Thesis On

Analysis of formalin content in marketed fish sample and development of a suitable removal technique

(This report presented in partial fulfillment of the requirements for the degree of Master of Pharmacy)

Submitted By:

ID: 163-46-136

Department of Pharmacy

Faculty of Allied Health Sciences

DAFFODIL INTERNATIONAL UNIVERSITY

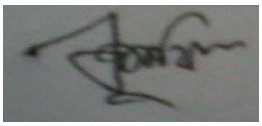
DHAKA, BANGLADESH

April 2018

DECLARATION

We hereby declare that, this project report is done by me under the supervision of **Md. Mustafezur Rahman**, Head of the department, Department of Pharmacy, Daffodil International University, impartial fulfillment of the requirements for the degree of Master of Pharmacy. I declaring that this Thesis is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for the award of Master or any degree.

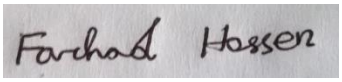
Supervised By:



_____

Md. Mustafezur Rahman
Head of the department
Department of Pharmacy
Faculty of Allied Health Science
Daffodil International University

Submitted By:



_____

Farhad Hossen
ID: 163-46-136
3th batch
Sec-A
Department of Pharmacy
Daffodil International University

ACKNOWLEDGEMENT

At the very beginning, we would like to express our sincere gratitude to Almighty, who has given us the chance to complete our project report in a very comfortable manner by keeping us sound and healthy all over the thesis working period.

We would like to express our deepest appreciation to all those who provided us the possibility to complete this project work. Whatever we have done is only due to such guidance and assistance and we would not forget to thank them. A special gratitude we give to our teacher & guide Md. Mustafezur Rahman, Head of the department, Pharmacy Department, Daffodil International University, whose contribution in stimulating suggestions and encouragement, helped me to coordinate my project work successfully.

We are thankful to and fortunate enough to get constant encouragement, support and guidance from all Teachers of Department of pharmacy, which helped us in successfully completing our project work.

We thank and appreciations also go to our colleague in developing the project and people who have willingly helped me out with their abilities.

We also thank the laboratory assistance Mr. Younus to help us all over the project work, without his help it could be difficult for us to complete our project work.

Lastly, we thank, our parents and friends for their constant encouragement without which this project would not be possible.

DEDICATION

DEDICATED

TO MY PARENTS

AND

ALMIGHTY ALLAH

ABSTRACT

Fish is an important food stuff and source of protein all over the world. In Bangladesh fisheries sector contributes a lot in case of earning foreign currency and meeting domestic need of animal protein. There has been a lot of controversy around the various poisons finding their way into our food. Use of formaldehyde on marketed fish is a very common practice in Bangladesh to preserve fish. Formaldehyde has serious harmful effect on public health including cancer, kidney failure and others diseases. The study was performed to determine the presence of formaldehyde on marketed fish as well as develop a removal technique of the formaldehyde from fish. The use of the Nash test, in conjunction with TCA extraction, for measuring formaldehyde in fish muscle is one of the most qualitative methods. This was done by means of a "recovery factor" which took into consideration the percent of formaldehyde added to the muscle extracted by the TCA solution. The percentage of added formaldehyde that was recovered varied with variations in the procedures used in preparing the muscle, making the extract, and carrying out the Nash test. In this study I used formalin kit to detect the presence of formaldehyde in different fish species and samples of each species those were collected from different places. I found that Bata, Tilapia, Rui fishes contain formaldehyde ranging from (5.26 to 1.88) mg per 10gm sample. By using only vinegar, we can remove 60 to 99 percent of formaldehyde content from some species as well as by using vinegar and heat we can remove 98 to 99 percent of formaldehyde content from other species of fish. In this study we also performed control test to check the amount of formaldehyde that produced naturally in the preserved fish. In case of Shorputi and Rui the amount was 0.098 and 0.084 mg per 10 gm sample. The present study suggested that fish from wet market contained a certain amount of added formaldehyde and fishes from both freshwater and marine sources shows to contain natural occurring formaldehyde in their muscle at different concentration. The present study also suggested that major portion of the formaldehyde in fish muscle can be removed through vinegar and heat. This research is very beneficial for the society in terms of public health and food safety.

TABLE OF CONTENT

List of Table

List of Figure

Chapter One

Introduction

1. Introduction:

Fish and seafood are an important part of a healthy diet and are considered as the biggest source of protein. Bangladesh is considered one of the most suitable regions for fisheries in the world, with the world's largest flooded wetland and the third largest aquatic biodiversity in Asia after China and India.

1.1 Fishes and current wet market status in Bangladesh

Being a riverine country, a lot of fishes are captured and also significant amount of fishes are produces through aquaculture to meet the food and nutritional needs of growing population every year. At present national average fish consumption is about 37 g/capita/day (13 kg year^{-1}) (DoF, 2003).

Fish is the main element of daily food chart for all Bangladeshi people. According to nutrition scientist an adult people should consume about 45.3 g proteins daily. Among them 15.1g will be animal protein. And for Bangladeshi people 80 of animal protein comes from fishes. So, fish has a great importance as nutritious food.

Bangladesh has a vast fisheries resource comprising of fresh, brackish and marine water. Those are inhabited by 296 fresh and brackish water species, 511 marine species, 14 exotic species of fish and 24 species of prawn. It is claimed that the total fish production has increased significantly over the last few decades but it is not sufficient to meet the growing demand of the country. So to meet the growing demand of the people, it is reported that more than 80 MT of fish and fishery products enter into Bangladesh every day through the Teknaf border from Myanma.

Bangladesh is a developing country. About 90% of animal protein in our diet comes from fish and livestock. Fish and fishery products are the country's third largest export commodity contributing 10% of its exchange earnings. In 2002-2003 Bangladesh earned US $324 million of which shrimp alone contributed 72% of the total by quantity and 89% by value.

By composition, fish contains fat free amino acids and water which is susceptible to spoilage by microorganisms and biochemical reactions during post mortem process. 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.

A survey conducted by Department of Fisheries, Dhaka University (Chowdhury *et al.* 2007) showed that initial selling price of imported Burmese rohu fish was the lowest (30 Tk/kg) and endpoint selling price (120 Tk/kg) referring the price increase of 90 Tk/kg while the other two types of rohu fishes had price increase of 105~110 Tk/kg for cultured rohuand 100~110 for captured rohu. The marketing of Burmese rohuinvolved less cost in icing, packaging and handling with the highest net income for the

fish traders i.e. paiker, aratdar and retailer while those cost was higher and net income was less in marketing of captured rohu. Aesthetics, freshness and cost of production were the factors behind the increased price of cultured and captured rohu fishes. In the
assembling and wholesale marketing process, the cost of icing, packaging and handling was less for Burmese rohu as they were found to be treated with formalin and other unknown type of preservatives and did not show signed of spoilage even with less ice and improper packaging and rough handling.
Neither the Department of Fisheries (DoF) nor the Bangladesh Standards and Testing Institution (BSTI) have any policy for import of fish. Although DoF strictly controls the quality of the fish and fish products for export under Fish and Fish Product Inspection and Quality Control Act'97, there is no provision or instruction or regulation to inspect or test the quality of imported fishes. The BSTI Ordinance 1985 (amended into an Act in 2003) gives instructions to test standards of 145 food products but fish and fish products are not included in the list. Thus, BSTI depend on the City Corporation Food Ordinance 1959, Pure Food Rules 1967 and DCC Ordinance 1985 (amended in 2005) during their recent drives in the fish markets for formalin treated fish.
Many different procedures have been proposed to determine formaldehyde in recent years. The Bangladesh Council of Scientific & Industrial Research (BCSIR) first develop a simple kit to detect presence of formalin in fish. The Department of Fisheries (DoF) imported a formaldehyde Meter (Z-300) which is basically to detect formaldehyde from air and application of this meter in fish has arisen ambiguity some. However, analysis of formaldehyde in aquatic products based on spectrophotometer is reported greatly as a simple, reliable and convenient method to determination of formaldehyde in water, food and environment. There is a possibility that fishermen may apply formalin in marine and freshwater fishes to prolong the freshness of the fish. However, the amount of formalin added into the fish may be low enough that it cannot be detected by existing method. Response surface methodology provides a scientific value for obtaining the optimum condition of retrieving the most formaldehyde from some marine and freshwater fishes. The spectrophotometric method using Nash's reagent and AOAC(**Association of Official Agricultural Chemists)** method (931.08) may be considered a reliable procedure to determine and to quantify the formaldehyde content in freshwater and marine fishes. Freshness is a property of fish that has a considerable influence on its quality (Connell, 1995). Now a day's consumer is becoming more conscious of the application of formaldehyde in fish and also its side effects. Therefore, it is important to determinate the formaldehyde content in the fish since it is claimed to be the major contaminant in some marine and freshwater fishes.

1.2 Formalin/Formaldehyde

Formalin is a clear, colorless, aqueous solution of 37 to 40 percent formaldehyde. The formula H-CHO and the boiling point of formalin is 91ºC to 101ºC. Formaldehyde is the simplest member of aldehyde family but a very reactive chemical, where the gaseous form is known as formaldehyde and the liquid form as formalin. Characteristically, formaldehyde is a colorless, strong-smelling, irritating, poisonous, and flammable gas and its chemical formula is CH_2OH which is also known as methanal, commonly produced by the oxidation of methanol. Formaldehyde is used as disinfectant and preservative.

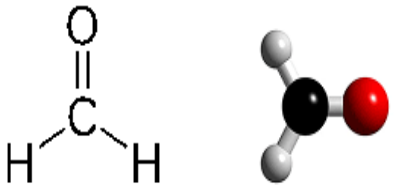


Figure 1.1: Structure of formaldehyde

The WHO derived a drinking-water guideline value for formaldehyde of 900 µg/litre based on a tolerable daily intake (TDI) value of 0.15 mg/kg bw/day calculated from a NOAEL of 15 mg/kg bw/day in a 2-year study in rats causing stomach irritation and papillary hyperplasia associated with severe irritation (WHO, 1993). An allocation of 20% of the TDI to drinking water was used to derive the guideline value. This guideline value was confirmed in a more recent evaluation (WHO, 2004).

International Agency for Research on Cancer (IARC) has classified formaldehyde as a Group 1 carcinogenic to humans (2004). According to the United States Environmental Protection Agency (EPA), maximum daily dose reference (RfD) for formaldehyde is 0.2 µg/g body weight per day (Wang et al., 2007). In 1985, Italian Ministry of Health has proposed formaldehyde values of 60 µg/g and 10 µg/g for *Gadidae* and crustaceans, respectively (Bianchi et al., 2007).

As formaldehyde is carcinogenic to human, it is important to investigate the content of formaldehyde in fish and seafood since they are claimed to be the major source of protein, and therefore providing more information to the production of safe and hygienic food. According to Malaysian Food Regulations 1985, Regulation 148 and 159 (2006), only smoked fish and meat are permitted to incidentally absorb formaldehyde during processing in a proportion not exceeding 5 µg/g.

However, for fresh fish, the permitted amount of formaldehyde present in fish is not specified. Formaldehyde may be formed during the ageing and deterioration of fish flesh. Besides natural formation of formaldehyde in fish and seafood by enzymatic reaction, other biochemical reactions can also occur such as oxidation of lipids as a result of microorganism activities. This will eventually result in physical damage of fish or production of chemical metabolites such as biogenic amines or other unpleasant compounds (Gram et al., 2002; Arashisar et al., 2004). Changes in pH, microbial numbers, and free amino acids have been proposed and/or used as indices of the freshness of iced aquatic species (Fatima and Qadri, 1985).

1.2.1 Harmful effect of formaldehyde

Some studies suggest that large formaldehyde exposures, for example from drinking formaldehyde solutions, are potentially lethal. Formaldehyde is converted to formic acid in the body, leading to a rise in blood acidity, slow breathing, hypothermia, and coma or death. People who have ingested formaldehyde require immediate medical attention. In the body, formaldehyde can cause proteins to irreversibly bind to DNA. Formaldehyde is listed as a probable human carcinogen. It is, therefore, important to make a detailed survey on the use of formalin in commercially important fishes.

Ingestion of as little as 30 mL (1 oz.) of formalin has been reported to cause death in an adult human being. Ingestion may cause corrosive injury to the gastrointestinal mucosa, with nausea, vomiting, pain, bleeding, and perforation. Corrosive injuries are usually most pronounced in the pharyngeal mucosa, epiglottis and esophagus. Systemic effects include metabolic acidosis, CNS depression and coma, respiratory distress, renal failure and associated cancer and tumor development.

Use of formalin in food is banned in Bangladesh; however, formalin contamination is reported to occur in table fish marketed in the country

1.2.2 Exposure of formaldehyde

Formaldehyde is a product of normal metabolism and is essential for the biosynthesis of certain amino acids in humans. The endogenous tissue levels of metabolically produced formaldehyde range from approximately 3 to 12 ng/g of tissue

Formaldehyde may be present in foods naturally or as a result of contamination. Concentrations of formaldehyde ranging from 3 to 26 mg/kg have been reported in a variety of food materials. Some

food additives, such as hexamethylenetetramine, have been reported to decompose gradually to formaldehyde in the presence of proteins or under acidic conditions. Daily intake of formaldehyde from food is difficult to evaluate; however, the World Health Organization (WHO) estimated it to be in the range of 1.5–14 mg/day (mean 7.75 mg/day) for an average adult estimated it to be 11 mg/day based on a North American diet.

The general population is exposed to formaldehyde mainly by inhalation. It has been estimated that individual smoking 20 cigarettes per day would receive from 0.38 to 1.0 mg/day by this route. Formaldehyde is released into the air from resin glues and plastic materials, and low air levels (parts per billion) may result from the photo-oxidation of fossil fuel-derived hydrocarbons. The overall daily inhalation exposure for an average adult has been approximated as 0.3– 2.1 mg (average 1 mg), with exposures as high as 5 mg/day. Assuming a contribution of approximately 9.4 mg/day from food, 1 mg/day from inhalation and 0.15 mg/day from water (worst-case scenario- 100 µg/L) an adult would receive 10.55 mg of formaldehyde per day.

1.2.3 Formalin exposure routes and possible effects on humans

- **Ingestion (swallowing):** Ingestion of pure formalin (10-40% formalin) could cause severe irritation and inflammation of the mouth, throat, and stomach, severe stomach pain will follow and possible loss of consciousness and death; Ingestion of dilute formalin will cause discomfort in the stomach and mouth
- **Inhalation (breathing):** Concentration of 0.5-2.0 mg/L may irritate the eyes, nose and throat. Concentrations of 3-5 mg/L may cause tearing of the eyes. Concentrations of 10-20 mg/L could cause difficulty in breathing, burning of the nose and throat, cough. Concentrations of 25-30 mg/L may cause severe respiratory tract injury. Concentrations of 100 mg/L is dangerous to life and health.
- **Skin (dermal):** Prolonged and repeated contact with formalin could cause numbness (lack of feeling) and a hardening or tanning of the skin.
- **Eye contact**: Formalin solution splashed in the eye can cause injuries from transient discomfort to severe such as loss of vision.
- **Carcinogenicity:** Formalin has the potential to cause cancer, repeated and prolonged exposure increases the risk of cancers of the lung, nasopharynx, oropharynx and nasal passage.

1.2.4 Current scenario of Bangladesh

Formalin use in foods is a crucial problem in Bangladesh currently. Local stores and supermarkets often sell fruits, fishes, and vegetables that have been treated with formalin to keep them fresh. However, in 2015, a Formalin Control Bill was passed in the Parliament of Bangladesh with a provision of life-term imprisonment as the maximum punishment and in addition 2,000,000 BDT as fine but not less than 500,000 BDT for importing, production or hoarding of formalin without license.

1.3 Vinegar/Acetic acid

Vinegar is a liquid consisting of about 5–20% acetic acid (CH3COOH), water, and other trace chemicals, which may include flavorings. The acetic acid is produced by the fermentation of ethanol by acetic acid bacteria. Vinegar is now mainly used as a cooking ingredient, or in pickling. As the most easily manufactured mild acid, it has historically had a great variety of industrial, medical, and domestic uses, some of which (such as its use as a general household cleaner) are still commonly practiced today.

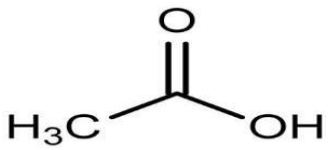


Figure 1.2: Structure of Vinegar/ Acetic acid

In our experiment we use vinegar or acetic acid to extract formaldehyde from muscle of the fish that was collected from different fish market of Dhaka city.

1.4 Nash test

The use of the Nash test in conjunction with TCA extraction, for measuring formaldehyde in fish muscle was made more quantitative and a very sensitive test that can detect small amount of formaldehyde content in the experimental sample. A procedure for the estimation of formaldehyde in biological materials was described by Nash in 1953. It is Based on the formation of diacetyldihydrolutidine from acetylacetone and formaldehyde in the presence of excess ammonium salt. The Nash method is simply a colorimetric method for measuring free or loosely bound formaldehyde.

1.5 Formalin extraction from fish muscle

Formaldehyde, however react readily and in some cases, almost irreversibly, with protein, amines, amino acids, and many other organic compounds. In biological materials, much of this bound formaldehyde is not available to react with the Nash reagent; therefore, this test is not a satisfactory measure of the total amount of formaldehyde that is preset in animal tissue unless it is used in conjugation with some method of extraction that release the more firmly bound formaldehyde. That's why we use a strong acid TCA (trichloro acetic acid) for the extraction of the formaldehyde and then detect the total amount of formaldehyde using the Nash reagents.

1.6 Aim of the experiment

- The main aim of the experiment is to determine the present formalin adulteration condition of the fish market of Dhaka city.
- Quantitatively determine the formaldehyde content in each types of fish sample.
- To determine the amount of formaldehyde content that can be remove by heating method.
- To determine the amount of formaldehyde content that can be remove by vinegar and heat.

Chapter Two

Literature Review

2. Literature Review

2.1 Title: Determination of formaldehyde content by spectrophotometric method in some fresh water and marine fishes of Bangladesh.

Author(s): Niloy Jaman, Md. Sazedul Hoque, Subhash Chandra Chakraborty, Md. Enamul Hoq, Hari Pada Seal

Abstract: The present study was conducted for quantitative analysis of formaldehyde presence in some important freshwater and marine fish species by spectrophotometric method using Nash reagent in conjunction with TCA extraction. The freshwater fish rohu, Labeo rohita ; tilapia, Oreochromis nilotica; Thai koi, Anabas testudineus; kachki, Corica soborna; and marine fish loyitta, Harpodon nehereus; chhuri, Lepturacanthus savala from local markets and from freshly caught samples were evaluated for determination of formaldehyde concentration. Formaldehyde concentration obtained in fishes from three different wet markets of Mymensingh mechhua bazar was ranged between 1.4 and 7.35 µg/g. On the other hand, formaldehyde concentration in freshly caught fishes rohu, tilapia and Thai koi collected from ponds of Freshwater Station, Bangladesh Fisheries Research Institute (BFRI), Mymensingh showed natural formaldehyde in their muscle having values of 1.45; 1.85 and 2.60 µg/g, respectively. The marine fish viz. loyitta and chhuri collected from the landing center of BFDC at Cox's Bazar and investigation in frozen, thawed condition showed to contain naturally occurring formaldehyde as 3.9 and 1.55 µg/g, respectively. Spectrophotometrically determination of formaldehyde concentration showed highest value of 7.35 µg/g in market sample of kachki, and naturally occurring formaldehyde concentration showed higher value of 2.6 µg/g in Thai koi from freshwater and 3.9 µg/g in loyitta fish from marine source. The present study suggested that fish from wet market contained a certain amount of added formaldehyde and fishes from both freshwater and marine sources shows to contain natural occurring formaldehyde in their muscle at different concentration.

2.2 Title: A Comparative Study of Present Status of Marketing of Formalin Treated Fishes in Six Districts of Bangladesh.

Author(s): Rafiad Islam, Shahin Mahmud, Abdul Aziz, Animesh Sarker, Marufa Nasreen.

Abstract: The fish is an important food stuff and source of protein all over the world. In Bangladesh, fisheries sector contributes a lot, in the case of the earning foreign currency and meeting domestic need of the animal proteins. To fulfill the domestic need of protein and fish, Bangladesh imports fish and fish products from the neighboring county. In many studies, it was proved that most of the imported fishes are contaminated with formalin, which is the highly hazardous and carcinogenic chemical. Information was collected from the fish retailers and consumers on the marketing of the formalin treated fishes through survey using prescribed questionnaire at 18 different fish markets in 6 different districts of Bangladesh. It was found that most of the commercially imported fishes are highly contaminated with formalin. On the other hand, local big fishes, such as rui (*Lobeo Rohita*), catla (*Catla catla*) and mrigal (*Cirrhinus cirrhosus*) etc., are also formalin contaminated partially, but not all the fishes. All the local small fishes are free from the formalin contamination. In this study, it was found that, among the 939 fish samples collected from the different fish markets of the six districts, 213 fishes (22.68%) were directly contaminated with formalin. The contamination rate is so much higher in the big city like Dhaka (36.78%) and lower in the small town like Jamalpur districts (13.33%). This study also indicated that, all the village markets were totally free from the formalin contamination. As the fish traders used formalin to increase the shelf life of the fishes, it was also observed that, the shelf life of the local fishes or formalin free fishes was much higher and the organoleptic characteristics were much more excellent than the formalin contaminated local or imported fishes. The price of the imported fishes was also lower than the local fishes. It was also clear that, the organoleptic characteristics of the imported fishes and formalin contaminated fishes were greatly different than the local fishes, which were not satisfactory to the consumers. The study also indicated that, the overall hygienic practice and sanitary conditions of the markets and the fish traders/retailers were very poor, not satisfactory. This survey also revealed that, all the traders or retailers who were mixed formalin with the fishes, knew about the bad effects of the formalin.

2.3 Title: Measurement of Formaldehyde in Fish Muscle Using TCA Extraction and the Nash Reagent

Author(s): C. H. Castell, Barbara Smith.

Abstract: The use of the Nash test, in conjunction with TCA extraction, for measuring formaldehyde in fish muscle was made more quantitative. This was done by means of a "recovery factor" which took into consideration the percent of formaldehyde added to the muscle extracted by the TCA solution. The average recovery from 15 different samples of cod muscle was 51.3% with an SD of 5.6. Because of differences between species in capacity to bind formaldehyde, it would appear that a different "recovery factor" may be required for each species of fish. As heating muscle increases its ability to bind formaldehyde, recovery factors developed for use with raw muscle are not applicable to the same muscle after it has been cooked. The percentage of added formaldehyde that was recovered varied with variations in the procedures used in preparing the muscle, making the extract, and carrying out the Nash test.

Chapter Three

Experimental Design

3. Experimental Design

3.1 Collection of Sample

Fish samples were collected from different markets such as Sukrabad bazaar, Subhanbag kancha bazaar, west Raja bazaar, Badda kacha bazar and Mirpur kacha bazar. In various types of fishes we found formaldehyde contamination. These fishes were carried out to the laboratory for determine and removal of formaldehyde from these sample and these are namely given below:

- Tilapia (*Oreochromis nilotica),*
- Shorputi (*Puntius sarana*),
- Taki (*Channa punctata*),
- Kachki (*Corcica suborna*),
- Rui (*Labeo rohita*),
- Ilish (*Tenualosa ilisha),*
- Mola (*Amblypharyngodon microlepis)*
- Bata (*Labeo bata*)
- Mrigol (*Cirrhinus cirrhosis*)
- Taki (*Channa punctate*)
- Puiya (*Lepidocephalichthys berdmorei*)

Figure 2.1: Mrigol

Figure 2.2: Rui

Figure 2.3: Shorputi

Figure 2.4: Telapia

Figure 2.5: Mola

Figure 2.6: Kachhki

Figure 2.7: Bata

Figure 2.8: Tanki

Figure 2.9: Ilish

Figure 2.10: Puiya

Figure 2: Various fish sample

3.2 Materials use for this project work are

3.2.1 Instrumental material

- UV-Visible spectrophotometer
- SS-tray
- Oven
- Knife
- Beaker
- Test-tube
- Measuring cylinder
- Volumetric flask
- Pipette
- Analytical balance
- pH meter
- Thermometer

The experiment was conducted in the laboratory of department of Pharmacy, Daffodil International University.

Figure 3.1: UV-Visible spectrophotometer

Figure 3.2: Oven

Figure 3.3: Analytical balance

Figure 3.4: Analytical thermometer

Figure 3.5: pH meter

Figure 3: Various instrument used for the experiment

3.2.2 Chemical reagents

- Trichloro acetic acid (TCA)
- Potassium hydroxide
- Hydrochloric acid
- Ammonium acetate
- Acetic acid
- 2,4-pentanedione
- Distilled water

Figure 4.1: Chemical reagent and equipment used

3.2.2.1 Chemical reagent preparation

- **TCA (6%):** 6 percent Trichloro acetic acid were prepared by measuring 6 gm of TCA crystal and dissolve in up to 100 ml distilled water.
- **KOH**: Potassium hydroxide (0.1 N) is prepared by measuring 0.562 gm of potassium hydroxide pellets and dissolve in up to 100 ml distilled water
- **HCl:** Hydrochloric acid is prepared by diluting 12N of HCL using distilled water to prepare 0.1N HCl solution.
- **Nash reagent:** Nash reagent is prepared by dissolving 15 gm of ammonium acetate in distilled water, then add 0.2 ml of 2,4-pentanedione and 0.3 ml of acetic acid and make up to 100 ml using distilled water. The Nash reagent is light sensitive that's why it is should keep in amber glass bottle. The Nash reagent activity is deteriorated gradually after 2 to 3 days of preparation. So, it should be use for 2 to 3 days.

Figure 4.2: Prepared chemical reagents

3.2.2.2 Trichloro acetic acid (TCA) activity

Formaldehyde react readily and, in some cases, almost irreversibly, with protein, amines, amino acids, and many other organic compounds. So, of this bound formaldehyde is not available to react with the Nash reagent. That why for the extraction of formaldehyde we use an strong acid TCA that break down the fish tissue and make the bound formaldehyde to release in the solution. Then we can determine the total amount of formaldehyde in the solution with the help of Nash reagent So, we can say that TCA have a great role in determining the content of formaldehyde.

3.2.2.3 Nash reagent activity

When the Nash reagent is added in the experimental solution that contain formaldehyde then Hantzsch reaction between acetylacetone, ammonia and formaldehyde proceeded so readily at pH 6 that it afforded a good method of estimating microgram amounts of the formaldehyde. The yellow color due to the formation of diacetyl-dihydro-lutidine ($C_{11}H_{15}NO_2$) is fully developed in two hours at room temperature.

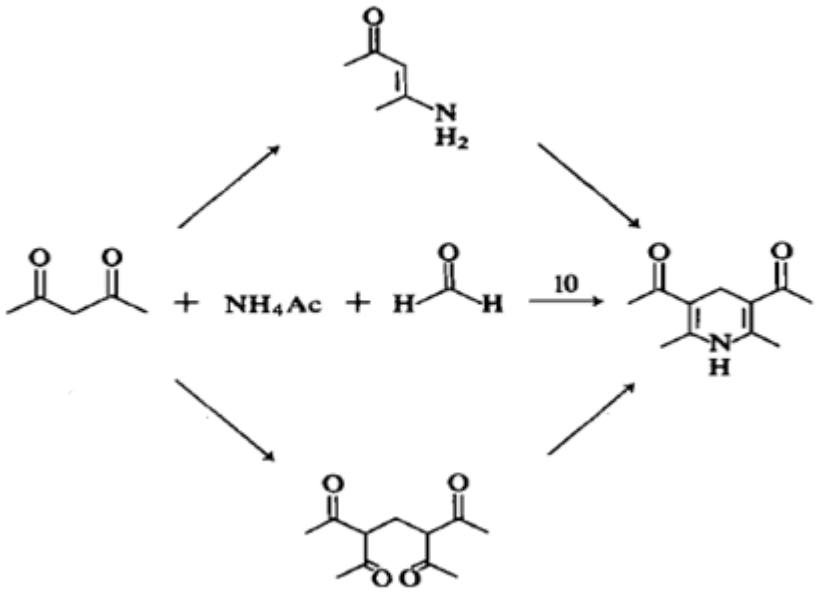


Figure 5: Reaction of Nash reagent with formaldehyde

3.3 Qualitative detection of formalin using Formalin Detection Kit for Fish

Qualitative detection of formalin was performed by the formalin detection kit for fish developed by Bangladesh Council of Scientific and Industrial Research (BCSIR). This kit contains three different solutions labeled as solution 1, 2 and 3.

First, the samples (cut fish parts) were washed with small quantity of water and a portion of washed out and 2ml water was taken in a test tube.

Using a dropper incorporated in the kit. 15 drops of solution 1 was added in the test-tube containing washed out water. After well stirring, the solution was allowed react for 30 second. 15 drops of solution 2 was added in the same test-tube. After waiting for 30 seconds, solution 3 was added, change in color pink or red indicated the presence of formalin whereas unchanged color indicated the sample is free from formalin.

6

Figure 6: Formalin kit

3.4. Standard curve establishment

A standard curve is prepared for the detection of formaldehyde in the experimental sample. For preparing standard curve:

- First took 1 ml of 37% formaldehyde solution in a 100-ml volumetric flask (flask-1) and make up to 100 ml using distilled water.
- Then from the flask (flask-1) again take 1 ml formaldehyde solution in another 100-ml volumetric flask and make up to 100 ml using distilled water and this is our stock solution.
- Now by diluting from the stock solution following concentration solutions: 0.370, 0.463, 0.555, 0.648, 0.740, 0.925, 1.110, 1.295, 1.388, 1.480 µg/ml are made.
- Now pipette 5ml of solution from each diluting solution in separate 50-ml volumetric flask
- Then add 2ml of NASH reagent in each volumetric flask
- Then heat in a water bath for 10 min at $60^{O}C$ temperature for better reaction
- After heating take their absorbance at 415nm of wavelength in a UV-Visible spectrophotometer.
- In a test tube pipette 5ml distilled water and add 2ml NASH reagent which is use as blank.
- These data is use to prepare a standard curve in Microsoft excel and using the standard curve we can quantitatively determine the amount of formaldehyde content in our fish sample that was collected for our experiment.
- The Nash test is a very sensitive method for the determination of small amount of formaldehyde.

In the table below the concentration of diluted stock solution and their following absorbance is given:

Concentration	Absorbance
0.370 µg/ml	0.031
0.463 µg/ml	0.036
0.555 µg/ml	0.042
0.648 µg/ml	0.048
0.740 µg/ml	0.053
0.925 µg/ml	0.064
1.110 µg/ml	0.074
1.295 µg/ml	0.086
1.388 µg/ml	0.092
1.480 µg/ml	0.097

Table 1: Standard formalin conc. and their corresponding absorbance

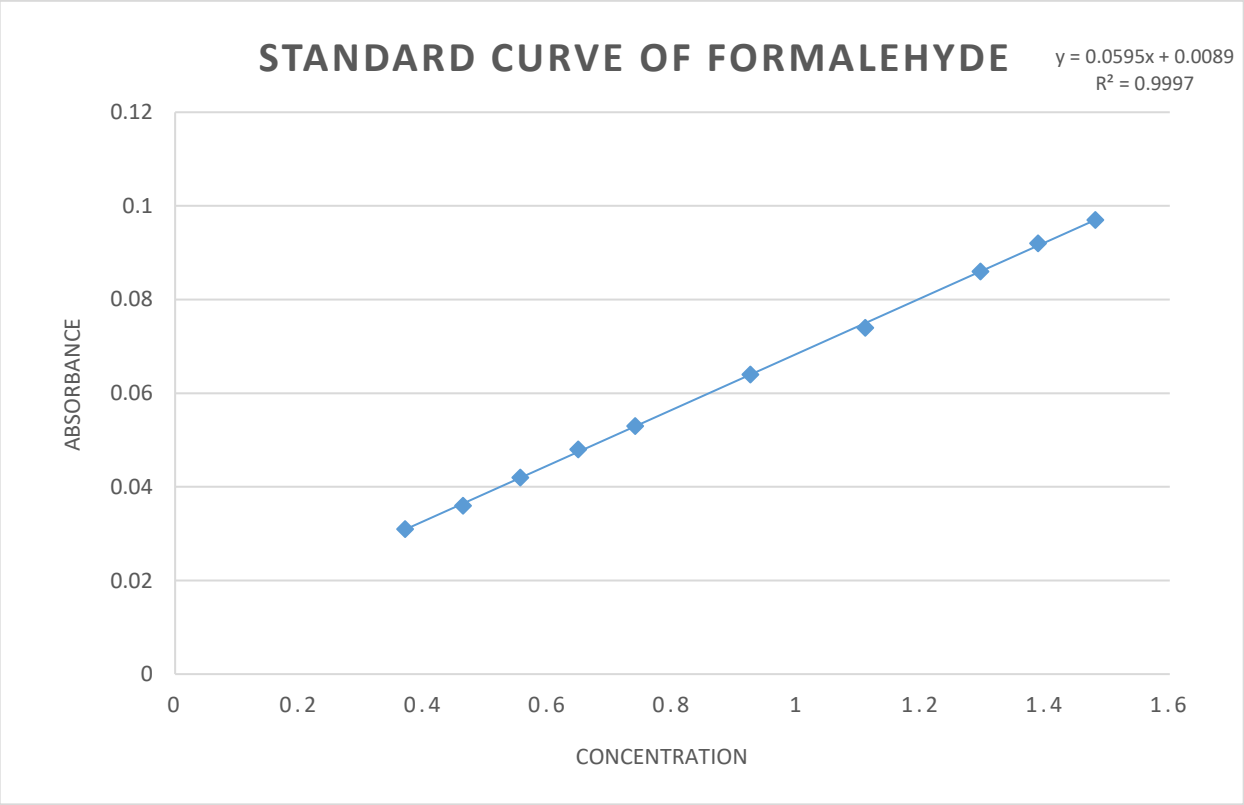


Figure 7: Standard curve of formaldehyde

3.5 Preparation of sample

3.5.1 Sample bought from market that contaminated with formaldehyde

- First the fish sample is cut into small pieces and a portion is kept for further experiment.
- Then the flesh portion of the fish is being separated.
- Then the separated flesh is taken in to a blender and blend for 10 minutes for homogenous mixing and after proper blending the blend form a paste like appearance.
- The blend sample was kept in the refrigerator for further use.

3.5.2 Fresh fish Sample bought from market in which formalin added in the laboratory

- First two different concentration of solution of formalin are 185 ppm per ml and 1850 ppm per ml is prepared
- Then some fresh fish sample is soak in the 185 ppm per ml formalin solution and some in the 1850 ppm per ml formalin solution and keep for about 1hours
- Then the fish sample is taken out from the solution and cut into small pieces, from then a portion is kept for further experiment.
- Then the flesh portion of the fish is being separated.
- Then the separated flesh is taken in to a blender and blend for 10 minutes for homogenous mixing and after proper blending the blend form a paste like appearance.
- The blend sample was kept in the refrigerator for further use.

Figure 8: Separation of fish flesh

Figure 9: Blend fish sample

3.6 Determination of formaldehyde content found in fish sample

First 10 gm of sample was weighted then add 35 ml water and mix properly. Then 60 ml of 6% tri-chloro-acetic acid was added for extraction of formaldehyde from the fish flesh. The extracted solution was then filtered by a Whatman No.1 of filter paper.

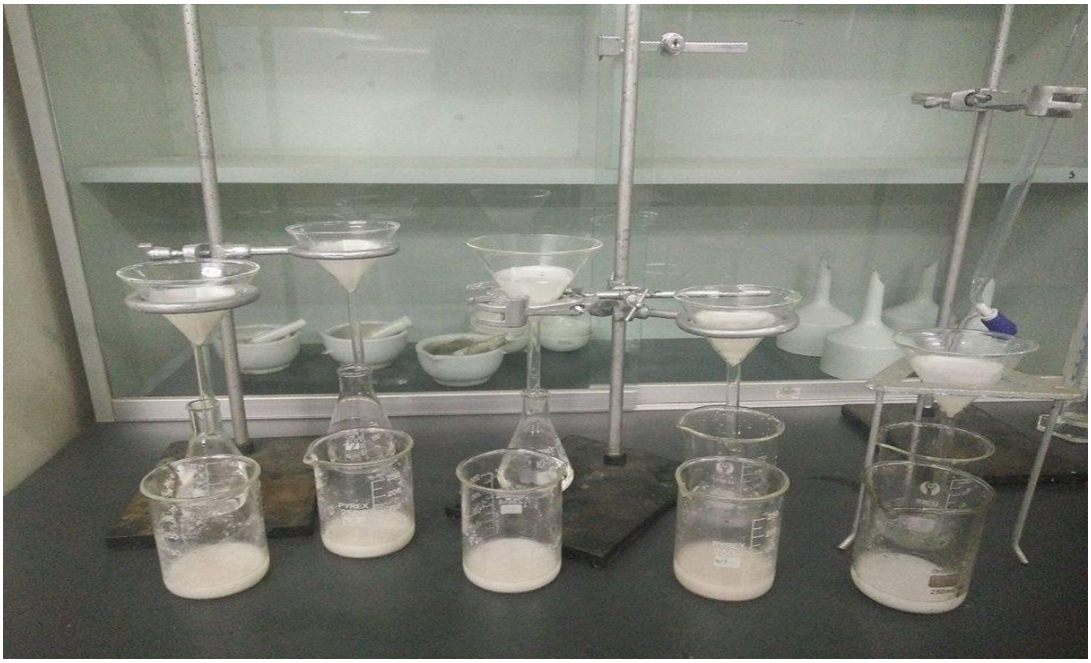


Figure 10: Filtration of fish flesh after add TCA

After filtration pH of the solution was determined by a pH meter. Due to the addition of tri-chloro-acetic acid the pH value of the sample was reduced and the pH of the filtered solution adjusted in between 6.00-7.00 by using Potassium hydroxide (KOH) and Hydrochloric acid (HCl).

Then 5 ml of sample solution was taken in a 50 ml of volumetric flask. 2 ml of previously prepared Nash's reagent was added as an indicator. Then it was heated in a water bath at 60^0 C for 30 minutes. Then the absorbance of the sample solution was determined by a UV-Visible spectrophotometer at 415 nm of wavelength. Triplicate of the absorbance was made for each sample and recorded for further calculation.

By using the standard curve the concentration of formaldehyde content in per 10 gm sample is calculated from their corresponding absorbance.

Figure 11: Filtrate of sample heat in water bath with Nash reagent

3.7 Removal procedure of formaldehyde through heat

10 grams of blended fishes were taken into 500 ml beaker from each type of fishes and then 35 ml water was added in each beaker and mix properly. Then each beaker was inserted into the oven at different temperature and kept them for 1 hours. After 1 hours, the heated sample was taken out from the oven and Then 60 ml of 6% tri-chloro-acetic acid was added for extraction of formaldehyde from the fish flesh. The extracted solution was then filtered by a Whatman No.1 of filter paper. After filtration pH of the solution was determined by a pH meter. Due to the addition of tri-chloro-acetic acid the pH value of the sample was reduced and the pH of the filtered solution adjusted in between 6.00-7.00 by using Potassium hydroxide (KOH) and Hydrochloric acid (HCl).

Then 5 ml of sample solution was taken in a 50 ml of volumetric flask. 2 ml of previously prepared Nash's reagent was added as an indicator. Then it was heated in a water bath at 60^0 C for 30 minutes. Then the absorbance of the sample solution was determined by a UV-Visible spectrophotometer at 415 nm of wavelength. The sample reading was placed in the standard curve for the calculation of formaldehyde content of the sample.

3.8 Removal procedure of formaldehyde through vinegar and heat

At first, the fish sample is soaked in 60ml white vinegar for one hour. Then the fish sample is cut into small pieces. Then the flesh portion of the fish is being separated. The separated flesh is taken in to a blender and blends for 10 minutes for homogenous mixing and after proper blending the blend form a paste like appearance. The blend sample was kept in the refrigerator for further use.

10 gm of blend sample was weighted followed by addition of 35 ml water and mix properly. Then 60 ml of 6% tri-chloro-acetic acid was added for extraction of formaldehyde from the fish flesh. The extracted solution was then filtered by a filter paper. After filtration, pH of the solution was determined by a pH meter. Due to the addition of tri-chloro acetic acid the pH value of the sample was reduced and the pH of the filtered solution adjusted in between 6.00-7.00 by using Potassium hydroxide (KOH) and Hydrochloric acid (HCl). Then 5 ml of sample solution was taken in a 50 ml of volumetric flask. 2 ml of freshly prepared Nash's reagent was added and heated in a water bath at 60^o C for 30minutes.Then the absorbance of the sample solution was determined by a UV-Visible spectrophotometer at 415 nm of wavelength. Triplicate of the absorbance was made for each sample and recorded for further calculation. By using the standard curve the concentration of formaldehyde content in per 10 gm sample is calculated from their corresponding absorbance.

3.9 Control Test

Naturally formaldehyde is produced in preserved fish. In our study we also perform the control test of formaldehyde for different fish samples. For this test, an alive and fresh fish sample was collected from fish market and preserved it in a refrigerator for 2 days. Then, the fish sample was cut into small pieces and a portion was kept for further experiment. The flesh portion of the fish is being separated. Then the separated flesh is taken in to a blender and blends for 10 minutes for homogenous mixing and after proper blending the blend form a paste like appearance. 10 gm of paste sample was weighted then add 35 ml water and mix properly. Then 60mlof6%tri-chloro-aceticacidwasaddedforextractionofformaldehydefromthefishflesh. The extracted solution was then filtered by a Whatman No.1 of filter paper. After filtration pH of the solution was determined by a pH meter. Due to the addition of tri-chloro- acetic acid the pH value of the sample was reduced and the pH of the filtered solution adjusted in between 6.00-7.00 by using Potassium hydroxide (KOH) and Hydrochloric acid (HCl).

Then 5 ml of sample solution was taken in a 50 ml of volumetric flask. 2 ml of previously prepared Nash's reagent was added as an indicator. Then itwas heated in a waterbath at 60^0 C for 30 minutes. Then the absorbance of the sample solution was determined by a UV-Visible spectrophotometer at 415 nm of wavelength. Triplicate of the absorbance was made for each sample and recorded for further calculation.

By using the standard curve, the concentration of formaldehyde content in per 10 gm sample is calculated from their corresponding absorbance.

Chapter Four

Result & Discussion

4. Result and discussion

4.1 Amount of formaldehyde content found in fish sample

The amount of formaldehyde content found in per 10 gm sample of ten different fish sample bought from different market place and fresh sample in which we add formalin in the laboratory is given in the table below:

Sl. No.	Name of the fish sample		Formalin added status	Amount of formalin content in per 10gm sample (mg)
	Local name	*Scientific name*		
1	Tilapia	*Oreochromismos sambicus*	Contaminated from market	4.01 mg
			Add in lab (soak in1850 ppm per ml solution)	3.61 mg
2	Bata	*Labeo bata*	Contaminated from market	5.89 mg
			Add in lab (soak in1850 ppm per ml solution)	6.33 mg
3	Shurputi	*Puntius sarana*	Contaminated from market	3.79 mg
			Add in lab (soak in1850 ppm per ml solution)	5.13 mg
4	Kachki	*Corcicasu borna*	Contaminated from market	4.36 mg
			Add in lab (soak in1850 ppm per ml solution)	3.74 mg
5	Ilis	*Tenualosa ilisha*	Contaminated from market	3.47 mg
			Add in lab (soak in1850 ppm per ml solution)	3.91 mg
6	Rui	*Labeo rohita*	Contaminated from market	3.46 mg
			Add in lab (soak in1850 ppm per ml solution)	2.97 mg
7	Mola	*Amblypharyngodon microlepis*	Contaminated from market	3.51 mg
			Add in lab (soak in1850 ppm per ml solution)	2.77 mg

Table 2: Content of formalin found in the experimental sample

From the above table we can see that the content of formaldehyde that found after analysis in the marketed sample that was previously contaminated with formalin and also the formalin content of fresh fish sample after soak in a formalin solution (1850 ppm per ml) for about 1 hours.

4.2 Determination of the amount of formaldehyde that can be removed by heat

4.2.1 Removal of formalin from Tilapia fish by heat

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
4.01 mg	100°C	1 hr.	1.152 mg	2.858 mg	71.27%
	110°C	1 hr.	1.103 mg	2.907 mg	72.45%
	120°C	1 hr.	0.940 mg	3.070 mg	76.56%
	130°C	1 hr.	0.782 mg	3.228 mg	80.50%
	140°C	1 hr.	0.731 mg	3.170 mg	81.77%
	150°C	1 hr.	0.620 mg	3.390 mg	84.54%
	160°C	1 hr.	0.573 mg	3.437 mg	85.71%
	170°C	1 hr.	0.401 mg	3.609 mg	90.00%
	180°C	1 hr.	0.302 mg	3.708 mg	92.47%
	190°C	1 hr.	0.230 mg	3.780 mg	94.26%
	200°C	1 hr.	0.193 mg	3.817 mg	95.19%
	210°C	1 hr.	0.140 mg	3.870 mg	96.51%
	220°C	1 hr.	0.113 mg	3.897 mg	97.20%
	230°C	1 hr.	0.087 mg	3.923 mg	97.83%
	240°C	1 hr.	0.061 mg	3.949 mg	98.48%
	250°C	1 hr.	0.042 mg	3.968 mg	98.95%

Table 3.1: Amount of formalin content remove from Tilapia by heat that was previously added from market

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
	100°C	1 hr.	0.910 mg	2.700 mg	74.79 %
	110°C	1 hr.	0.801 mg	2.809 mg	77.81%
	120°C	1 hr.	0.723 mg	2.887 mg	79.97%
	130°C	1 hr.	0.602 mg	3.008 mg	83.32%
	140°C	1 hr.	0.570 mg	3.040 mg	84.21%
	150°C	1 hr.	0.523 mg	3.087 mg	85.51%
	160°C	1 hr.	0.482 mg	3.128 mg	86.66%
	170°C	1 hr.	0.415 mg	3.195 mg	88.50%
	180°C	1 hr.	0.346 mg	3.264 mg	90.41%
	190°C	1 hr.	0.232 mg	3.378 mg	93.57%
	200°C	1 hr.	0.180 mg	3.430 mg	95.01%
	210°C	1 hr.	0.156 mg	3.454 mg	95.67%
	220°C	1 hr.	0.109 mg	3.501 mg	96.98%
	230°C	1 hr.	0.072 mg	3.538 mg	98.01%
	240°C	1 hr.	0.059 mg	3.551 mg	98.37%
	250°C	1 hr.	0.048 mg	3.562 mg	98.67%

Table 3.2: Amount of formalin remove from Tilapia fish by heat that added in fresh fish at the laboratory

From the above table, we see that,

By means of heat at various temperature we can remove about 70 % of formalin when the sample heat at 100°C temperature, at 150°C temperature we can remove about 85% of formalin, at 200°C temperature we can remove about 95% formalin from the fish sample and we can remove almost 99% formalin from the Tilapia fish sample when we heat at 250°C temperature.

4.2.2 Removal of formalin from Bata fish by heat

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
5.89 mg	100°C	1 hr.	4.760 mg	1.130 mg	19.19%
	110°C	1 hr.	4.310 mg	1.580 mg	26.83%
	120°C	1 hr.	4.060 mg	1.830 mg	31.07%
	130°C	1 hr.	3.540 mg	2.350 mg	39.90%
	140°C	1 hr.	3.210 mg	2.380 mg	45.19%
	150°C	1 hr.	2.960 mg	2.930 mg	49.75%
	160°C	1 hr.	2.740 mg	3.150 mg	53.48%
	170°C	1 hr.	2.450 mg	3.440 mg	58.40%
	180°C	1 hr.	2.210 mg	3.680 mg	62.48%
	190°C	1 hr.	1.710 mg	4.190 mg	71.14%
	200°C	1 hr.	1.270 mg	4.620 mg	78.44%
	210°C	1 hr.	0.960 mg	4.930 mg	83.70%
	220°C	1 hr.	0.530 mg	5.360 mg	91.00%
	230°C	1 hr.	0.280 mg	5.610 mg	95.25%
	240°C	1 hr.	0.098 mg	5.792 mg	98.34%
	250°C	1 hr.	0.073 mg	5.817 mg	98.76%

Table 4.1: Amount of formalin content remove from Bata fish by heat that was previously added from market

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
6.33 mg	100°C	1 hr.	4.520 mg	1.810 mg	28.59%
	110°C	1 hr.	4.170 mg	2.160 mg	34.12%
	120°C	1 hr.	3.840 mg	2.490 mg	39.34%
	130°C	1 hr.	3.560 mg	2.770 mg	43.76%
	140°C	1 hr.	3.370 mg	2.960 mg	46.76%
	150°C	1 hr.	3.120 mg	3.210 mg	50.71%
	160°C	1 hr.	2.780 mg	3.550 mg	56.08%
	170°C	1 hr.	2.430 mg	3.900 mg	61.61%
	180°C	1 hr.	2.100 mg	4.230 mg	66.82%
	190°C	1 hr.	1.880 mg	4.450 mg	70.30%
	200°C	1 hr.	1.310 mg	5.020 mg	79.30%
	210°C	1 hr.	0.961 mg	5.369 mg	84.82%
	220°C	1 hr.	0.476 mg	5.854 mg	92.48%
	230°C	1 hr.	0.199 mg	6.131 mg	96.86%
	240°C	1 hr.	0.082 mg	6.248 mg	98.70%
	250°C	1 hr.	0.073 mg	6.257 mg	98.85%

Table 4.2: Amount of formalin remove from Bata fish by heat that added in fresh fish at the laboratory

From the above table, we see that,

By means of heat at various temperature we can remove about 20 % of formalin when the sample heat at 100°C temperature, at 150°C temperature we can remove about 50% of formalin, at 200°C temperature we can remove about 80% formalin from the fish sample and we can remove almost 99% formalin from the Bata fish sample when we heat at 250°C temperature.

4.2.3 Removal of formalin from Shorputi fish by heat

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
3.79 mg	100°C	1 hr.	3.38 mg	0.41 mg	10.82%
	110°C	1 hr.	3.14 mg	0.65 mg	17.15%
	120°C	1 hr.	2.97 mg	0.82 mg	21.64%
	130°C	1 hr.	2.61 mg	1.18 mg	31.13%
	140°C	1 hr.	2.29 mg	1.50 mg	39.58%
	150°C	1 hr.	2.01 mg	1.78 mg	46.97%
	160°C	1 hr.	1.83 mg	1.96 mg	51.71%
	170°C	1 hr.	1.74 mg	2.05 mg	54.09%
	180°C	1 hr.	1.66 mg	2.13 mg	56.20%
	190°C	1 hr.	1.28 mg	2.51 mg	66.27%
	200°C	1 hr.	0.82 mg	2.97 mg	78.36%
	210°C	1 hr.	0.75 mg	3.04 mg	80.21%
	220°C	1 hr.	0.61 mg	3.18 mg	83.91%
	230°C	1 hr.	0.29 mg	3.50 mg	92.34%
	240°C	1 hr.	0.23 mg	3.56 mg	93.93%
	250°C	1 hr.	0.14 mg	3.65 mg	96.30%

Table 5.1: Amount of formalin content remove from Shorputi fish by heat that was previously added from market

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
5.13 mg	100°C	1 hr.	4.36 mg	0.77 mg	15.01%
	110°C	1 hr.	4.17 mg	0.96 mg	18.71%
	120°C	1 hr.	3.82 mg	1.31 mg	25.54%
	130°C	1 hr.	3.49 mg	1.64 mg	31.97%
	140°C	1 hr.	3.14 mg	1.99 mg	38.79%
	150°C	1 hr.	2.61 mg	2.52 mg	49.12%
	160°C	1 hr.	2.23 mg	2.90 mg	56.53%
	170°C	1 hr.	1.96 mg	3.17 mg	61.79%
	180°C	1 hr.	1.80 mg	3.33 mg	64.91%
	190°C	1 hr.	1.39 mg	3.74 mg	72.90%
	200°C	1 hr.	1.07 mg	4.06 mg	79.14%
	210°C	1 hr.	0.88 mg	4.25 mg	82.85%
	220°C	1 hr.	0.63 mg	4.50 mg	87.72%
	230°C	1 hr.	0.57 mg	4.56 mg	88.89%
	240°C	1 hr.	0.32 mg	4.81 mg	93.76%
	250°C	1 hr.	0.19 mg	4.94 mg	96.30%

Table 5.2: Amount of formalin remove from Shorputi fish by heat that added in fresh fish at the laboratory

From the above table, we see that,

By means of heat at various temperature we can remove about 10 % of formalin when the sample heat at 100°C temperature, at 150°C temperature we can remove about 50% of formalin, at 200°C temperature we can remove about 80% formalin from the fish sample and we can remove almost 96% formalin from the Shorputi fish sample when we heat at 250°C temperature.

4.2.4 Removal of formalin from Kachki fish by heat

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
4.36 mg	100ºC	1 hr.	3.02 mg	1.24 mg	28.44%
	110ºC	1 hr.	2.75 mg	1.61 mg	36.93%
	120ºC	1 hr.	2.33 mg	2.03 mg	46.56%
	130ºC	1 hr.	1.99 mg	2.37 mg	54.36%
	140ºC	1 hr.	1.54 mg	2.82 mg	64.68%
	150ºC	1 hr.	1.31 mg	3.05 mg	69.95%
	160ºC	1 hr.	1.12 mg	3.24 mg	74.31%
	170ºC	1 hr.	1.00 mg	3.36 mg	77.06%
	180ºC	1 hr.	0.93 mg	3.43 mg	78.67%
	190ºC	1 hr.	0.81 mg	3.55 mg	81.82%
	200ºC	1 hr.	0.69 mg	3.67 mg	84.17%
	210ºC	1 hr.	0.42 mg	3.94 mg	90.37%
	220ºC	1 hr.	0.28 mg	4.08 mg	93.58%
	230ºC	1 hr.	0.11 mg	4.25 mg	97.48%
	240ºC	1 hr.	0.09 mg	4.27 mg	97.94%
	250ºC	1 hr.	0.06 mg	4.30 mg	98.62%

Table 6.1: Amount of formalin content remove from Kachki fish by heat that was previously added from market

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
3.74 mg	100°C	1 hr.	2.81 mg	0.93 mg	24.87%
	110°C	1 hr.	2.53 mg	1.21 mg	32.35%
	120°C	1 hr.	2.41 mg	1.33 mg	35.56%
	130°C	1 hr.	2.12 mg	1.62 mg	43.32%
	140°C	1 hr.	1.93 mg	1.81 mg	48.40%
	150°C	1 hr.	1.64 mg	2.10 mg	56.15%
	160°C	1 hr.	1.17 mg	2.57 mg	68.71%
	170°C	1 hr.	1.00 mg	2.74 mg	73.26%
	180°C	1 hr.	0.93 mg	2.81 mg	75.13%
	190°C	1 hr.	0.86 mg	2.88 mg	77.01%
	200°C	1 hr.	0.44 mg	3.30 mg	88.24%
	210°C	1 hr.	0.21 mg	3.53 mg	94.39%
	220°C	1 hr.	0.11 mg	3.63 mg	97.06%
	230°C	1 hr.	0.09 mg	3.65 mg	97.59%
	240°C	1 hr.	0.07 mg	3.67 mg	98.13%
	250°C	1 hr.	0.06 mg	3.68 mg	98.40%

Table 6.2: Amount of formalin remove from Kachki fish by heat that added in fresh fish at the laboratory

From the above table, we see that,

By means of heat at various temperature we can remove about 25 % of formalin when the sample heat at 100°C temperature, at 150°C temperature we can remove more than 50% of formalin, at 200°C temperature we can remove about 85% formalin from the fish sample and we can remove almost 98% formalin from the Kachki fish sample when we heat at 250°C temperature.

4.2.5 Removal of formalin from Ilish fish by heat

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
3.47 mg	100°C	1 hr.	2.41 mg	1.06 mg	30.55%
	110°C	1 hr.	2.34 mg	1.13 mg	32.56%
	120°C	1 hr.	2.00 mg	1.47 mg	41.36%
	130°C	1 hr.	1.82 mg	1.65 mg	47.55%
	140°C	1 hr.	1.68 mg	1.79 mg	51.59%
	150°C	1 hr.	1.52 mg	1.95 mg	56.20%
	160°C	1 hr.	1.35 mg	2.12 mg	61.10%
	170°C	1 hr.	1.30 mg	2.17 mg	62.54%
	180°C	1 hr.	1.22 mg	2.25 mg	64.84%
	190°C	1 hr.	0.96 mg	2.51 mg	72.33%
	200°C	1 hr.	0.76 mg	2.71 mg	78.10%
	210°C	1 hr.	0.68 mg	2.79 mg	80.40%
	220°C	1 hr.	0.66 mg	2.81 mg	80.98%
	230°C	1 hr.	0.53 mg	2.94 mg	84.73%
	240°C	1 hr.	0.29 mg	3.18 mg	91.64%
	250°C	1 hr.	0.10 mg	3.37 mg	97.12%

Table 7.1: Amount of formalin content remove from Ilish fish by heat that was previously added from market

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
3.91 mg	100°C	1 hr.	2.43 mg	1.48 mg	37.85%
	110°C	1 hr.	2.19 mg	1.72 mg	43.99%
	120°C	1 hr.	2.06 mg	1.91 mg	48.85%
	130°C	1 hr.	1.92 mg	1.99 mg	50.90%
	140°C	1 hr.	1.83 mg	2.08 mg	53.20%
	150°C	1 hr.	1.69 mg	2.22 mg	56.78%
	160°C	1 hr.	1.57 mg	2.34 mg	59.85%
	170°C	1 hr.	1.49 mg	2.41 mg	61.89%
	180°C	1 hr.	1.41 mg	2.50 mg	63.94%
	190°C	1 hr.	1.32 mg	2.59 mg	66.24%
	200°C	1 hr.	1.16 mg	2.75 mg	70.33%
	210°C	1 hr.	1.04 mg	2.87 mg	73.40%
	220°C	1 hr.	0.86 mg	3.05 mg	78.01%
	230°C	1 hr.	0.52 mg	3.39 mg	86.70%
	240°C	1 hr.	0.31 mg	3.60 mg	92.07%
	250°C	1 hr.	0.14 mg	3.77 mg	96.42%

Table 7.2: Amount of formalin remove from Ilish fish by heat that added in fresh fish at the laboratory

From the above table, we see that,

By means of heat at various temperature we can remove more than 30 % of formalin when the sample heat at 100°C temperature, at 150°C temperature we can remove more than 56% of formalin, at 200°C temperature we can remove about 70% formalin from the fish sample and we can remove almost 97% formalin from the Ilish fish sample when we heat at 250°C temperature.

4.2.6 Removal of formalin from Rui fish by heat

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
3.46 mg	100°C	1 hr.	2.94 mg	0.52 mg	15.03%
	110°C	1 hr.	2.71 mg	0.75 mg	21.67%
	120°C	1 hr.	2.66 mg	0.80 mg	23.12%
	130°C	1 hr.	2.17 mg	1.29 mg	37.28%
	140°C	1 hr.	2.05 mg	1.41 mg	40.75%
	150°C	1 hr.	1.98 mg	1.48 mg	42.77%
	160°C	1 hr.	1.81 mg	1.65 mg	47.69%
	170°C	1 hr.	1.59 mg	1.87 mg	54.05%
	180°C	1 hr.	1.42 mg	2.04 mg	58.96%
	190°C	1 hr.	1.17 mg	2.29 mg	66.18%
	200°C	1 hr.	1.01 mg	2.45 mg	70.81%
	210°C	1 hr.	0.92 mg	2.54 mg	73.41%
	220°C	1 hr.	0.79 mg	2.67 mg	77.17%
	230°C	1 hr.	0.61 mg	2.85 mg	82.37%
	240°C	1 hr.	0.39 mg	3.07 mg	88.73%
	250°C	1 hr.	0.13 mg	3.33 mg	96.24%

Table 8.1: Amount of formalin content remove from Rui fish by heat that was previously added from market

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
2.97 mg	100°C	1 hr.	2.46 mg	0.51 mg	17.17%
	110°C	1 hr.	2.21 mg	0.76 mg	25.59%
	120°C	1 hr.	2.13 mg	0.84 mg	28.28%
	130°C	1 hr.	2.00 mg	0.97 mg	32.67%
	140°C	1 hr.	1.82 mg	1.15 mg	38.72%
	150°C	1 hr.	1.67 mg	1.30 mg	43.77%
	160°C	1 hr.	1.48 mg	1.49 mg	50.17%
	170°C	1 hr.	1.31 mg	1.66 mg	55.89%
	180°C	1 hr.	1.16 mg	1.81 mg	60.94%
	190°C	1 hr.	1.03 mg	1.94 mg	65.20%
	200°C	1 hr.	0.87 mg	2.10 mg	70.71%
	210°C	1 hr.	0.63 mg	2.34 mg	78.79%
	220°C	1 hr.	0.43 mg	2.54 mg	85.52%
	230°C	1 hr.	0.29 mg	2.68 mg	90.24%
	240°C	1 hr.	0.22 mg	2.75 mg	92.59%
	250°C	1 hr.	0.10 mg	2.87 mg	96.63%

Table 8.2: Amount of formalin remove from Rui fish by heat that added in fresh fish at the laboratory

From the above table, we see that,

By means of heat at various temperature we can remove about 15 % of formalin when the sample heat at 100°C temperature, at 150°C temperature we can remove more than 40% of formalin, at 200°C temperature we can remove about 70% formalin from the fish sample and we can remove almost 96% formalin from the Rui fish sample when we heat at 250°C temperature.

4.2.7 Removal of formalin from Mola fish by heat

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
3.51 mg	100°C	1 hr.	2.79 mg	0.72 mg	20.51%
	110°C	1 hr.	2.58 mg	0.93 mg	26.50%
	120°C	1 hr.	2.43 mg	1.08 mg	30.77%
	130°C	1 hr.	2.32 mg	1.19 mg	33.90%
	140°C	1 hr.	2.16 mg	1.35 mg	38.46%
	150°C	1 hr.	2.03 mg	1.48 mg	42.17%
	160°C	1 hr.	1.86 mg	1.65 mg	47.01%
	170°C	1 hr.	1.52 mg	1.99 mg	56.70%
	180°C	1 hr.	1.33 mg	2.18 mg	62.11%
	190°C	1 hr.	1.00 mg	2.51 mg	71.51%
	200°C	1 hr.	0.67 mg	2.84 mg	80.91%
	210°C	1 hr.	0.47 mg	3.04 mg	86.61%
	220°C	1 hr.	0.32 mg	3.19 mg	90.88%
	230°C	1 hr.	0.21 mg	3.30 mg	94.01%
	240°C	1 hr.	0.084 mg	3.426 mg	97.61%
	250°C	1 hr.	0.072 mg	3.438 mg	97.95%

Table 9.1: Amount of formalin content remove from Mola fish by heat that was previously added from market

Heating					
Amount of formaldehyde found before heat per 10 gm. sample (mg)	**Temp**	**Time**	**Formalin found after heat per 10 gm. sample (mg)**	**Amount of formaldehyde remove by heat (mg)**	**Removal of formalin (%)**
2.77	100°C	1 hr.	2.13	0.64	23.10
	110°C	1 hr.	1.94	0.83	29.96
	120°C	1 hr.	1.73	1.04	37.55
	130°C	1 hr.	1.69	1.08	38.99
	140°C	1 hr.	1.58	1.19	42.96
	150°C	1 hr.	1.47	1.30	46.93
	160°C	1 hr.	1.22	1.55	55.96
	170°C	1 hr.	`1.06	1.71	61.73
	180°C	1 hr.	0.71	2.06	74.37
	190°C	1 hr.	`0.53	2.24	80.87
	200°C	1 hr.	0.31	2.46	88.81
	210°C	1 hr.	0.20	2.57	92.78
	220°C	1 hr.	0.15	2.62	94.58
	230°C	1 hr.	0.062	2.708	97.76
	240°C	1 hr.	0.038	2.732	98.63
	250°C	1 hr.	0.031	2.739	98.88

Table 9.2: Amount of formalin remove from Mola fish by heat that added in fresh fish at the laboratory

From the above table, we see that,

By means of heat at various temperature we can remove about 20 % of formalin when the sample heat at 100°C temperature, at 150°C temperature we can remove more than 40% of formalin, at 200°C temperature we can remove more than870% formalin from the fish sample and we can remove almost 98% formalin from the Mola fish sample when we heat at 250°C temperature.

4.3 Determination of the amount of formaldehyde that can be removed by soak in vinegar and then by heat

4.3.1 Removal of formalin from Tilapia fish by soak in vinegar and then by heat

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
4.01 mg	1.322 mg	2.688 mg	67.03%	100°C	1 hr.	0.670 mg	83.29%
				110°C	1 hr.	0.035 mg	99.12%
				120°C	1 hr.	0.031 mg	99.23%

Table 10.1: Amount of formalin content remove from Tilapia fish by soak in vinegar and then by heat that was previously added from market

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
3.61 mg	0.994 mg	2.616 mg	72.47%	100°C	1 hr.	0.270 mg	92.52%
				110°C	1 hr.	0.029 mg	99.20%
				120°C	1 hr.	0.023 mg	99.36%

Table 10.2: Amount of formalin remove from Tilapia fish by soak in vinegar and then by heat that added in fresh fish at the laboratory

From the above table, we see that,

By soak in vinegar we can remove about 70% formalin from the sample. When we heat the sample at 100°C temperature we can remove more than 80% formalin and by heating at 120°C temperature we can remove about 99 % formalin from the Tilapia fish.

4.3.2 Removal of formalin from Bata fish by soak in vinegar and then by heat

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
5.89 mg	0.873 mg	5.017 mg	85.18%	100°C	1 hr.	0.113 mg	98.08%
				110°C	1 hr.	0.062 mg	98.95%
				120°C	1 hr.	0.038 mg	99.35%

Table 11.1: Amount of formalin content remove from Bata fish by soak in vinegar and then by heat that was previously added from market

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
6.33 mg	1.004 mg	5.326 mg	84.14%	100°C	1 hr.	0.207 mg	96.73%
				110°C	1 hr.	0.085 mg	98.66%
				120°C	1 hr.	0.044 mg	99.30%

Table 11.2: Amount of formalin remove from Bata fish by soak in vinegar and then by heat that added in fresh fish at the laboratory

From the above table, we see that,

By soak in vinegar we can remove about 85% formalin from the sample. When we heat the sample at 100°C temperature we can remove more than 95% formalin and by heating at 120°C temperature we can remove about 99 % formalin from the Bata fish.

4.3.3 Removal of formalin from Shorputi fish by soak in vinegar and then by heat

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
6.33 mg	0.504 mg	5.926 mg	92.04%	Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
				100°C	1 hr.	0.0076 mg	99.80%

Table 12.1: Amount of formalin content remove from Shorputi fish by soak in vinegar and then by heat that was previously added from market

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
5.13 mg	0.10 mg	5.03 mg	98.05	Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
				100°C	1 hr.	0.0091 mg	99.82%

Table 12.2: Amount of formalin remove from Shorputi fish by soak in vinegar and then by heat that added in fresh fish at the laboratory

From the above table, we see that,

By soak in vinegar we can remove more than 90% formalin from the sample. When we heat the sample at 100°C temperature we can remove more than 99% formalin from the Shorputi fish.

4.3.4 Removal of formalin from Kachki fish by soak in vinegar and then by heat

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
4.36 mg	0.319 mg	4.041 mg	92.68%	100°C	1 hr.	0.132 mg	96.92%
				110°C	1 hr.	0.061 mg	98.60%
				120°C	1 hr.	0.043 mg	99.01%

Table 13.1: Amount of formalin content remove from Kachki fish by soak in vinegar and then by heat that was previously added from market

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
3.74 mg	0.194 mg	3.546 mg	94.81%	100°C	1 hr.	0.095 mg	97.46%
				110°C	1 hr.	0.048 mg	98.72%
				120°C	1 hr.	0.032 mg	99.14%

Table 13.2: Amount of formalin remove from Kachki fish by soak in vinegar and then by heat that added in fresh fish at the laboratory

From the above table, we see that,

By soak in vinegar we can remove more than 90% formalin from the sample. When we heat the sample at 100°C temperature we can remove about 97% formalin and by heating at 120°C temperature we can remove about 99 % formalin from the Kachki fish.

4.3.5 Removal of formalin from Ilish fish by soak in vinegar and then by heat

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
3.47 mg	1.23 mg	2.24 mg	64.55%	100°C	1 hr.	0.43 mg	87.61%
				110°C	1 hr.	0.17 mg	95.10%
				120°C	1 hr.	0.031 mg	99.10%

Table 14.1: Amount of formalin content remove from Ilish fish by soak in vinegar and then by heat that was previously added from market

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
3.91 mg	1.17 mg	2.74 mg	69.02%	100°C	1 hr.	0.240 mg	93.62%
				110°C	1 hr.	0.098 mg	97.49%
				120°C	1 hr.	0.029 mg	99.25%

Table 14.2: Amount of formalin remove from Ilish fish by soak in vinegar and then by heat that added in fresh fish at the laboratory

From the above table, we see that,

By soak in vinegar we can remove more than 60% formalin from the sample. When we heat the sample at 100°C temperature we can remove more than 87% formalin and by heating at 120°C temperature we can remove about 99 % formalin from the Ilish fish.

4.3.6 Removal of formalin from Rui fish by soak in vinegar and then by heat

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
3.46 mg	0.094 mg	3.366 mg	97.28%	Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
				100°C	1 hr.	0.0363 mg	98.95%

Table 15.1: Amount of formalin content remove from Rui fish by soak in vinegar and then by heat that was previously added from market

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
2.97 mg	0.079 mg	2.891 mg	97.34%	Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
				100°C	1 hr.	0.0236 mg	99.20%

Table 15.2: Amount of formalin remove from Rui fish by soak in vinegar and then by heat that added in fresh fish at the laboratory

From the above table, we see that,

By soak in vinegar we can remove more than 97% formalin from the sample. When we heat the sample at 100°C temperature we can remove more than 99% formalin from the Rui fish.

4.3.7 Removal of formalin from Mola fish by soak in vinegar and then by heat

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
3.51 mg	1.34 mg	2.17 mg	61.82%	100°C	1 hr.	0.260 mg	92.59%
				110°C	1 hr.	0.120 mg	96.58%
				120°C	1 hr.	0.053 mg	98.49%

Table 16.1: Amount of formalin content remove from Mola fish by soak in vinegar and then by heat that was previously added from market

Amount of formalin found before soak in vinegar (mg)	Amount of formalin found after soak in vinegar (mg)	Amount of formalin remove by vinegar (mg)	Removal of formalin by vinegar (%)	Heating			
				Temp	Time	Amount of formalin found after heat (mg)	Removal of formalin by heat (%)
2.77 mg	0.84 mg	1.93 mg	69.68%	100°C	1 hr.	0.097 mg	96.50%
				110°C	1 hr.	0.073 mg	97.36%
				120°C	1 hr.	0.041 mg	98.52%

Table 16.2: Amount of formalin remove from Mola fish by soak in vinegar and then by heat that added in fresh fish at the laboratory

From the above table, we see that,

By soak in vinegar we can remove more than 60% formalin from the sample. When we heat the sample at 100°C temperature we can remove more than 90% formalin and by heating at 120°C temperature we can remove about 98 % formalin from the Mola fish.

4.4 Removal of formalin from fresh fish sample after soak in 185 ppm per ml formalin solution

4.4.1 Amount of formalin found after soak different fish sample in 185 ppm per ml formalin solution for 1 hours

Sl. No.	Name of the fish sample		Bought from	Amount of formalin content found in per 10gm sample
	Local name	Scientific name		
1	Shorputi	*Puntius sarana*	Local market	0.361 mg
2	Tilapia	*Oreochromis mossambicus*	Local market	0.401 mg
3	Kachhkie	*Corica soborna*	Footpath	1.54 mg
4	Rui	*Labeo rohita*	Local market	0.868 mg
5	Ilish	*Tenualosa ilisha*	Local market	0.340 mg
6	Puiya	*Lepidocephalichthys berdmorei*	Local market	0.563 mg
7	Taki	*Channa punctata*	Footpath	0.418 mg
8	Mola	*Amblypharyngodon microlepis*	Footpath	1.15 mg
9	Mrigol	*Cirrhinus cirrhosis*	Local market	0.402 mg
10	Bata	*Labeo bata*	Local market	0.688 mg

Table 17: Formaldehyde content found in different fish sample when we soak fresh sample in 185ppm per ml formalin solution

From the above table,

We can see that the amount of formalin determines by soaking formalin free marketed sample in to a formalin solution of 185 ppm per ml. Different fish sample absorbed the formalin from formalin solution at different rate.

4.5 Determination of the amount of formaldehyde can be removeable by heat that we added in the laboratory by soak in (185 ppm per ml) formalin solution

Sl. No.	Name of sample	Amount of formalin found before heating	Heating			Amount of formalin remove by heat	% Removed by heat
			Temp.	Time	Formalin found after heat		
01	Shorputi	0.361 mg	100°C	1 hr.	0.0089 mg	0.3521 mg	97.53 %
			200°C	1 hr.	0.0042 mg	0.3568 mg	98.84 %
02	Tilapia	0.401 mg	100°C	1 hr.	0.0819 mg	0.3191 mg	79.58 %
			200°C	1 hr.	0.028 mg	0.373 mg	93.02 %
03	Kachhkie	1.54 mg	100°C	1 hr.	0.292 mg	1.248 mg	81.03 %
			200°C	1 hr.	0.080 mg	1.46 mg	94.80 %
04	Rui	0.868 mg	100°C	1 hr.	0.0051 mg	0.8629 mg	99.41 %
			200°C	1 hr.	0.0034 mg	0.8646 mg	99.61 %
05	Ilish	0.340 mg	100°C	1 hr.	0.0616 mg	0.2784 mg	81.88 %
			200°C	1 hr.	0.0545 mg	0.2855 mg	83.97 %
06	Puiya	0.563 mg	100°C	1 hr.	0.0361 mg	0.5269 mg	93.59 %
			200°C	1 hr.	0.0354 mg	0.5276 mg	93.71 %
07	Taki	0.418 mg	100°C	1 hr.	0.0316 mg	0.3864 mg	92.44 %
			200°C	1 hr.	0.0127 mg	0.4053 mg	96.96 %
08	Mola	1.15 mg	100°C	1 hr.	0.134 mg	1.016 mg	88.34 %
			200°C	1 hr.	0.108 mg	1.042 mg	90.61 %
09	Mrigol	0.402 mg	100°C	1 hr.	0.0304 mg	0.3716 mg	92.44 %
			200°C	1 hr.	0.0060 mg	0.396 mg	98.51 %
10	Bata	0.688 mg	100°C	1 hr.	0.0097 mg	0.6783 mg	98.59 %
			200°C	1 hr.	0.0048 mg	0.6832 mg	99.30 %

Table 18: Formaldehyde content found in different fish sample after heat at different temperature and determine the % removal of formalin content

From the above table, we see that,

- We can remove on an average about 90 percent of formaldehyde content from the fish sample when heating the sample at 100°C temperature for 1 hours.
- We can remove about on an average about 95 percent of formaldehyde content from the fish sample when heating the sample at 200°C temperature for 1 hours.

4.6 Control test

SI. NO.	Name of the sample	Amount of formaldehyde found
01	Shorputi	0.098
02	Rui	0.084

Table 19: Control test of formaldehyde for preserved fish

From the above table, we see that,

Formalin is naturally produced in preserved fish. In case of Shorputi and Rui fish the amount of naturally produced formaldehyde is 0.098 and 0.084 mg per 10gm sample.

5. Discussion

From all above data we can see that many of the marketed sample are contaminated with formalin and by means of heat we can remove a large portion of formalin from various fish sample. We can remove about 30% of formalin when we heat the sample at 100^{o}C temperature. When we heat the sample at 150^{o}C temperature it can be remove more than 50% formalin for most of the sample. By heating these sample at 200^{o}C temperature we are able the remove 80% of formalin from most of the sample and at 250^{o}C temperature we can remove more than 90% of formalin from the fish sample.

When we soak the formalin contaminated fish sample in vinegar and after that by heating about 120^{o}C temperature we are able to remove 99% of formalin from the fish sample.

When we soak fresh sample in lower concentrate formalin solution then we can remove the absorbed formalin content on an average 90% by heating at 100^{o}C temperature.

Though fresh fish contain some naturally occurring formalin content inside them but this is in minor level and most of the portion of this formalin is removed by heating at 100^{o}C temperature.

Chapter Five
Conclusion

6. Conclusion

The present study revealed that, the presence of formaldehyde in market samples of Rui, Tilapia, Kacchki, and some others from different markets of Dhaka city with the different ranges. Based on the findings by different authors, the present situation of fish adulteration in wet markets is a fact which presently shows improving due to public awareness, government initiatives against formalin use in markets. This experiment based on Nash test in conjunction with TCA extraction method.

In this study, I found that the formaldehyde content in various fish sample can be removeable about 10 to 70 percent, when we heat it about 100^{o}C temperature. The primary aim of the study is to remove almost 100 percent formaldehyde content from the sample by heating method. When I increase the temperature about 200^{o}C then I get about 70 to 95 percent removal of formaldehyde content from various fish sample. When I further increase the temperature about 250^{o}C then I get about 99 percent removal of formalin from various fish sample. So, from my study I can say that it is not possible to remove 100 percent formaldehyde content from the fish sample. But it is possible to remove a major fraction of formaldehyde content from the fish sample by the heating process. Again, by heating the formalin contaminated fish sample after soak in vinegar for 1 hours it is possible to remove 99 percent of formalin at 100^{o}C to 120^{o}C temperature. From the control test I get that fresh fish sample also contain formalin naturally in their body. But I also see when we soak fish sample in low concentrate formalin solution then most of the absorbed formalin content can be removed by heating at about 100^{o}C. Thus, I also can says that major portion of the naturally occurring formalin content in fish sample is get removed from fish by normal cooking procedure. Finally, I can say that a minor portion of formaldehyde content is remain in the sample which will reduce the harmful effect of formaldehyde in our body. But the most important thing is in order to control or reduces formaldehyde content in fish and other food, several measures such as raise public awareness, banned unauthorized export and import of formaldehyde, government steps and overall ethical development of mass people should be necessary.

Chapter Six
References

7. Reference

1. Niloy Jaman, Md. Sazedul Hoque, Subhas Chandra Chakraborty, Md. Enamul Hoq, Hari Pada Seal "International journal of fisheries and aquatic studies" Vol 2 ,Issue 6, 2015, page no: 94 – 98. Accesses date: 9-10-2017
2. Riaz Uddin, Moin Ibna Wahid, Tasbira Jesmeen, Naz Hasan Huda, Kumar Bishwajit Sutradhar "Stamford Journal of Pharmaceutical Science", Vol 4,(2011) Page No: 49-52. Accesses date: 7-11-2017
3. Bruce Jon Compton and William C. Purdy "The mechanism of the reaction of the Nash and the Sawicki aldehyde reagent" issue 3,1980, page no: 2207- 2211, Accesses date: 10-11-2016
4. C. H. Castell, Barbara Smith "journal of the Fisheries Research Board of Canada" 1973, Vol. 30, page number: 91-98, Access date: 7-11-2017
5. Kibria, G. (2007) Formalin and Fish Trade in Bangladesh—Human and Environmental Risks for http://www.sydneybashi-bangla.com Access date: 9-11-2017
6. Rafiad Islam, Shahin Mahmud, Abdul Aziz, Animesh Sarker, Marufa Nasreen "A Comparative Study of Present Status of Marketing of Formalin Treated Fishes in Six Districts of Bangladesh" issue 13 January 2015 page no: 124 – 134 access date: 12-11-2017
7. Haque and Mohsin "Intensity of Formalin Use for Consumable Fish Preservation in Dhaka City,Bangladesh", vol:4 (2009), page no:51-53, access date: 12-11-2017
8. Noordiana, Fatimah and Farhana "Formaldehyde Content and Quality Characteristics of Selected Fish and Seafood from Wet Markets" vol-18 (2011), page no: 125-136, access date: 12-11-2017
9. Yeasmin, T., Reza, M.S., Khan, M.N.A., Shikha, F.H. and Kamal, M. "Present Status of Marketing of Formalin Treated Fishes in Domestic Markets at Mymensingh District in Bangladesh*"* vol:1 (2010), page no:21-24. Access date: 12-11-2017
10. MoFL (2011) Ministry of Fisheries & Livestock, Government of the People's Republic of Bangladesh. http://www.mofl.gov.bd access date: 12-11-2017
11. https://en.wikipedia.org/wiki/List_of_fishes_in_Bangladesh, access date: 12-11-2017
12. Dr. Naomi Mirza, http://m.maya.com.bd/en/article/general-health/food-for-thought-how-to-detoxify-food access date:12-11-2017

13. Dr. Naomi Mirza, http://www.thedailystar.net/food-for-thought-25605, access date: 12-11-2017
14. http://www.nature.com/nature/journal/v170/n4336/abs/170976a0.html, access date: 12-11-2017
15. http://files.foodmate.com/2013/files_2990.html, Access date: 12-11-2017
16. http://www.academia.edu/6516923/The_Colorimetric_Estimation_of_Formaldehyde_by_Means_of_the_Hantzsch_Reaction access date: 12-11-2017
17. https://en.wikipedia.org/wiki/Formaldehyd, access date 12-11-2017
18. http://www.chemspider.com/Chemical-Structure.24777129.html, access date: 12-11-2016
19. https://www.tedpella.com/msds_html/18508msd.htm, access date: 05-01-2018.
20. https://www.sciencedirect.com/science/article/pii/S2468550X16300260 access date: 05-01-2018.
21. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/415.pdf , access date: 05-01-2018.
22. https://www.canada.ca/content/dam/canada/health-canada/migration/healthy-canadians/publications/healthy-living-vie-saine/water-formaldehyde-eau/alt/water-formaldehyde-eau-eng.pdf, access date: 05-01-2018.
23. Squire, R.A. and Cameron, L.L. An analysis of potential carcinogenic risk from formaldehyde. Regul. Toxicol. Pharmacol., 4: 107–129 (1984),
24. U.S. Environmental Protection Agency. Method 554. Determination of carbonyl compounds in drinking water by DNPH derivatization and high performance liquid chromatography (HPLC). Environmental Monitoring Systems Laboratory, Cincinnati, OH (1992).
25. World Health Organization. Formaldehyde. Environmental Health Criteria 89, International Programme on Chemical Safety, Geneva (1989).
26. Owen, B.A., Dudney, C.S., Tan, E.L. and Easterly, C.E. Formaldehyde in drinking water: comparative hazard evaluation and an approach to regulation. Regul. Toxicol. Pharmacol., 11: 220–236 (1990).
27. Kitchens, J.F., Castner, R.E., Edwards, G.S., Harward III, W.E. and Macri, B.J. Investigation of selected potential environmental contaminants: formaldehyde. EPA-560/2-76-009, U.S. Environmental Protection Agency, Washington, DC (1976)

28. Grosjean, E., Williams II, E.L. and Grosjean, D. Ambient levels of formaldehyde and acetaldehyde in Atlanta, Georgia. J. Air Waste Manage. Assoc., 43: 469–474 (1993).
29. Urea–Formaldehyde Foam Insulation Information and Coordination Centre. UFFI. Report on the national testing survey to the Board of Review, Ottawa (1981), cited in reference 5.
30. Department of National Health and Welfare. An investigation of formaldehyde gas levels in houses in St. John's, Newfoundland. Prepared under contract by Geortec Limited. Publication No. 82-EHD-83, Environmental Health Directorate, Ottawa (1982).