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FINAL REPORT OF THE MINOR RESEARCH PROJECT

"DETECTION AND QUANTIFICATION OF FORMALDEHYDE CONTENT IN FOOD FISHES FROM LOCAL MARKETS"

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EXECUTIVE SUMMARY OF FINAL REPORT OF THE MINOR RESEARCH PROJECT

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I. INTRODUCTION

Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. World per capita fish supply reached a new record high of 20 kg in 2014. Fish continues to be one of the most-traded food commodities worldwide with more than half of fish exports by value originating in developing countries (FAO, 2016).

The nutritive and medicinal values of fishes have been recognised from the time immemorial. It is proved that fish protein comprise all the ten essential amino acid in desirable strength for human consumption. The four major constituents in the edible portion of fish are water, protein, lipids (fat or oil) and ash (minerals). Carbohydrates, vitamins, nucleotides, other non-protein nitrogenous compounds are also present in small quantities in fish muscle. Though quantitatively minor components, they play vital roles in maintaining the system and thus are essential for growth and development of the organisms. The health benefits of consuming fish as a source of omega-3 fatty acid have been well established. It can reduce cholesterol levels and the incidence of stroke and can protect against cardiovascular diseases, improve cognitive development in children and slow cognitive decline in the elderly (Fernandes and Venkatraman, 1993; Ashie *et al*., 1996; Ismail, 2005).

Fish and other seafoods are highly perishable and can only be kept fresh in ice for eight to fourteen days depending on the species. In order to keep the freshness of fish and seafoods, fisherman and fish vendors tend to use formalin as preservation agent. Formalin is usually used for the preservation of tissues. It makes the fishes stiff and keeps them fresh for longer period of time. Inadequate freezing facilities and ice factories and time consuming transport force the fish traders to resort to such malpractice. Available reports suggest that formalin is sometimes added or sprayed to the fishes by the fish traders while transporting to domestic marketing chain to prevent spoilage and increase shelf life (Yeasmin*et al*., 2010). Owing to the increasing demand for fishes, the world market farmers continue to use formalin and other chemicals to maximized harvest efficiency of the fish, although this may be detrimental to human health (Andem *et al*.,2015).

Formaldehyde is a naturally occurring organic compound with formula CH_2O (HCHO). It is the simplest of aldehydes with systematic name methanal. Formaldehyde is a colourless, strong smelling gas .It was first reported in 1859 by the Russian Chemist Aleks anrButlerov (Butlerow, 1859).

Formalin is a 37-40 percent aqueous solution of dissolved formaldehyde CH_2O.It was used in fish culture by Leger (1909) to control costia on trout. Formalin was brought into common usage by Fish (1940) to control ectoparasitic diseases of hatchery trout and salmon.

Formaldehyde is metabolized naturally in our bodies by normal metabolism and can also be found in the air, natural food, some skin care products as well as preservatives in processed food, especially in dried and frozen food.If the amount of formaldehyde is small, it does not harm health.However, it can cause minor to serious problems such as pain, vomiting, coma and possible death when large doses of formaldehyde is taken. Formaldehyde has an acceptable daily intake (ADT) of 0.2mg/kg body weight set by the United States Environmental Protection Agency (Nash, 1953).

In the food industry, formaldehyde is used as antibacterial agent and preservative in food processing.It is widely used for its bleaching effects and also as a preservative in order to prevent spoilage by microbial contamination. Formaldehyde is also used as a preservative in dried foods,fish, certain oils, fats and disinfectants for containers.In sea food and crustaceans, formaldehyde is known to form post mortem from the enzymatic reduction of trimethylamineoxide (TMAO) to formaldehyde and dimethylamine (Sotelo *et al*.,1995).

Formaldehyde is a major byproduct of the manufacturing industry (Heck *et al*., 1990), a common environment hazard (Flyvholun and Andersen 1993; Tang *et al*.,2009; International Agency for Research on Cancer, 2012), and a product of the cellular metabolism of many methylated compounds.

Formaldehyde is listed as a probable human carcinogen (Yeasmin, 2010). Studies relating to formaldehyde have focused almost exclusively on its toxicology in animals and humans. The carcinogenic properties and detrimental effects of formaldehyde exposure on growth and reproductive development have been described and summarized extensively (Golden *et al* ., 2006; Tang *et al* ., 2009;Zhang *et al* ., 2009;Szende and Tyihak, 2010;Duong *et al* ., 2011; Tulpule and Dringen,2013).Formaldehyde is also highly toxic to microbes and it has widespread application as a disinfectant for sterilization.

The use of synthetic chemicals such as formaldehyde as a means of increasing agricultural productivity has posed a serious treat and great consequences to the water bodies (Chitmamaticand Nunsong, 2009). The application of formalin to fish found to kill bacteria, fungi and others harmful microorganism posed a great problem to human after the consumption of the affected fishes (Gaafer *et al.*, 2010). Owing to the increasing demand for fishes, the world market farmers continue to use formalin and other chemicals, to maximize harvest efficiency of the fish, although this may be detrimental to human health (Okomoda *et al.*, 2010). Ayuba *et al*.(2013) showed that increased concentration of formalin on aquatic water reduce the amount of oxygen circulated, causes respiratory distress among the fishes, loss of balance, gulping for air, vertical movement of fishes, excessive accumulation of mucus and death. Fish is a major test organism in ecotoxicological studies because of their link to man in the food chain (Oshode *et al.*, 2008). Also, they are particularly useful for the assessment of waterborne and sediment deposited toxins where they may provide advanced warning of the potential danger of new chemicals and the possibility of environment pollution.

Uses of formaldehyde

Formaldehyde is a commonly used chemical compound that exists in various forms and at room temperature, is a colourless distinctive strong and even pungent smelling, flammable and gaseous substances. Formaldehyde has been used in a number of industries for various purposes such as for the manufacturing of building materials like pressed wood products, ply woods etc. It is produced from cigarette smoke, fuel burning application and kerosene space heaters. Additional uses in household products include additive for permanent press, an ingredient in glues and as a preservative in medical laboratories as embalming fluid and as a sterilizer. Since formaldehyde is a byproduct of combustion and other inherent processes, it can be found in significant concentrations and in various environments. Most formaldehyde enters commerce as formalin.

Harmful effects of formaldehyde

Formaldehyde is highly toxic to all animals regardless of the method of intake. Ingestion of 30ml of a solution containing 37% formaldehyde has been reported to cause death in an adult man. At concentrations above 0.1ppm in air formaldehyde can irritate the eyes and mucus membrane resulting in watery eyes. Formaldehyde inhaled at this concentration may cause headaches, a burning sensation in the throat and difficulty in breathing and can trigger asthma symptoms. Formaldehyde is known to be toxic for human being at high concentrations above 0.08% to be irritating to the respiratory tract, eye, and skin (Chun *et al.*, 2007).

Potential health effects of formaldehyde include sore throat, coughing, shortness of breath, headache, vomiting, blurred vision, and diarrhoea (Tang *et al*., 2009). Formaldehyde has also been known to cause contact dermatitis both as irritant and allergen. Irritant contact dermatitis occurs when too much formaldehyde has been exposed to skin. Symptoms of irritant contact dermatitis include redness and scaling of the skin. Allergic contact dermatitis occurs when the skin comes in contact with formaldehyde and produces an immune system response, potentially causing redness or itching of the skin.

Regular consumption of formalin glazed fish increases chance of malignancy and neurological impairment or brain functions. The International Agency for Research on Cancer (IARC) says there was sufficient evidence for carcinogenicity in humans. Based on the available evidences, some of these expert agencies have evaluated the cancer causing potential of formaldehyde. On 10 June 2011, the US National Toxicology Program formally described formaldehyde to be a known carcinogen. The most common result of chronic poisoning caused by formalin was damaged kidneys and cancer. Formalin may cause uncontrolled cell growth or cancer in stomach, lung and respiratory system if anyone consumes fish contaminated with it. A study showed mice exposed to formalin with concentration of six to fifteen parts per million for two years developed squamous cell carcinoma in the nostril.

Formaldehyde is an essential metabolic intermediate in mammalian cells that is produced during the normal metabolism of amino acid such as serine,glycine, methionine and choline. Most inhaled formaldehyde is deposited and absorbed in the upper respiratory tract, with which the substance first comes into contact (Heck *et al*., 1985).In humans, due to oral and nasal breathing, depositions and absorption occur in the nasal passages, oral cavity, trachea and bronchus (Monticello *et al.,* 1991).

The US Environment Protection Agency (EPA) has established a maximum daily dose reference (RFD) of 0.2mg kg^{-1} body weight per day for formaldehyde. At exposure increasingly greater than the RFD, the potential for adverse health effects increases (Wang *et al*., 2007).

Formaldehyde in seafood

Fishery products are of great importance for global human nutrient (Feldhusen,2000). However, a number of biological, chemical and physical hazards were reported associated with sea food contamination (Huss *et al.,* 2000) and microbiological sources of food borne diseases .Chemicals contamination in food can include natural toxicants such as mycotoxins (Melchert and Pabel,2004), marine toxins (Vale *et al*., 1999) and naturally occurring substances. Among them, great attention has been paid towards volatile toxic aldehyde like formaldehyde. WHO

reported that highest concentration of formaldehyde was found in marine fish (WHO, 2002). Bianchi *et al* .(2007) studied that during storage, fish belonging to the Gadidae family have high formaldehyde concentration in four cases out of 14 exceeding the value of 60 mg kg^{-1} proposed by the Italian Ministry of Health. Moreover, variable formaldehyde levels were observed among four species of squids, which was generally far higher in viscera than in muscle of frozen squid (Zhu *et al*., 2007)

Formaldehyde has been used as food additive in processed seafood such as herring and caviar in some countries (WHO, 2002). Besides, formalin is used as an agent to control external parasitic infection in fish. Formalin effectively kills parasites on gills, skin and fins. It is extremely effective against most protozoans, as well as some of the larger parasite such as monogenetic trematodes. Formalin also was employed as aquatic fungi (Saprolegniaceae) often causes disease problems for fish culturists (Schnick, 1991; Rach *et al.*, 2005). In addition, high concentrations of formalin are used to control fungi on fish eggs. However, formalin usage for aquaculture was not approved in Australia,Europe and Japan due to its association with oncogenesis (Schninck *et al.*, 1997)

Recently, several media outlets have reported on formalin preserved fish across Asia. In Hong Kong, noodle fish are regularly found to be contaminated with formalin (Aminah *et al*. 2013). The Malaysian government have information that imported fish like cod, salmon, and tuna have formalin in them. Fishes are dipped in formalin before being transported from fishing ports to the inland markets in Sri Lanka. In Tanzania, Indonesia, China, and Ghana, the use of formalin to store fish is widespread (Goon *et al*., 2014). Many studies done by the Bangladesh government show evidence of high formalin levels in imported freshwater fish, while the amounts in local varieties of the same species are almost negligible (Hossain *et al*., 2008). High formalin content in imported freshwater fish definitely confirmed this illegal practice of formalin preservation (Bianchi *et al.*, 2007).

Several published reports support intentional addition of formaldehyde by vendors to marketed fishes to improve shelf life and visual appearance. However, little is known about the quality aspects of fishes contaminated with formalin. Prolonged dietary formalin exposure could be potentially lethal even in low amounts. The present work was planned and executed with the following objectives:

1. To ascertain the quality of fishes based on sensory characters
2. To quantify the concentration of formaldehyde and biogenic amines from fish muscle
3. To evaluate the formaldehyde content in fishes from local markets

4. To understand the microbial load in fishes preserved with different concentrations of formaldehyde.

2. MATERIALS AND METHODS

i. Collection of samples:

Samples were collected from local markets of Alappuzha District viz. Aroor, Cherthala, Kalavoor, Alappuzha and Thottappally. Regular sampling was done fortnightly for from August 2014 to May 2016. Radom samples including fin fishes and shell fishes were collected. The samples were stored separately in polythene bags in an ice box and transported to the laboratory. The samples were stored in deep freezer until further analysis. Apart from fresh samples, frozen and canned samples were purchased from supermarkets to evaluate the formaldehyde content.

ii. Species identification:

The collected fish and shell fish samples were identified up to species level using standard keys. Fishes were identified using Munro (2000) and FAO (1984). Prawns were identified following FAO (1984) and CMFRI (2013).

iii. Measurement of pH:

5g of the tissue was homogenised in a tissue homogeniser using little distilled water. pH was measured using digital pH meter.

iv. Estimation of formaldehyde content:

Formaldehyde content in the collected samples was determined by calorimetric method from muscle tissue. The method used for the estimation is the Hantzsch reaction using Nash reagent (Nash, 1952) Castell and Smith (1953) and Ng (1987). Working standard solutions were prepared from the stock solutions at concentrations 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg/lit. Absorbance was measured in a UV-VIS spectrophotometer at 415nm. The blank, standards, and test solutions were treated in the same manner.

v. Estimation of TMA and DMA:

Picrate salt formation method of Dyer as modified by Tozawa *et al.*, 1971 was used for the determination of TMA. The procedure proposed by Dyer and Mounsey (1945) was used for the estimation of DMA.

vi. **Microbial load in fishes treated with formaldehyde:** Analysis of formaldehyde content in formalin (37-40%) treated fishes and its effect on microbial load was done in a commonly available freshwater fish, *Oreochromis mossambicus*. The study was carried out between January and June 2017. Following are the methodologies employed for carrying out the work.

a. Collection of samples:

Tilapia fishes for the present study were collected from live fish vendors. Live samples from same batches were used for the analysis. Fishes of size 8 to 10cms were used for the study.

b. Analysis of formaldehyde content:

To analyse the dynamics of formaldehyde content in formalin treated fishes, six batches of 10 fishes each were kept in different formaldehyde concentrations in separate trays of 1 litre capacity. Formaldehyde solutions were prepared having concentrations viz. 0.01%, 0.25%, 0.50%, 0.75% and 0.1%. Each solution was poured in separate trays (Trays 1 to 5) and 10 fishes were transferred simultaneously into each tray. Similarly a control tray was maintained in which distilled water without formaldehyde was used for introducing the fishes. At the beginning of the experiment, estimation of formaldehyde content in fresh fish was done. Time of introduction of fishes into the experimental trays was noted. At every 2hours ie. 2hours, 4 hours and 6 hours, fishes from each tray were analysed to determine the formaldehyde content.

c. Analysis of microbial load:

Preparation of culture blanks and culture media: 100ml saline water (0.5% for *O.mossambicus*) was dispensed into conical flasks as diluents for each sample and 9ml of these diluents or 9ml of saline water was dispensed into test tubes for serial dilutions. The diluents were sterilized and stored. Nutrient agar was freshly prepared in conical flasks for core plate method, sterilized and stored for future purpose.

Preparation of the experimental containers: Six trays were arranged, one is taken as control that contained one litre saline water without formaldehyde. Other trays contained one litre of sterilized saline water (0.5%). From tray one to tray five, different concentration of 37% of formaldehyde was added (0.01%, 0.025%, 0.05%, 0.75% and 0.1%). Each tray contained five fishes and one fish was taken for initial procedure.

Collection of fish skin surface and preparation of serial dilutions: Using sterile dissecting tools, samples were taken from the fish (skin surface from both side). Skin surface samples were added into 100ml sterile saline water in the conical flask and thoroughly mixed to make

10^{-2} dilution. For further dilution (10^{-3}) 1ml water was taken from the conical flask and was added into 9ml saline water in the test tube and thoroughly mixed and from this dilution another 1ml is pipetted out into another 9ml tap water in the test tube and 10^{-4} dilution was prepared. Samples (skin surface from both side of each fish) were collected from each trays in an interval of two hours. Likewise samples from second hour, fourth hour and sixth hour were taken for microbial analysis.

Inoculation into the nutrient agar: 0.5ml of inoculum was pipetted into sterile petri dishes from 10^{-3} and 10^{-4} dilutions. This was done in duplicates and also labelled sequentially (Plate A and Plate B). Using pour plate method, about 20ml of sterilized molten nutrient agar medium (an ear bearable temperature was considered) was poured into the inoculum added petri dishes. Both the inoculum and agar medium was mixed by gentle swirling in clockwise and anticlockwise direction and allowed to solidify. All poured petri dishes were incubated in inverted position at 27^{o}C for 24-72hours. Microbial counts were taken during 24hours, 48hours and 72hours interval.

Calculation: Area of square piece of skin was calculated by placing it on a graph paper. Microbial load was counted and expressed as Colony Forming Units (CFU)/ml using the following equation:

$$\text{CFU/ml} = \frac{No.of\ colonies}{Inoculum\ volume\ x\ Surface\ area\ of\ skin}$$

3. RESULTS

a. Sensory Analysis: The observations made on sensory characters viz. general appearance, colour of gills, eyes etc. showed that the samples collected from local markets were categorised under fresh condition (Table.1).

Table.1. Sensory characters of fishes collected for the study

Quality parameter	Character	Score
General appearance	Skin	Bright
	Blood spot on gill cover	None
	Stiffness	Stiff in rigor mortis

	Belly	Firm
	Smell	Fresh
Eyes	Clarity	Clear
	Shape	Normal
Gills	Colour	Characteristic, red

b.pH value: pH value of samples varied from 6.4 to 7.5. Mean pH values were 6.62±0.22 (Mean ± SD).

c. Estimation of Formaldehyde content:

For the determination of formaldehyde content, 50 samples each of marine fin fishes viz. *Sardinella longiceps, Rastrelliger kanagurta, Nemipterus japonicus, Stolephorus indicus*, estuarine fishes viz. *Johnius soldado, Thrissocles hamiltonii*, freshwater species viz. *Labeo rohita, Oreochromis mossambicus* and shrimps viz. *Fenneropenaeus indicus, Penaeus monodon* and *Parapenaeopsis stylifera* were analysed. Among the fin fishes, formaldehyde content was highest in *R. kanagurta* (0.199±0.286µg/g) and in shrimps, *P. stylifera* recorded high content of formaldehyde (0.2267±0.042µg/g) (Fig.2)

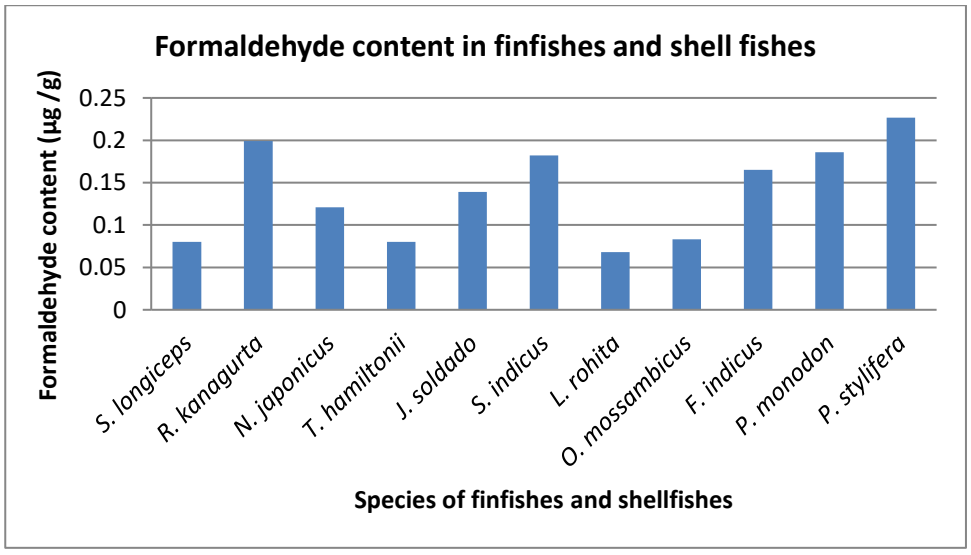

Fig. 1. Mean formaldehyde content in different species of fin fishes and shell fishes

Formaldehyde content in frozen fishes collected from the market varied from 0.928 to 0.147µg/g (Fig. 2). The highest formaldehyde concentration was recorded in *Mene maculata*

followed by *Leiognathus equulus, Mugil cephalus, Sphyrarena obtusata, Dussumieria elopsoides, Anodontostoma chacunda* and *Rastrelligerkanagurta.*

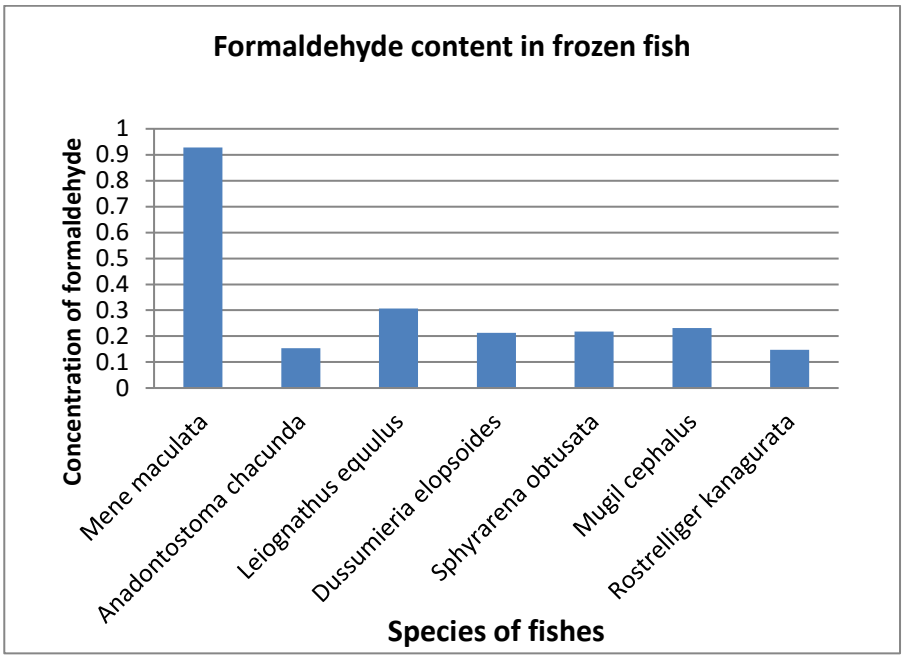

Fig. 2. Mean formaldehyde content in frozen fish samples

Formaldehyde content in canned tuna was found to vary from 0.308 to 1.093µg/g.The mean concentration was found to be 0.762±0.360 µg/g.

d. TMA and DMA:

Six species were used for the estimation of TMA and DMA viz. *Rastrelliger kanagurta* (Indian Mackerel), *Nemipterus japonicus* (Pink Salmon), *Megalaspis cordyla* (Finletted mackerel), *Escualosa thoracata* (White Sardine), *Caranx melampygus* (Bluefin Trevally) and *Scomberomorus commersoni* (Narrow banded Spanish Mackerel)

Mean TMA-N content in fishes varied from 0.854±0.050mg/100g in seer fish to 4.58±0.875 mg/100g in Finletted mackerel (Fig. 3). Mean TMA-N in mackerel, pink perch, white sardine and bluefin trevally were 1.602±0.469, 0.944±0.038, 1.614±0.115 and 1.292±0.067 mg/100g respectively.

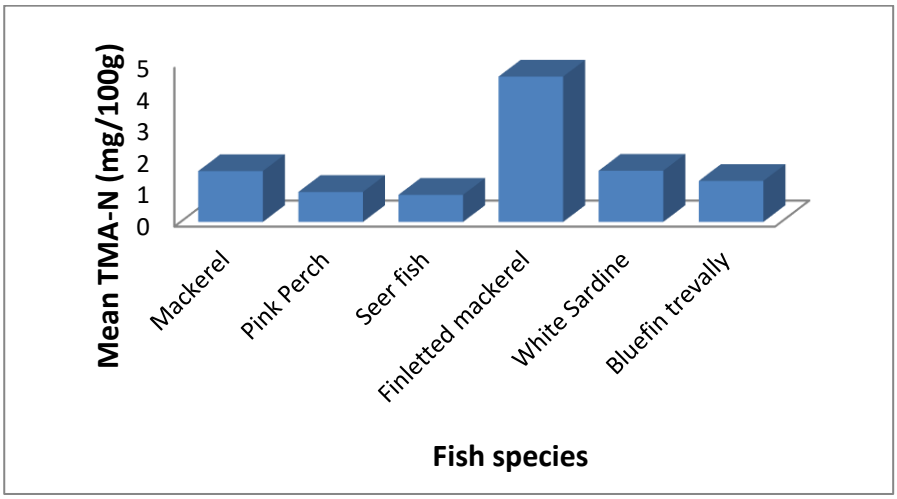

Fig. 3. Mean TMA-N concentration in different fish species

Mean DMA-N concentration in different fish species varied from 0.872±0.059in seer fish to 5.766 ±0.516mg/100g in filleted mackerel (Fig. 4).

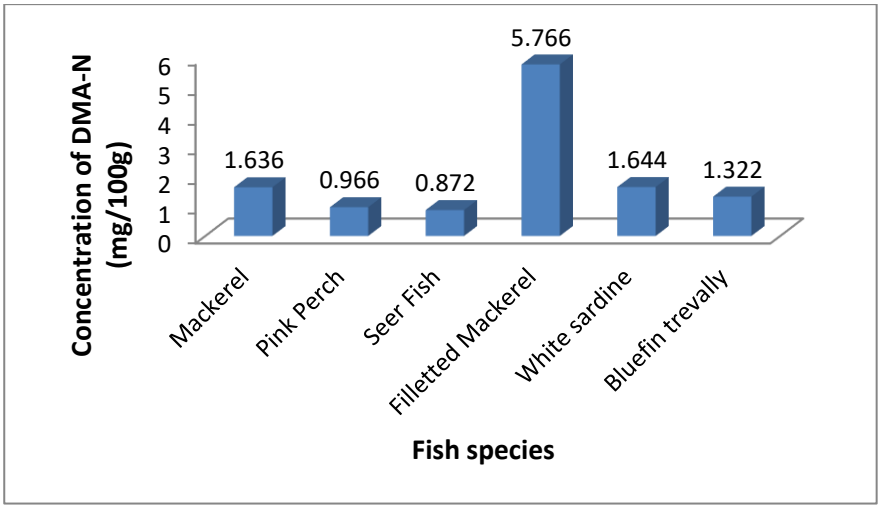

Fig.4. Mean DMA-N content in different fish species

e. Formaldehyde content in formalin treated fishes:

Formaldehyde content in fishes in control (tray without formaldehyde) was found to vary from 0.971 µg /g to 1.048µg/g. At two hours, 4hrs and 6hrs the mean formaldehyde content was 1.071, 1.048 and 1.032 µg/g respectively.In the tray containing formalin concentration 0.01% , mean concentration of formaldehyde in fish muscles varied from 0.97 to 1.187 µg/g. Mean formaldehyde content was 1.187, 1.110 and 1.079 µg/g at 2, 4 and 6 hours respectively.

At 0.025% formalin treatment, fish muscles showed formaldehyde content that ranged from 0.97 to 1.56 µg/g . At 2hrs, 4hrs and 6 hrs mean formaldehyde content was 1.156, 1.125 and 1.048 µg/g respectively. In the tray with 0.05% formalin content, fish muscles contained a mean formaldehyde concentration of 1.403, 1.295 and 1.079 respectively at 2hours, 4 hours

and 6 hours respectively. Formaldehyde content of fishes in tray 4 (0.075% formalin) varied from 0.97 to 2.267 µg/g. Formaldehyde content in flesh was 1.804 and 1.264 µg/g at 4hours and six hours respectively.In the tray with highest formalin content of 0.1%, mean concentration of formaldehyde in muscles of fishes was found to vary from 0.971 to 2.730µg/g. At 2hours, 4hrs and 6hours, mean formaldehyde content was 2.174, 2.730 and 1.588 µg/g respectively.

When fishes were treated with formaldehyde, their assimilation into muscles wasfound to vary with the treated concentrations. In the control there was a slight increase in formaldehyde concentration till the sixth hour of treatment. In trays 1 to 5, formaldehyde content in fish muscles increased till 2nd hour and then showed a decline until 6th hour.

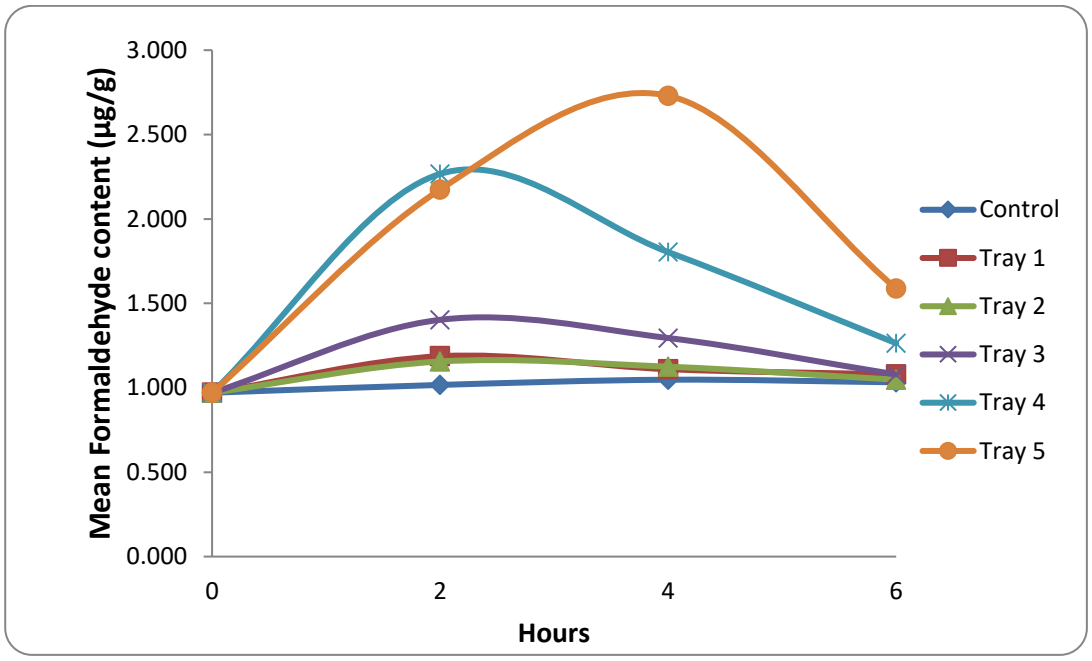

Fig. 5.Time dependent change in formaldehyde content in fishes

However, in tray 5, formaldehyde content in fishes increased until 4th hour and later showed a decline at 6th hour. At all formalin treated trays, concentration of formaldehyde in fish muscle was highest at the 2nd hour except in tray 5 where maximum concentration was recorded at 4th hour of treatment (Fig.5).

Microbial load in fishes: Initial microbial load in *O.mossambica* was found to vary from 189×10^{-4} to 255×10^{-3} CFU/ml. The tray without formaldehyde showed the maximum microbial growth in *viz.*195.5×10^{-4} to 258×10^{-3} , 263×10^{-4} to 305×10^{-3}, 225.5×10^{-4} to 295×10^{-3}CFU/ml at 2hours, 4hours and 6 hours respectively Microbial load in samples at the beginning of the experiment in fresh fishes ranged from 189×10^{-4} to 255×10^{-3} CFU/ml. Tray I with formaldehyde concentration 0.01% showed microbial growth ranging 1.5×10^{-4} to $2.5\times10^{-}$

^{3},24×10^{-4} to 77.5×$10^{-3}$and 26.5×10^{-4} to 119×10^{-3}CFU/ml at 2hours, 4hours and 6hours of treatment. At formalin concentration 0.025%, microbial load at 2hours, 4hours and 6hours was found to be 1.5×10^{-4} to 2×10^{-3} , 1.5×10^{-4} to 2.5×10^{-3} and 1.5×10^{-4} to 4.5×10^{-3} CFU/ml respectively.

Tray III with formaldehyde concentration 0.050% showed microbial growth from 0.5×10^{-4} to 1×10^{-3}, 1×10^{-4} to 3×$10^{-3}$and 1×10^{-4} to 4.5×10^{-3}CFU/ml at 2, 4 and 6 hours respectively. Tray IV with formaldehyde concentration 0.075% has microbial growth 0.5×10^{-4} to 1×10^{-3} , 0.5×10^{-4} to 1×10^{-3}, 0.5×10^{-4} to 1.5×10^{-3} CFU/ml at 2hours, 4 hours and 6 hours of treatment. Tray 5 with formaldehyde concentration 0.1% has microbial growth 0.5×10^{-4} to 0.5×10^{-3} , 0.5×10^{-4} to0.5×10^{-3} , 1×10^{-4} to 1.5×10^{-3}CFU/ml at 2 ours, 4 hours and 6 hours respectively.

Lowest microbial growth was observed in the tray V (1×10^{-4} to 1.5×10^{-3}) which was treated with 0.1% formaldehyde. Highest microbial growth was observed in tray I (26.5×10^{-4} to 119×10^{-3}) which was treated with 0.01% formaldehyde. In the control tray, microbial load was found to increase till 6th hour of treatment (Fig. 6).

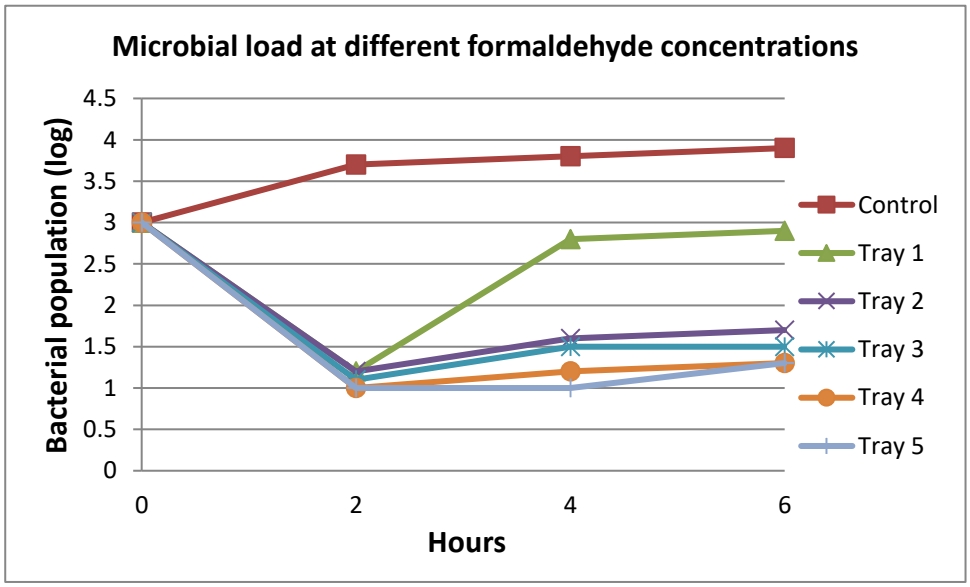

Fig. 6 . Microbial load in fishes treated with different concentrations of formalin

From the result it is clear that the microbial population in the control fish had increased in huge numbers compared to the initial bacterial growth. The tray I showed, declined in growth of microbes when compared to control but higher load when compared to trays 2, 3, 4 and 5. This may due to the lower concentration of formaldehyde in tray1. The microbial load in tray 2.3.4 and 5 decreased with increase in concentration of formaldehyde. Tray V showed a dramatic decrease in growth of microbes when compared to initial and control values.

From this experiment, it is clear that 0.1% of formaldehyde is found to be effective in minimizing the microbial load. Formaldehyde content had great restriction on microbial growth even in the sixth hour of experiment.

Correlation was performed between concentration of formalin in flesh and microbial load observed at different time intervals in each treatment. In control a perfect positive correlation was observed in between formalin concentration and microbial load (r = 0.9449). Perfect negative correlations were observed in all the trays with treatment of formalin on fish. The correlation coefficient values (r) observed were -0.80396, -0.93121, -0.82775, -0.78879 and -0.85859 in trays 1 (0.01% formalin), 2 (0.025% formalin), 3 (0.050% formalin), 4 (0.075% formalin) and 5 (0.1% formalin) respectively.

5. DISCUSSION

Fish is a high-value crop with increasing consumer demand. They are prone to rapid post-harvest spoilage because of their composition (Ismail, 2005). The freshness of this extremely perishable food item influences considerably its acceptability to the consumer (Sikorski *et al.* 1976; Shenouda 1980; Connel 1995). Inadequate preservation facilities and lengthy transport from distant places lead to considerable decreases in fish quality by the time the products reaches consumers (Haque and Mohsin, 2009). In this scenario, many fish traders use formalin to prevent spoilage and increase shelf life (Yeasmin *et al*., 2010).

Formaldehyde is an important cellular metabolite. In the bacterial world, formaldehyde is generated by methanotrophs and methylotrophs during the oxidation of short-chain hydrocarbons such as methane or methanol. Many ordinary foods such as fish naturally contain small amount of formaldehyde. which is been naturally produced in the body. However, excess formaldehyde has been reported in many fishes as adulterated by different channels during marketing (Niloy, 2015).

Formaldehyde is a toxic substance that can kill bacteria and viruses as well as cause damage to human cells. Food manufactures som times add Formaldehyde to foods such as fish, meat, milk to extend shelf life. Studies conducted at different wet markets in Dhaka city (Hossain *et al.,* 2008; Haque and Mohsin, 2009 and Yeasmin *et al*., 2010) rationalizes the incidence of adding formalin

The concentration of formaldehyde has been detected as result of post-mortem change in the tissues of various fish species such as cod (*Gadus macrocephalus*), Alaskan Pollock (*Theragrachaco gramma*), blue shrimp(*Penaeus stylirostris*) and pacific shrimp (*Pandalus jordani*) (Amano *et al*., 1963; Flores and Crawford, 1973; Hose and Lighter,1980). Endogenous

formaldehyde residues ranging from 0.1-31.8µg/g were detected in several species including *Anguilla japonica* (Ueno *et al*., 1984), striped bass (*Morone saxariles*) (Xu and Rogers, 1995) and Nile tilapia (*Tilapia nilotica*).

Noordina *et al.* (2011) found out that Bombay duck has significantly higher concentration of formaldehyde content collected from Kewatkhali market of Bangladesh. Hossain *et al*., 2008 examined the formaldehyde content in Rui fish *(Labeo rohita*) and found that imported fish contained more (13.40±0.52nmol/mg of fish protein) formaldehyde than fresh fish (3.95± 0.4nmol/mg of fish protein from local market. Noordian *et al*., 2011 reported formaldehyde content ranging from 0.38 to 15.75µg/g from 10 species collected from wet market in Malaysia.

Jung *et al*., 2001 revealed that TMAO is much more available in marine fish than in fresh water fish. Uzairu *et al*.,(2010) investigated formaldehyde levels in some manufactured regular foods and found that the values were all below the standard range of 0-0.8% .Sotelo *et al*., (1995) found that formaldehyde accumulated during frozen storage ,reacted with protein and subsequently caused protein denaturation of the muscle.

Comparing the above reports, content of formaldehyde was found to be lower in various fish species in the present study. In the present study, mean concentration of formaldehyde was highest in *R. kanagurta* was 1.99 µg/ g which was in the accepted range set by USEPA. However, samples of *R. kanagurta* and *N. japonicus* exceeding this limit were obtained during the analysis. The mean formaldehyde concentration among shellfishes was highest in *P.stylifera* (0.2267±0.0418 µg/g) followed by *P.monodon* (0.1858±0.0259µg/g) and *F.indicus* (0.1650±0.0644µg/g). The mean formaldehyde concentration was highest in clam which is 1.8190±0.3628µg/g. The mean formaldehyde concentration of both prawns and clams were in the accepted formaldehyde range set by USEPA. Thus the prawns and clams collected from the market can be considered safe for consumption because of the low formaldehyde content. Among frozen fishes, highest formaldehyde concentration was recorded in *Mene maculate (0.928) followed* by *followed* by *Leiognathus equulus, Mugil cephalus, Sphyraena obtusata, Dussumieria elopsoides Anodontostoma chacunda, Rastrelliger kanagurta.*

According to Yasuhara and Shibamoto (1995), fishes are not considered palatable if the formaldehyde level is 10-20mg/kg. An accepted daily intake of 0.2mg/kg body weight has been set by the United States Environment Protection Agency Xuang *et al*.(2009). Formaldehyde is a normal cellular metabolite and levels in mammalian cells range from 1.5 to 15 µg/g (Feinman, 1988)

Siskorski *et al., 1982* reveals that formaldehyde produced naturally in th fish muscle by either bacteria or enzyme reaction became covalently bonded for a cross- linkage among peptide chains. Tunhun *et al.,* 1982 reported that there was no adverse health effects on humans due to the formaldehyde contaminated fish consumption based on the risk of calculation. According to them the fish from market can be considered safe for consumption because of low formaldehyde content. Furthermore, frying and boiling the fish can potentially reduce the formaldehyde content.

In the present study, it was found that mean formaldehyde content in fresh specimen of *Oreochromis mossambicus* was 0.971 µg /g. It is clear that after the death of fish formaldehyde content slightly as evident in the control tank. This can be due to the production of formaldehyde from TMAO. However, when compared to above values it is clear that market fish contain high amount formaldehyde (1.4 and 7.35 µg/g.) and the concentration will be higher than 0.1 %. This may due to the addition or may due the normal development of formaldehyde in fishes. But when compared to the normal value (0.971 µg /g to 1.032 µg/g) of formaldehyde from the control fishes, it is clear that there occur illegal addition of formaldehyde. Present study indicate that fish treated with 0.1 % of formaldehyde absorb only 2.730 µg/g and when time pass this concentration shows decreasing trend. Mezbah *et al*. (2014) conducted a work similar to the present work and in their study they aimed to detect and quantify formaldehyde in fresh and formaldehyde treated fish samples. Rohu fish (Labeo rohuta) was used to conduct the experimental study. Formaldehyde solutions of different concentrations were applied on the fish samples. The residue formaldehyde in formaldehyde treated fish samples were quantified with time. Naturally produced formaldehyde in the untreated fish samples was quantified at different time intervals. The shelf lives of the fish samples, with or without formaldehyde treatment, were also investigated in this study.

The present study showed that when fishes were treated with formaldehyde, their assimilation into muscles was found to vary with the treated concentrations. In the control there was a slight increase in formaldehyde concentration till the sixth hour of treatment. In trays 1 to 5, formaldehyde content in fish muscles increased till 2^{nd} hour and then showed a decline until 6^{th} hour. In a similar study, small amounts of naturally occurring formaldehyde was detected and quantified in fresh fish samples, the concentration of naturally occurring formaldehyde increased slowly in fresh fish sample with respect to time. The concentrations of residual formaldehyde in formaldehyde treated fish samples were found to be very low (< 4 ppm) compared to that of the treating solutions concentrations (400 – 800 ppm). The results indicate that only a fraction of the applied formaldehyde was adsorbed by fish protein.

However, the formalin treated fish samples demonstrated longer shelf life compared to that of the non-adulterated fish samples. The antibacterial/antifungal nature of the formaldehyde solution and the existing residual formaldehyde in the formaldehyde treated fish samples may cause the longer shelf life. When comparing above results with the present study it clear that why the formaldehyde concentration decrease in each tray when time passes and this is because only a fraction of the applied formaldehyde was adsorbed by fish protein. Study of microbial load revealed that lowest microbial growth was observed in the tray V (1×10^{-4} to 1.5×10^{-3}) which was treated with 0.1% formaldehyde. Highest microbial growth was observed in tray I (26.5×10^{-4} to 119×10^{-3}) which was treated with 0.01% formaldehyde. In the control tray, microbial load was found to increase till 6^{th} hour of treatment.

Formaldehyde penetrates cells quickly but its reaction with protein starts slowly (Rooban., 2012). This may be the reason of gradual decrease of formaldehyde content in fish samples. Besides, the residual formaldehyde may hinder the growth of microorganisms on the fish samples. The concentration of residual formaldehyde existing in formaldehyde treated fish flesh, may also contribute to protein fixation. Moreover, formaldehyde acts as an antibacterial and antifungal agent; therefore, treating fish samples with formaldehyde solutions may denature the microorganism on the fish skin or in fish fillet, and delay the spoilage of formalin treated fish samples (Mezbah *et al* 2014).

6. CONCLUSION

Fishes are considered as the cheapest source of animal protein and it has been an important component in the diet of populations from under developed and developing countries. Fish is a highly perishable commodity. Freshness is a property of fish that has considerable influence on its quality. Loss of freshness followed by spoilage is the result of a complex combination of microbiology, chemical and physical process.

Spoilage begins as soon as the fish dies. Spoilage is the result of a series of complicated changes that take place in the dead fish mainly by the enzyme , bacteria and by chemical oxidation. It is not possible to keep the fish fresh for prolonged periods, but it is possible to produce a product which closely resembles to fresh by using freezing and cold storage. Recently, many chemicals including formaldehyde is being added illegally but for long term preservation it is not illegal such as in labs, museum formaldehyde is used as an effective preservative. But consumption of formaldehyde contained fishes can cause potential health hazards.

Market samples including fresh fin fishes and shell fishes and products like frozen and canned fishes were found to contain considerable amount of formaldehyde. Mean

formaldehyde content in the fresh *O. mossambicus* was found to be 0.97 ±0.014 µg/g. Formaldehyde content in fishes in control (tray without formaldehyde) was found to vary from 0.971 µg /g to 1.048 µg/g. At two hours, 4hrs and 6hrs the mean formaldehyde content was 1.071, 1.048 and 1.032 µg/g respectively. Highest formaldehyde content was recorded in the tray with formalin content of 0.1%. Mean concentration of formaldehyde in muscles of fishes was found to vary from 0.971 to 2.730 µg/g. At 2hours 4hrs and 6hours, mean formaldehyde content was 2.174, 2.730 and 1.588 µg/g respectively.

When fishes were treated with formaldehyde, their assimilation into muscles was found to vary with the treated concentrations. In the control there was a slight increase in formaldehyde concentration till the sixth hour of treatment. This is attributed to the formation of formaldehyde in fish tissue from TMAO. In trays 1 to 5, formaldehyde content in fish muscles increased till 2^{nd} hour and then showed a decline until 6^{th} hour. However, in tray 5, formaldehyde content in fishes increased until 4^{th} hour and later showed a decline at 6^{th} hour. At all formalin treated trays, concentration of formaldehyde in fish muscle was highest at the 2^{nd} hour except in tray 5 where maximum concentration was recorded at 4^{th} hour of treatment. Decrease in the formaldehyde concentration in muscles of treated fish might be due to the adsorption of formaldehyde by fish protein.

Assessment of microbial load indicated that the tray without formaldehyde showed the maximum microbial growth in *Oreochromis mossambicus* (195.5×10^{-4} to 258×10^{-3} , 263×10^{-4} to 305×10^{-3}, 225.5×10^{-4} to 295×10^{-3} CFU/ml at 2hours, 4hours and 6 hours respectively. Microbial load in samples at the beginning of the experiment in fresh fishes ranged from 189×10^{-4} to 255×10^{-3} CFU/ml. Lowest microbial growth was observed in the tray V (1×10^{-4} to 1.5×10^{-3} CFU/ml) which was treated with 0.1% formaldehyde. Highest microbial growth was observed in tray I (26.5×10^{-4} to 119×10^{-3}CFU/ml) which was treated with 0.01% formaldehyde. In the control tray, microbial load was found to increase till 6^{th} hour of treatment. Correlation was performed between concentration of formalin in flesh and microbial load observed at different time intervals in each treatment. In control a perfect positive correlation was observed in between formalin concentration and microbial load (r = 0.9449). Perfect negative correlations were observed in all the trays with treatment of formalin on fish. The correlation coefficient values (r) observed were -0.80396, -0.93121, -0.82775, -0.78879 and -0.85859 in trays 1 (0.01% formalin), 2 (0.025% formalin), 3 (0.050% formalin), 4 (0.075% formalin) and 5 (0.1% formalin) respectively.

The study provides an important information about the presence of formaldehyde content in market samples and the influence of formaldehyde on the microbial load of Tilapia and also the concentration of formaldehyde in the body to reduce microbial growth. A very small

quantity of 0.1% is sufficient to cause a decrease in the activity of microbes and thereby improve the shelf life. Thus concentrations at this level remain unnoticed by the consumers when fishes are illegally treated with formaldehyde by the vendors.

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