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***Research Article** (Open Access)*

A STUDY ON FORMALIN ADULTERATION IN TWO COMMERCIAL MARINE FISHES (*SARDINELLA LONGICEPS* AND *SYNAGARIS JAPONICUS)* FROM CHENNAI MARKET, TAMIL NADU, INDIA

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ABSTRACT
Recently, the illegal usage of formalin by fish farmers and vendors in various Indian markets to extend the shelf life of seafood became an emerging issue. The current study was assessed to verify the presence or absence of formalin from two distinct retail marketplaces, Viz, M.G.R. Nagar and Perambur in Chennai. Two marine fresh fish samples, Oil Sardine (*Sardinella longiceps)* and Blackmouth splitfin (*Synagaris japonicus*) were collected to estimate the concentration of formalin by using Spectrophotometric Method and done the histopathological study in fishes' muscle after exposed to 1% of formalin in various interval period (0hrs, 6hrs,12hrs,20hrs and 24hrs). The result in test of formalin reveals that Oil Sardine collected from Perambur contained formalin Conc. of 0.91µg/g and from M.G.R. Nagar contained formalin Conc. of 0.8µg/g. Blackmouth splitfin collected from Perambur contained formalin Conc. of 0.65µg/g and from M.G.R. Nagar contained formalin Conc. of 0.55µg/g. The Histopathological changes occur that the formalin adulteration muscle tissues at 0 hour is almost similar to that of control sample in appearance, Slight decrease of the organoleptic characteristics at 6 hours, tissue structure of musculature, Unfavorable changes in muscle fibers at 20 and 24 hours. The histopathological study clearly shows the gradual spoilage of the muscles in 24 hours. Overall, the study deduced that formalin adulteration is not only harmful for human health but also affected the textural properties of fish considerably. Those people dealing with formalin over a long period of time are likely to be afflicted with health issues such as blindness, asthma, lung cancer and so on.

Keywords: *Sardinella longiceps, Synagaris japonicus, Formalin Test and Histopathological Study*

INTRODUCTION

Fish is widely accepted as essential nutrient required in human diet because of its high palatability, low cholesterol, tender flesh and cheaper source. The pattern of fish intake was increasing recently, probably due to the widespread awareness of health benefits correlated with fish consumption (Sadiku and Oladimeji, 1991). Fish is a highly nutritious food supplying 16% of animal protein provides omega-3 polyunsaturated fatty acids (ω-3 PUFAs) with essential micronutrients (Kangsen *et al.,* 2021). Fresh fish is one of the most perishable foods, therefore keeping it at peak quality is challenging task to maintain the freshness of the fish in the supply chain since, in most cases, the locations where fish are produced and consumed are kept apart. Fish can be preserved using a variety of techniques, including chilling, freezing, canning, and smoking, but each has its own merits and demerits (Hoque *et al.*,2016). Fresh fish was given chemicals like formalin to extend its shelf life significantly above the short-term preservation techniques used today due to mass production and a lack of post-harvest management infrastructure (Sahu *et al.,* 2018).

Formalin has been shown to have a carcinogenic effect, which is well-established; yet, its impact on the quality of muscles has not been fully studied. The fish were treated with varying quantities of formalin, and the results showed that the content rose up to thirty times at higher concentrations (Hoque *et al.,* 2018). After investigation on *in vivo* toxicity on rats, Kundu *et al.* (2020) reported that the protective mucus layer

detachment from the secretory layer was caused by granulation and disintegration of the layer. This confirms that eating carps contaminated with formaldehyde on a regular basis raises the risk of developing major health issues such lung, nasopharyngeal, oropharyngeal, and stomach cancers. It is very difficult to fully eliminate formalin from fish once it has been intentionally added.

A recent issue in illegal use of formaldehyde by fish marketers and fishermen to prolong the preservation of fish during sales and transportation and found formaldehyde traces on the ice used in newly marketed fish and interstate fish consignments (FSSAI 2018). In addition to producing allergy and dermatitis, exposure to this chemical increases the risk of developing respiratory and brain tumours (Hoque *et al.,* 2018). Additionally, (Kashyap *et al.,* 2024) found that formalin-treated fish had significantly fewer essential and non-essential amino acids, which may have made the fish an incomplete supply of protein

Fish flesh may also develop formaldehyde while it is being stored, which accelerates the degeneration of the flesh. Previous research suggest that the duration and temperature of frozen storage are the primary determinants of the formaldehyde content of fish meat (Badii and Howell 2002). According to Tumun *et al.* 1996 reports, formaldehyde has been found in fish, and it may form different stable chemical interactions with proteins in fish tissues, which could accelerate the degeneration of the flesh. Therefore, it is generally accepted that chemical bonding is the mechanism by which formaldehyde forms in situ. (Kundu *et al.,* 2020) This implies that analytical methods are required to identify the presence of free, reversibly bound, and irreversibly bound formaldehyde in fish flesh. The formaldehyde fraction that can be extracted using diluted perchloric acid at a specific temperature is known as free formaldehyde (Kumar *et al.* 2010). Overall, the study deduced that formalin adulteration is not only harmful for human health but also affected the textural properties of fish considerably (Parameshwari *et al.,* 2021).

According to (Jinadasa *et al.,* 2022), the most effective ways to lower the health hazards caused by formalin adulteration are to boil fish to a core temperature of 70°C and wash it with tap water. The overall hygienic practice and sanitary conditions of the markets and the fish traders/retailers were very poor, not satisfactory and all the traders or retailers who were mixed formalin with the fishes, knew about the bad effects of the formalin (Rafiad Islam, *et al.,* 2015). Despite the fact that the fish adulterer dealers employ formalin at lesser concentrations, no very logical assay was really seen. Based on information gathered from a few sources, an approximate amount of formalin was combined with water to adulterate fish, and it was believed that the formalin content was close to 1% (Khan *et al.,* 2012). Histopathological assessment has been increasingly recognized as an effective tool for evaluating the environmental effects (Amthul Azeez, *et al.,* 2020). In both lab and field investigations, histopathological alterations have been extensively employed as biomarkers to assess the health of fish exposed to pollutants. The ability to examine particular target organs is one of the main benefits of utilizing histopathological biomarkers in environmental monitoring (Marina *et al.,* 2007).

In order to prevent fish muscle degradation, the current method was designed to support the goals for determining the histopathological changes over time and the strength of 1% formalin leads tissue quality of formalin is totally degraded in 24 hours. The fish samples collected from different markets were studied for histopathological changes at different time intervals.

MATERIALS AND METHODS

Fish Collection:

There are many fish markets are available in and around Chennai. As there were many, we selected the fish sample collection from Major Fish retail market at Perambur and M.G.R. Nagar in Chennai. From each market three samples of Oil Saradine (Sardinella *longiceps)* and Blackmouth *(Synagaris japonicus*) type of commercial fishes were purchased. The collected fish samples with an average length and weight of *Sardinella longiceps* is 10.13±0.15 and 41.67±0.36 *and Synagaris japonicus is* 10.73±0.21 and 54.21±0.47 respectively were immediately taken to the laboratory. During transit, each fish sample was wrapped in a polythene bag and placed in an ice box until it arrived at the laboratory to maintain freshness of the samples. The ICAR-CIFT formalin detection kit was used to detect the qualitative of formalin upon

arrival. As it showed positive result the samples were kept in the freezer about 2-3 days for the quantitative analysis on the fish samples was done using the spectrophotometric method.

Evaluation of Formalin:

The concentration of formalin content in fish muscle was determined in *Sardinella longiceps and Synagaris japonicus* using Nash reagent (Castell and Smith 1973) and Trichloroacetic acid (TCA) extract developed by Benjakul *et al.* 2003. used as an indicator to detect the absorbance of formaldehyde. Nash's reagent was mixed of dissolving ammonium acetate (15 g), acetyl acetone (0.3 mL), acetic acid (0.2 mL) and the volume were raised to 100 mL of distilled water was kept in a dark-glass bottle covered with aluminum foil for 24 hours because it is a light sensitive. A 0.1 N NaOH and 0.1 N HCl were used to alter the pH of the distillate to 7.0 by pH meter. Initially, scales, skin and bones of the fish were removed and then muscle of fish samples was taken into blender for homogenization and blended for 10 minutes. 30 g of blended sample of each fish was mixed with 60 mL of 6% TCA solution and homogenized uniformly using a mortar and pestle. The supernatant was then filtered by using Whatman No.1 of filter paper. 5mL of filtrated extraction was collected to adjust the pH around 7 by using NaOH or HCl and stored in deep freezer for 30 minutes. Later the sample was taken out from freezer and added 2 mL of previously prepared Nash's Reagent was mixed and was kept at 60^0 C water bath for 20-30 minutes. The absorbance of this mixture was determined by UV/v spectrophotometer at 415 nm. Triplicate of the absorbance was made for each sample and recorded for further calculation. Finally, the concentration of formalin was determined from the standard curve that was prepare in the range of 0-0.8 µg/ mL

***Muscle pH estimation*:**

The pH of fish sample measurements was taken according to Yeasmin *et al.* (2010). After homogenizing of 4 g of fishes' muscle was mixed in 20 ml of distilled water. pH meter was calibrated with standard buffer solution of pH 4.2 and 9.2 for measuring the pH of mixed sludge was determined with a pH meter (pH TUTOR, Eutec).

***Protein solubility*:**

Analysis of protein solubility was followed according to (Robinson and Hogden, 1940) was determined using phosphate buffer (15.6 mM Na2 HPO4 , 3.5 mM KH2 PO4 , containing 1.1 M Potassium chloride, pH 7.5). The fish muscles were homogenized in phosphate buffer at 9000 rpm for 2 min using homogenizer and then centrifuged (9000 × g) at 4ºC for 15 min. The amount of soluble protein was measured in the pellet homogenate before centrifugation and the supernatant that was collected during the centrifugation. The protein solubility was calculated as:

Protein solubility = (amount of soluble protein before centrifugation/ amount of soluble protein after centrifugation) × 100

Water holding capacity (WHC):

According to (Jauregui *et al.*, 1981) WHC for fish samples were measured by weighing 3 g of fish muscles sample was placed with Whatman No. 1 filter paper between two layers. Sample was placed at the bottom of 50 mL centrifuge tubes and centrifuged at 5000 × g for 10 min at 4°C. After centrifugation, the sample was removed and reweighed. WHC was calculated as follows

WHC (%) = (W1-W2)/W1 × 100

Where, W1 is the initial weight (g) and W2 is the final weight.

Average of three replicates was reported as value of WHC

***Histopathological Study*:**

Permanent slides were prepared following the methods by Naser and Farhana, (2005) and Naser and Mustafa, (2006) modified from Humason (1979). A group of fish were treated with 1% formalin for 1 hour and kept exposed in open air. After treating with formalin, muscle samples were collected from dorsal side of the fishes at 0 hour, 6 hour, 12 hour, 16 hour, 20 hour and 24 hour observation for histopathological test. Fresh fish was tested as control. For histopathological preparation the different fishes muscles such as *Sardinella longiceps* and *Synagaris japonicus* of both control and experimental groups were excised and immediately fixed in Bouid fluid. The tissue was the processed as per the procedure followed in Pearse

(1980) with Hematoxylin and Eosin stains. Photo-micrographs were taken to substantiate observation and the results are discussed.

Statistical analysis was performed with data obtained were analyzed by one-way analysis of variances (ANOVA) using the computer package SPSS software.

RESULTS

TEST OF FORMALIN

The concentration of formalin found from the muscle of fish (*Sardinella longiceps*) Collected from Perambur market was 0.91 ug/g done in triplicate, whereas the fish collected from M.G.R. Nagar market shows the concentration of formalin was 0.8ug/g in triplicates greater than the acceptable limit (>0,5). Table 1. The concentration of formalin found from the muscle of fish (*Synagaris japonicus*) Collected from Perambur market was 0.65 ug/g done in triplicate, whereas the fish collected from M.G.R. Nagar market shows the concentration of formalin was 0.55ug/g in triplicates greater than the acceptable limit (>0.5). Fig 1

Table 1: Concentration of formalin in *Sardinella longiceps* and *Synagaris japonicus* collected from local fish market

S. No.	Fish samples	Market area	Quantity	Length of fish (cm)	Weight of fish (gms)	Concentration of formalin (µg/gm)	Acceptable limit
1	*Sardinella longiceps*	Perambur	3	10.13±0.15	41.67±0.36	0.91±0.015	>0.5
		M.G.R	3	10.19±0.1	38.19±0.81	0.83±0.01	
2	*Synagaris japonicus*	Perambur	3	10.73±0.21	54.21±0.47	0.66±0.011	
		M.G.R	3	10.58±0.18	51.33±0.9	0.56±0.021	

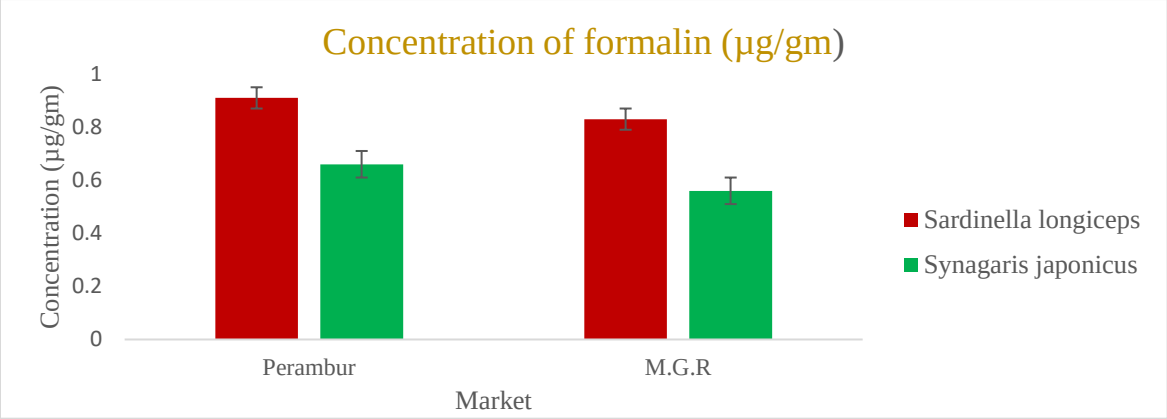

Figure 1: Concentration of formalin in *Sardinella longiceps* and *Synagaris japonicus*

EFFECT OF FORMALIN TREATMENT ON QUALITY OF FISHES:

The initial formalin content in the untreated fish was examined for effect of fish quality. And also, the fishes were treated by dipping in 1% formalin solution for 24 hours. The pH of the untreated *Sardinella longiceps and Synagaris japonicus* fishes from Perambur and M.G.R. Market was 6.79±1.12, 6.91±1.01, 6.77±0.84

and 7.08±1.07 which decreased to 6.33±0.17, 6.42±0.11, 6.37±0.14, 6.48±0.17 in treated formalin fishes respectively seen in Figure 2. The Water Holding Capacity (WHC) of the untreated *Sardinella longiceps and Synagaris japonicus* fishes from Perambur Market was 68.12±0.61and 69.73± 0.83 were reduced to 66.92±1.53 and 67.13± 0.46 where as in M.G.R. Market were 70.16±0.42 and 69.94±1.05 to 67.76±1.28 and 68.17±1.51 respectively which found to be lessen after formalin treatment as shown in Figure 4. The initial Protein Solubility of untreated fishes were 78.84±1.41, 81.77±1.09, 80.11±1.02 and 83.84±1.47 diminish to 75.91±1.02, 79.54±1.29, 78.01±1.25 and 81.34±1.89 respectively in Figure 4 due to the fishes treated with 1% of formalin solution as shown in Table 2 and Table 3. The correlation between the fish quality parameters is significant at the 0.05 level ($p > 0.05$) in treated formalin as shown in Table 4.

Table 2: Initial Formalin Content untreated fish quality parameters

Parameters	Perambur		M.G. R	
	Sardinella longiceps	*Synagaris japonicus*	*Sardinella longiceps*	*Synagaris japonicus*
pH	6.79±1.12	6.91±1.01	6.77±0.84	7.08±1.07
WHC (%)	68.12±0.61	69.73± 0.83	70.16±0.42	69.94±1.05
Protein Solubility (%)	78.84±1.41	81.77± 1.09	80.11±1.02	83.84±1.47

Value are means ± SD, n = 3, p > 0.05

Table 3: Effect of 1% formaldehyde treatment of fish quality parameters

Parameters	Perambur		M.G. R	
	Sardinella longiceps	*Synagaris japonicus*	*Sardinella longiceps*	*Synagaris japonicus*
pH	6.33±0.17	6.42±0.11	6.37±0.14	6.48±0.17
WHC (%)	66.92±1.53	67.13± 0.46	67.76±1.28	68.17±1.51
Protein Solubility (%)	75.91±1.02	79.54± 1.29	78.01±1.25	81.34±1.89

Value are means ± SD, n = 3, p > 0.05

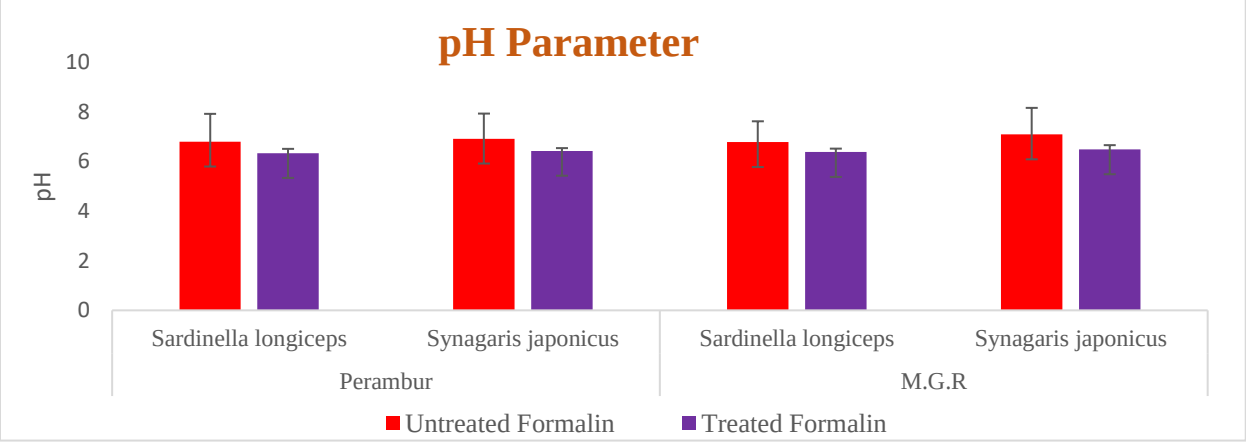

Figure 2: Test of formalin by pH parameter in selected Fishes

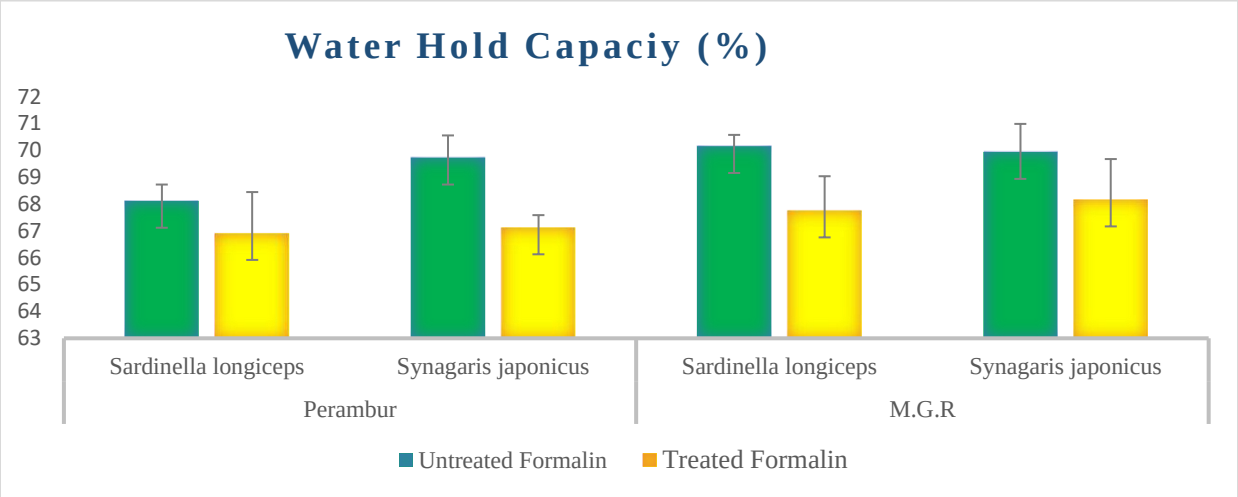

Figure 3: Test of formalin in selected Fishes by Water Hold Capacity

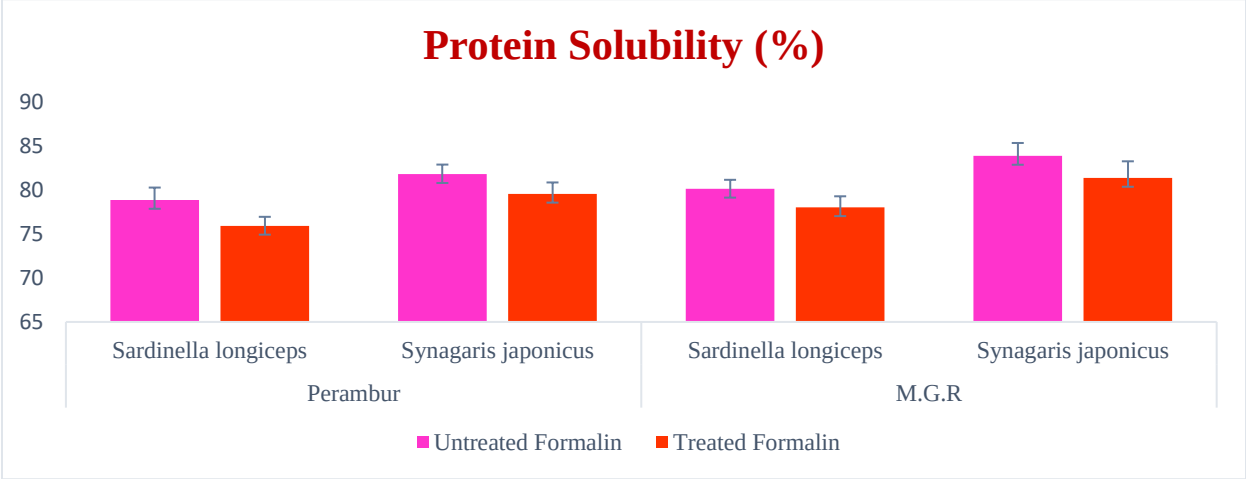

Figure 4: Protein Solubility test done in selected Fishes

Table 4: Correlation of fish quality parameters in both sample fishes treated with 1% formalin

Parameter	*pH (Sardinella longiceps)*	*pH (Synagaris japonicus)*	*WHC (*Sardinella longiceps*)*	*WHC (Synagaris japonicus)*	*PS (Sardinella longiceps)*	PS (*Synagaris japonicus*)
pH (Sardinella longiceps)	1	.946**	.371**	.597**	.951*	.893**
pH (*Synagaris japonicus*)	.946**	1	.645*	.707**	0.999	.981*
WHC (Sardinella longiceps)	.371**	.645*	1	.738**	0.635	0.739
WHC (*Synagaris japonicus*)	.597**	.707**	.738**	1	.708**	.564**
PS (Sardinella longiceps)	.951*	0.999	0.635	.708**	1	.930*
PS (*Synagaris japonicus*)	.893**	.981*	0.739	.564**	.930*	1

***. Correlation is significant at the 0.01 level (2-tailed).*

**. Correlation is significant at the 0.05 level (2-tailed).*

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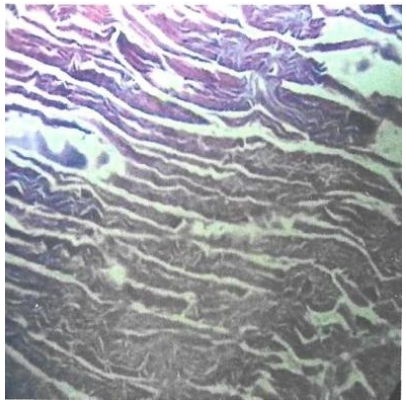
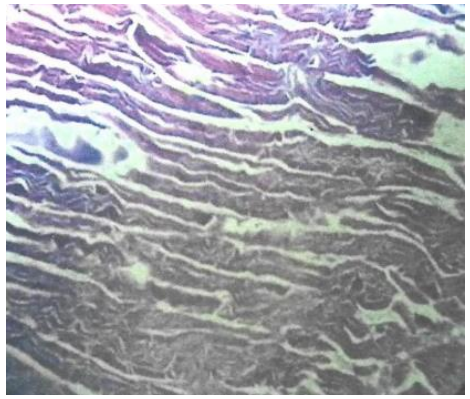
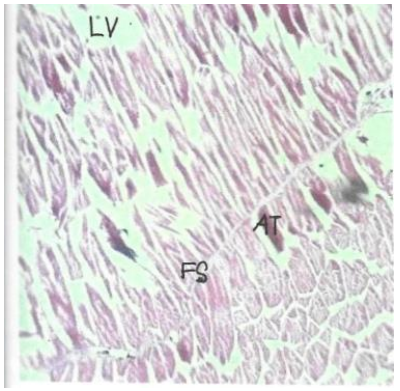


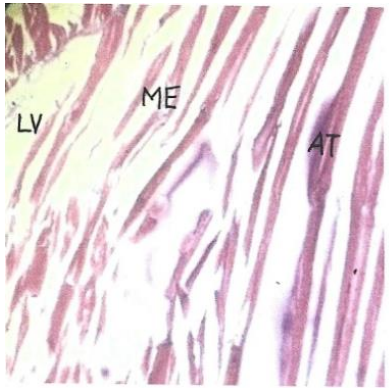
T.S. of control (fresh) fish muscle at 0 Hour



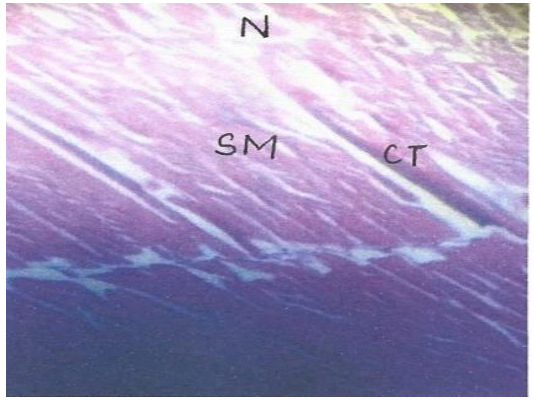
T.S. of 1% formalin adulterated fish muscle at 0 Hour



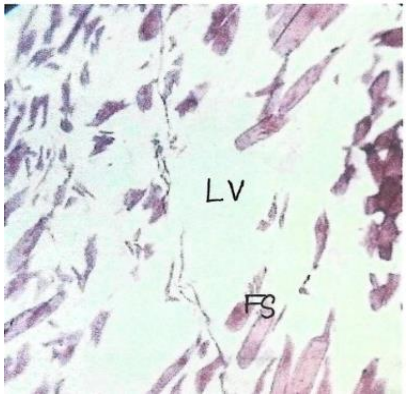
T.S. of 1% formalin adulterated fish muscle at 6 Hour



T.S. of 1% formalin adulterated fish muscle at 12 Hour

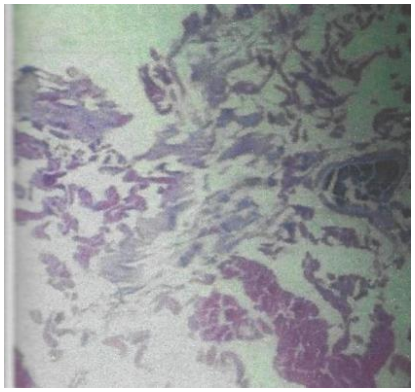


T.S. of 1% formalin adulterated fish muscle at 20 Hour

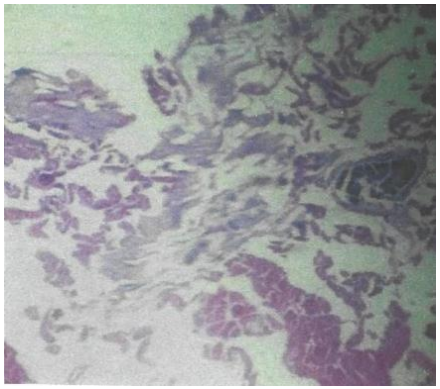


T.S. of 1% formalin adulterated fish muscle at 24 Hour

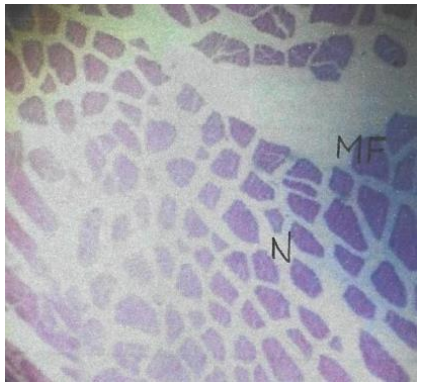
PLATE 1: Histopathological changes in *Sardinella longiceps*
LV- Large Vacuoles, FS- Fibre Splitting , AT-Atrophic Fibre, ME- Mild Effacement , SM- Smooth Tissue, N- Necrosis, CT- Connective Tissues



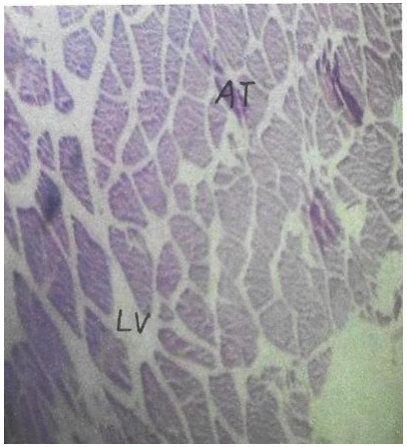
T.S. of control (fresh) fish muscle at 0 Hour



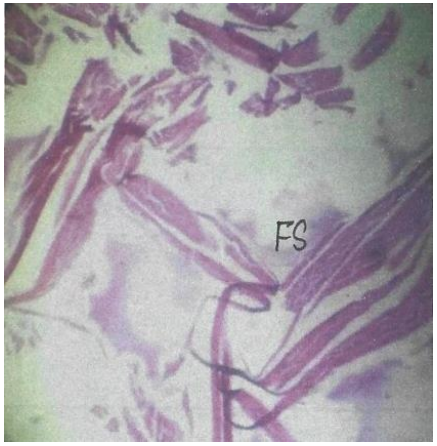
T.S. of 1% formalin adulterated fish muscle at 0 Hour



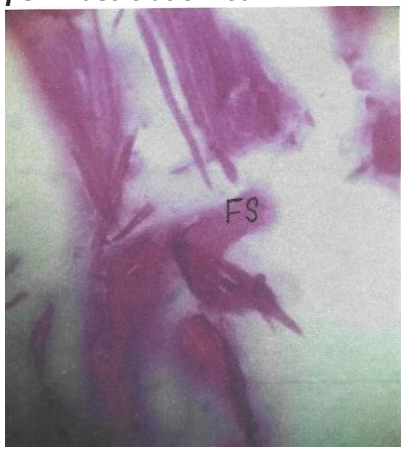
T.S. of 1% formalin adulterated fish muscle at 6 Hour



T.S. of 1% formalin adulterated fish muscle at 12 Hour



T.S. of 1% formalin adulterated fish muscle at 20 Hour



T.S. of 1% formalin adulterated fish muscle at 24 Hour

PLATE 2: Histopathological changes in *Synagaris japonicus*
LV- Large Vacuoles, FS- Fibre Splitting, AT-Atrophic Fibre, ME- Mild Effacement, N- Necrosis

HISTOPATHOLOGICAL STUDIES

Histopathological changes in muscle of 1% formalin adulteration in two commercially accessible fishes (*Sardinella longiceps* and *Synagaris japonicus*) were shown in Plate 1 and Plate 2. Histopathological slides show that the muscle tissue contaminated with formalin at 0 hours looks almost exactly like the control sample. Muscles appeared fresh and no obvious physical changes took place. The muscle fibres were visible in flawless condition. The peripheral and inner muscle fibres did not vary with respect of their interrupted sequences in the muscle fibres showed in both control and treated 1% formalin adulterated tissue sample at 0 hour. The sample that was examined after 6 hours, 12 hours, 20 hours and 24 hours showed modifications in the musculature's tissue structure. Gradual degradation was noticed in the consecutive slides. Slight lessen of the organoleptic characteristics was showed in the muscle sample after 6 hours of exposure to air (Plate - and -). At later of observational point, the rate of desolation increased. There were several adverse changes observed in the muscle fibers of 20 hour and 24-hour samples. Desolation and destruction of membrane was observed. The myofibril bundles had a lot of excess cellular space and were asymmetrically arranged. The histological analysis amply demonstrates how the fish muscles gradually deteriorated over the duration of 24 hour

DISCUSSION

Fish and sea food are a good source of protein and thus are a vital part of a healthy diet (Ashie *et al.,* 1996). Importantly, fat, free Amino acids and water contained within the fish are susceptible to fast spoilage during post mortem process (Fernandes and Venkatraman, 1993, Ismail, *et al*., 2005). Therefore, keeping fish fresh in ice for more than one week is difficult and also depends upon the species chosen the issue becomes more important to a country like India. when imports of fish are necessary because the nation's regional fish supply is weak to fulfil demand (Cristina Bosetti, *et al*,. 2008). Lately, many reports in public media have focused to the problem of Wholesalers and Vendors treating fish, with formaldehyde solution (Formalin) to preserve shelf life in order to reduce their cost and increase revenues at the price of public health (Joshi, *et al.,* 2015).

Formaldehyde is a member of aldehyde family which occurs in the gaseous form whereas the liquid form is formalin made up to 37% formaldehyde by weight. Formalin (37–40% formaldehyde) is one of the most effective, widely used chemical in pisciculture for its antiparasitic, antifungal and prophylactic activities (Zhao, X. O. and Zhang, Z. Q. 2009). It is used in paints, cleaning products and textile industry, as well. Due to its antiparasitic, antifungal, and preventive properties, formalin (which contains 37–40% formaldehyde) is one of the most useful and extensively used chemicals in pisciculture. It is utilized in the textile, paint, and cleaning product industries (Ramazan Mert *et al.*, 2014)

Fish is traditionally preserved by chilling, freezing, and ice cooling; however, these methods invariably reduce the fish's freshness and incur additional costs. Thus, in order to maximize their earnings, traders are continuously looking for labor and money saving ways to extend the fish's shelf life (A. Kundu *et al.* 2020*)*. Driven by this objective, they started submerging the fish in toxic substances like sodium benzoate and formalin solution. The "International Agency for Research on Cancer" classifies formalin, a transparent, colourless solution with 40% formaldehyde (FA) in water, as a Class I carcinogen. Long-term FA inhalation can cause respiratory issues as well as nose, throat, and eye irritation.

Formalin is utilized to improve product appearance and postpone spoiling despite these hazards (P. Devaraj *et al. et al.*, 2021), even though doing so compromises safety regulations (Islam *et al.,* 2024). According to several studies the action of FA not only reduced the bacterial load but also improved the elasticity of the fish muscles, giving the impression of freshness (Jinadasa *et al.,* 2022). Numerous publications have described the extent to which this issue of this places like Southeast Asia, South Asia, and some portions of Africa. Remarkably, reports from Bangladesh, India, and Malaysia indicate that frightening proportions of fish samples in local markets have excessive formalin levels (Md. A. Khan *et al.*, 2023, A. Kakoti Jhorna Borah, *et al.,* 2024).

Formaldehyde is widely used in chemical industries and also as a disinfectant and preservative. Importantly, International Agency for Research on Cancer (IARC) has classified formaldehyde as a Group 1 Carcinogen to humans (IARC, 2004). Long-term exposure to formalin increases the risk of developing health problems such lung cancer, asthma, and blindness (Hossain *et al.,* 2011). Exposure to formaldehyde associated with freshwater fish consumption is a public health concern in Southern Bangladesh and needs further assessment and risk management strategies.

The Food Safety and Standard Authority of India (FSSAI) fixed an Ashco maximum limit for formaldehyde content with4 mg/kg for freshwater fish and 100 mg/kg for brackish and marine fish (FSSAI, 2019). In India, there is wide spread discussion on formalin adulteration in fish but it has not been estimated quantitatively. The concentration of formalin in the fish sample (*Sardinella longiceps* and *Synagaris japonicus*) collected from two different locations (Perambur and M.G.R Nagar market) was calculated as 0.91µg/g (*Sardinella longiceps*) and 0.65µg/g (*Synagaris japonicus*). The concentration of formalin in all species of all location is relatively low with general mean value at around 1%. In a similar study performed by the author (Bianchi *et al.*, 2007) was not investigate further when the formaldehyde was greater than 1% because the low concentration levels are not considered hazardous to health. It has observed >5% as the formaldehyde content in fresh fish samples (Bianchi *et al.,* 2007). Similarly, the research work has observed 0.38 to 15.75µg/g-1 formaldehyde in different fish species (Nordiana *et al.,* 2011).

In the present study of formalin concentration in stored fish match with their finding and it can be assumed that there was no deliberate formalin contamination to the fish. Interestingly. Even when held at 0°C, formaldehyde is formed, and after 6 days, the content rises to 134% (Paudel and Pandey, 2017). Therefore, there is a high chance of formaldehyde content increasing when stored for a long time period. While transporting from one country to other.

According to (Sanyal *et al.,* 2017), fresh fish (control) had a pH of 6.72, which dropped to 6.59 after being given 1% formalin. The protein denaturation may have caused the aggregations brought on by the formalin treatment, which resulted in a decrease in protein solubility was done according to (Morisasa *et al.* 2020). The significant drop in pH was also observed to occur at the same time as the solubility of proteins; this could be because the isoelectric pH of the muscle protein is moving. It revealed that the protein recovered from rohu fish treated with 5% formalin solution showed a comparable decrease in protein solubility. (T. Yeasmin *et al.* 2010)

Formaldehyde's strong reactivity causes cross links to form between myofibrillar proteins and formaldehyde, toughening the flesh and reducing its ability to hold water (Haard and Simpson, 2000). This lowers acceptability and functionality (Li *et al.,* 2007). In order to find the concentration of formalin in the fish tissue muscle, Histopathological studies were carried out in the present investigation. Statistical study that explains the strength of the two variables are related. Finding consistent correlations that may be used in everyday circumstances is one of the benefits of correlation studies. According to (Mehta, *et al.,* 2010), it can be used to quantify the statistical significance and strength of the relationship between two or more water quality variables and one variable that acts as the dependent variable. Histology is one method to assess the effects of contaminants on the health of fish in the environment and can be used to observe a causal link between exposure to hazardous substances and their biological responses (Phadmacanty *et al.,* 2023).

Histopathological changes in muscles of 1% formalin adulterer fishes (*Sardinella longiceps* and *Synagaris japonicus*). Histopathological slide demonstrate that the formalin adulterer muscle tissue at 0 hour is almost similar to that of control sample in appearance. At the start of the trial, there were no appreciable obvious quality changes in the tissue sample. Muscle looked fresh and no visible physical changes took place. The Muscle fibres appeared in intact condition. The peripheral and inner muscle fibers did not differ with respect of their interrupted sequences in the muscle fibers in both control and 0 hour formalin adhered tissue sample. Hossain *et al.,* (2008) shows in their research, that presence of formalin is prominent in most of the imported fishes from Myanmar and India.

The samples observed after 6 hours, 12 hours, 20 hours and 24 hours showed changes in tissue structure of musculature. There was a noticeable decline in quality between the subsequent slides. After six hours in the air, there was a small decline in the muscle sample's organoleptic features (Khan *et al.,* 2012). The rate of desolation was increased at later stages of observation. Apparently, even after 20 hours of exposure to the open air, the quality of the muscular tissue was judged to be satisfactory. The survey's findings also showed that there is a price difference between fish that is imported from other countries and fish that is produced locally in Bangladesh (Yeasmin, *et al.* 2010). In case of catla, rohu, mrigal and silver carp, the price of the locally produced fishes are quite high compared to those coming from India and Myanmar. Fish may generally be distinguished between those that are imported and those that are produced locally based on their size, colour, and other organoleptic characteristics.

According to the findings, customers typically choose fish that is produced locally over fish that is imported, and most of the time, stores let customers know whether a fish is imported or not. The retailers inform the consumers whether it was imported or not. The shelf life of the locally produced fish is much longer than those of imported fish. The survey also indicated that the overall hygienic condition of the retailers and sanitary conditions of the markets were very poor except KR and SP fish market, where both sanitary and hygiene conditions were found to be in acceptable condition. (Ahmed *et al.,* 2005) also found unhygienic condition of retail fish markets in Gazipur, Bangladesh. Yeasmin *et al.* (2010*)* also found unhygienic condition of domestic markets at Mymensingh district in Bangladesh.

In 2011, the National Toxicology Program, an interagency program of the Department of health and Human services, named formaldehyde as a known human Carcinogen in its 12th report in carcinogens (National Toxicology Program, 2011). Some fish dealers treat fish with formalin, despite the fact that it is dangerous and could pose a health risk to humans (Rafiad Islam *et al.,* 2015).

It was observed in a study conducted in Dhaka city (Haque and Mohsin, 2009) that almost 5% shops of total consumable fishes contain formalin treated fishes those are sold in fish markets. They found this intensity to vary market to market and species to species. They discovered that formalin had a significant effect on Rui fish, while Kirwan Bazar had the greatest concentration of fish that had received formalin treatment. The present investigation focused mostly on large fish species, indicating a significant percentage of formalin content in fish (Riaz Uddin *et al.,* 2011).

Unfavorable changes were observed in the muscle fibers of 20 hours and 24 hours samples. Desolation and destruction of membrane was visible. the myofibril bundles were irregular, loosely attached with huge extra cellular space, water binding capacity of the muscle was lower than all the other muscles observed in the experiment. The histopathological study clearly shows the gradual spoilage of the fish muscle in 24 hours' time.

Overall, the study deduced that formalin adulteration is not only harmful for human health but also affected the textural properties of fish considerably.

CONCLUSION

It is assume that formalin is added to the fish, especially those come from bordering countries by the local fish traders to keep the fish fresh for a long time, but as it is a carcinogenic chemical and has got the ability to produce serious health hazards like cancers of the lungs, nasopharynx, oropharynx and nasal passage to the population regulatory bodies should take necessary steps to minimize and stop formalin treatment of the fish. The current study involves a small number of samples to detect formalin in fish in local market of Chennai city. But it gives us a comprehensive picture to understand the extent and magnitude of the scenario.

From the above observation, it can be concluded that exposure to 1% formalin for 1 hour was not effective to prevent muscle decomposition and preserve fish muscle for longer than 20 hours period when exposed to open air. In fish market fish traders possibly preserve the fishes in relatively higher concentration of formalin for longer period to preserve fish for several hours without ice although the present study revealed

that the tested concentration of formalin delayed desolation of muscle tissue for 12 to 20 hours, in concern to the health hazard effect it should be avoided to use in fish.

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