cm
Middle width	10 cm	17 cm	10 cm	16 cm	10 cm	16 cm	10 cm	16 cm
Lower width	8 cm	9 cm	8 cm	9 cm	8 cm	9 cm	8 cm	9 cm

Table 7: Batch 3 - Liver sample 5 and Liver sample 1

Parameters	**1st day**		**30th Day**		**40th Day**	
Lavana	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra
Weight	412 gm	301 gm	289 gm	188 gm	290 gm	183 gm
Length	23 cm	21 cm	20 cm	12 cm	19 cm	12 cm
Breadth	12 cm	11 cm	11 cm	11 cm	11 cm	11 cm

Table 8: Batch 3 - Stomach sample 4 and Stomach sample 1

Parameters	**1st day**		**30th Day**		**40th Day**	
Lavana	Saindhava	Samudra	Saindhava	Samudra	Saindhava	Samudra
Weight	260 gm	315 gm	116 gm	166 gm	84 gm	140 gm
Length	32 cm	36 cm	29 cm	33 cm	27 cm	33 cm
Upper width	23 cm	23 cm	19 cm	19 cm	19 cm	19 cm
Middle width	14 cm	14 cm	10 cm	10 cm	10 cm	10 cm
Lower width	7 cm	7 cm	8 cm	8 cm	8 cm	8 cm

Table 9: Formalin - Liver sample 7 and Stomach sample 7

Parameters	**1st day**		**10th Day**		**20th Day**		**30th Day**		**40th Day**	
Organ	**Liver**	**Stomach**	**Liver**	**Stomach**	**Liver**	**Stomach**	**Liver**	**Stomach**	**Liver**	**Stomach**
Weight	460 gm	375 gm	440 gm	355 cm	425 gm	350 gm	417 gm	348 gm	416 gm	350 gm
Length	12 cm	35 cm	14 cm	26 cm	13 cm	24 cm	11.5 cm	30 cm	13 cm	26 cm
Breadth	22 cm	----	21 cm	-----	19 cm	-----	21 cm	-------	19 cm	-----
Upper width	----	25 cm	----	17 cm	---	17 cm	----	17 cm	---	17 cm
Middle width	----	12 cm	----	10 cm	---	10 cm	----	11 cm	---	11 cm
Lower width	----	8 cm	----	7 cm	---	7 cm	----	7 cm	---	7 cm

Observation on Color, Smell and Texture

Throughout the research, Liver and Stomach samples were brown to brownish. Initially, they emitted a meat-like odour, which later transitioned to a sweetish peculiar smell. By the end of the study, the formalin-preserved samples had a formalin odour.

The Liver samples were soft and smooth initially, but in the later stages, they became hard, rough, and dry with brittle ridges. Similarly, the Stomach samples were soft and rough initially, but over time, they turned rough, dry, and in some cases, brittle.

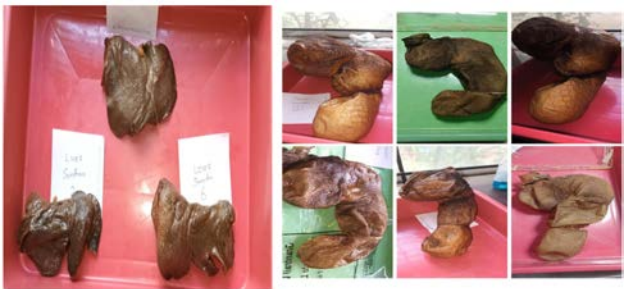

Figure 6: The outcome after application of resin

Observation after one year

All the samples' shapes and sizes (weight, length, and width) were almost the same as measured on the 40th day. The texture of the Liver samples remained consistent and became harder due to moisture loss. The Stomach samples became rougher and harder. All the samples emitted a resinous smell, while the formalin-preserved samples had a formalin odour at the end of the study.

Figure 7: Goat Stomach samples preserved with Saindhava and Samudra lavana

Figure 8: Goat Liver samples preserved with Saindhava and Samudra lavana

Phase 2: Result: No microorganisms were found in Samples and control.

Table 10: Colony Forming Units

Sample	Colony forming units per 1 mL
Saindhava- Liver 1	0.0 x 10^3
Samudra- Stomach 1	0.0 x 10^3
Samudra- Stomach 2	0.0 x 10^3
Saindhava- Stomach 4	0.0 x 10^3
Saindhava- Liver 2	0.0 x 10^3
Samudra- Stomach 6	0.0 x 10^3
Saindhava- Stomach 3	0.0 x 10^3
Saindhava- Stomach 5	0.0 x 10^3
Formalin- Stomach 7	0.0 x 10^3
Saindhava- Liver 3	0.0 x 10^3
Samudra- Liver 4	0.0 x 10^3
Samudra- Liver 5	0.0 x 10^3
Samudra- Liver 6	0.0 x 10^3
Formalin- Liver 7	0.0 x 10^3
Saline (Control)	0.0 x 10^3

Figure 9: Colony Forming Unit Test

DISCUSSION

Formalin as a preservativeA combination of dehydration, protein cross-linking and denaturation, loss of soluble components during diffusion and osmotic effect led to a slight reduction in weight and breadth of the Liver and Weight, breadth and length of the Stomach sample. Also, the water content was replaced by the formalin solution here.[10,11]

There was a slight increase in the length of the Liver sample. The combined effect of swelling of collagen fibres, changes in tissue turgor and relaxation of muscles and connective tissue during the preservation process may cause a slight increase in the length of the Liver. Colour changed from brown to pale in both samples because of the mixed effects of protein fixation, pigment changes, loss of blood, chemical reactions and dehydration. As the formalin has a strong odour, it replaces the smell of meat in both organs. [10,11]

The texture of the organs was smooth and soft. Formalin fixation does not involve processes that would cause significant mechanical damage to the tissues, like extreme heat or desiccation. So, the tissues might have retained their smooth and soft texture. Impressions or features on the stomach wall were not visible. The shape of the stomach was not maintained.

The media in which we preserved samples were unclear, showing colour change and tissue content. Fixation inhibits autolysis and putrefaction, hardens tissue, and facilitates manipulation. When formalin comes into contact with fresh tissue, the haemoglobin

becomes converted to the brownish-tan pigment haemitin. This conversion is observed when fresh tissue (usually a red-pink colour) is placed into 10% formalin at room temperature. These factors cause the above changes in the organ.[10,11]

Lavana (Salt) as a preservative

The moisture content was more pronounced in the organs preserved using Saindhava Lavana (Rock salt). This may be due to the properties of Saindhava Lavana, which is known to be laghu (light), snigda (oily), and sheetala (cooling). The Liver and Stomach's weight, length, and breadth were reduced to half of their actual. The combined effects of biochemical and physical changes are due to factors like osmosis and dehydration, protein denaturation, and cell collapse due to reduced turgor pressure and reduced interstitial spaces.[12,13]

The colour of samples became dark brown because of reactions like the Millard reaction, dehydration causing the increasing concentration of pigments and the colouring substances, and chemical reaction between NaCl and tissue component. Some halophilic (salt-loving) microbes might still survive and produce pigments as metabolic byproducts. As the Liver contains blood, the haemoglobin in red blood cells can break down into various coloured compounds, such as hemosiderin, which are brown. The iron in haemoglobin can form dark-coloured compounds upon degradation and oxidation. The smell was also reduced.[14]

The lack of a meat smell in these organs preserved with salt is primarily due to dehydration, which creates an environment unsuitable for the growth of odour-producing bacteria and fungi. Additionally, the chemical interactions of salt with proteins and other compounds in the tissue prevent the formation and release of volatile odorous molecules. These combined effects result in the absence of the typical meat smell. After the application of Cedarwood oil, the organ smelled of the oil.[12,13,15]

The texture was converted to hard, rough and wrinkles from smooth and softness. This is primarily due to dehydration, which removes water and causes tissues to contract and harden. Protein denaturation and aggregation, loss of turgor pressure, chemical interactions, and increased density further contribute to these textural changes, resulting in the observed hard, rough, and wrinkled appearance. Impressions or features on the stomach wall were visible. Processes like dehydration and contraction of tissues, protein denaturation, loss of turgor pressure, and compaction of tissue layers make the tissue firmer, denser, and more closely adhering to its underlying structures, resulting in more prominent and visible impressions.[12,13,15]

Liver samples from batches 2 of both Saindhava and Samudra lavana (Sea salt) and batch 3 preserved in Saindhava lavana (Rock salt) experienced preservation issues, which led to an unusual smell, texture changes, and improper preservation. The Liver sample of batch 2 was opened on the 20th and batch 3 on the 30th. The delay in opening and inspecting the samples likely contributed to the preservation issues. If the Liver samples were exposed to a humid environment or not kept adequately sealed, the salt could have reabsorbed moisture, which could transfer back into the Liver. Improper sealing of samples or the humid environment might have caused the decomposition of samples.

The Liver of batch 3 was slightly stiff and soft with proper shape. As the sample was opened on the 30th day, it didn't absorb all the moisture. So, it was not very hard and rough.

The Stomach sample of batch 3 was rough, dry and soft. Here, the salt of the sample was first changed on the 30th day, which didn't cause too much absorption of water and moisture from the sample, so the sample was still slightly soft.

The moisture content was lower in organs preserved using Saindhava lavana, resulting in lesser dryness than those preserved with Samudra lavana.

Key ingredients used in the procedure

Cedarwood oil is a yellow-coloured sticky oil obtained from the cedar trees. It contains bioactive constituents like alpha cedrene, beta cedrene, ketone/terpene, caryophyllene, cadinene, cedrol, and a group of sesquiterpenes. Cedarwood essential oil exhibits antiseptic, anti-microbial, antifungal, anti-inflammatory, insecticidal, and pesticidal properties. It also acts as a moisturizer.[16]

Plastination is a preservation process involving embedding tissue in synthetic polymers like silicon, polyester, epoxy, or resin. The method replaces water and fat in tissue with polymer, resulting in non-toxic, odourless, dry, and durable specimens that are easy to handle and examine. Epoxy, silicone, and polyester are commonly used polymers in this process. Resin application offers numerous benefits: specimens become dry, easy to handle, store, transport, and durable. It's non-hazardous, non-infectious, emits no fumes or fluids, making it safe for use.

CONCLUSION

Formalin's use as a preservative has known side effects, but embalming with formalin remains the preferred technique for preserving dead bodies due to its cost efficiency compared to salt preservation. Salt preservation, while costly, renders bodies hard and complicated for dissection due to moisture absorption. However, salt preservation shines in preserving organs, especially hollow ones like the stomach and intestine, if done for 30 days to prevent brittleness. Solid organs like lungs and Liver can be preserved for 40 days, with salt changed every 10 days and stored in sterilized containers. Salt-preserved organs can be hardened like rock and, when resin-coated, suitable for showcasing. They can also be painted to highlight external structures, nerve and blood supply, lobes, and other parts.

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