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FORMALIN CONTENT OF THREE ICED FISH SPECIES (MACKEREL, HORSE MACKEREL AND SADINELLA) CONSUMED IN CALABAR, NIGERIA

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ABSTRACT: *The objective of this study is to determine the concentration of Formalin in three common species of iced fish namely; Mackerel, Horse mackerel and Sadinella consumed in Calabar, Cross River State of Nigeria. The aim is to ascertain whether the iced fishes consumed in Calabar contains formalin, and its concentration is within the permissible limits of the World Health Organization (WHO) in order to safeguard the health of the people. To achieve this, the samples were collected from the central market (Watt market) in Calabar and prepared by wet digestion method using a modified Nash's reagent, and analysed using a UV spectrometer. The results of the analysis revealed that the average concentration of formalin in the three species of iced fish was 1.42, 1.83 and 2.20 mg/L for Mackerel, Horse mackerel and Sadinella respectively. Thus, the Formalin content of the fishes was in the order: Sadinella > Horse mackerel > Mackerel. These results indicate that the concentration of Formalin in the three species of iced fish consumed in the study area is low and within the permissible limit of WHO. Therefore, the consumption of these species of iced fish may not pose any health risk at the moment. However, effort has to be made by relevant authorities or agencies to ensure that Formalin-free ice fishes are not imported, produced or distributed for consumption in the State.*
KEYWORDS: formalin, concentration, iced fish, consumption, Calabar.

INTRODUCTION

Fish contributes to the growth and health of living body by providing important nutrients such as protein, lipids, minerals and other essential chemicals. Brain selective nutrients such as omega-3 fatty acids, iodine and iron are found in sea fish in a higher amount; these reduce the risk of subnormal brain development and also reduce the risk of heart disease, stroke, cancer and some other serious ailments (Cunnane and Stewart, 2010). Fish and seafood are important part of a healthy diet and one of the major sources of protein.

Fishes are susceptible to spoilage by microorganisms and biochemical reactions during post mortem process because they contain fat, free amino acids and water. As a result, fishes are very perishable and can only be kept fresh in ice for 8 to 14 days depending on the species. In order to keep the freshness of the fish, formaldehyde (formalin) is used as a preservative (Ashie *et al.*, 1996). Although the spoilage of fish can be prevented by applying different processing and preservation method namely; drying, salting, chilling, canning curing, freezing, microwave heating, etc. However, to maintain the freshness of fish and seafood, fishermen and fish vendors tend to carelessly use formalin (formaldehyde) as a preservation agent.

Formaldehyde is easily flammable, colourless and readily polymerized gas at ambient temperature. Formaldehyde is widely used in consumer goods to protect the products from spoilage by microbial contamination. It is often added to keep food pleasing to the consumers, but this chemical poses a hazardous threat to human health (Cui *et al.*, 2007). In food industries, formaldehyde is widely used in food processing for its bleaching effect and also as preservative in order to prevent the product from spoilage by microbial contamination especially seafood (Wang *et al.*, 2007). Formalin which contains 37% formalin formaldehyde has been used as a therapeutant to control ectoparasites and aquatic fungi disease events occurring at fish culture facilities (Rach *et al.*, 1997). Fishes are dipped in a formaldehyde bath for this purpose (Greg *et al.*, 2003). Its residues in the food for human consumption are prohibited because of possible carcinogenicity (Jung *et al.*, 2001). Formalin is the common form of liquid formaldehyde available in chemical shops. The IUPAC or systematic name of formaldehyde is Methanal and it is the first member of the homologue Alkanals, with CH_2O (H-CHO) as its molecular formula. Stoker *et al.* (2004) reported that it is usually found as 37-56% aqueous solution known as formalin.

According to FDA (1998), there are three commercial formaldehyde products approved by US Food and Drug Administration (FDA) that have similar formulations of about 37% formaldehyde used in US aquaculture as parasiticides viz; Parasite-S (for use on all finfish and penaeus shrimp; Western Chemical), Paracide-F (for use on bluegill, catfish, largemouth bass, salmon and trout; Argent Chemical Laboratories) and Formalin-F (for use on bluegill, catfish, largemouth bass, salmon and trout; Natchez Animal Supply. Based on the label recommendations, routine treatment concentrations of formalin ranged from15-250 mg/L for control of protozoan and monogenetic trematodes on fish and shrimp, and up to 2000 mg/L for control of fungi on fish eggs (Jung *et al.*, 2001). Formalin and dimethylamine is also produced from the enzymatic reduction of trimethylamine- N-oxide during frozen storage and it causes protein denaturation and muscle toughness (Bianchi *et al.*, 2007). It results in the loss of food quality because of unacceptable texture, undesirable flavor, odour, colour and its harm for consumers. Sotelo *et al.* (1995) have stated that formaldehyde accumulates during the frozen storage of some fish species, including cod, Pollack and haddock. The deterioration in the quality of frozen fish due to micro-organisms and various biochemical processes is nearly eliminated, but some enzymatic activities cause changes in its products. These changes are of great commercial importance, because they are limiting factor for the shelf-life of frozen seafood (Benchman, 1996).

High level of formaldehyde (e.g. 10-20 mgkg) in fish may not be considered as palatable as human food source (Yasuhara and Shibamoto, 1995). An acceptable daily intake of 0.2 $mgkg^{-1}$ body weight have been set by the United State Environment Protection Agency (USEPA), whereas values of 60 $mgkg^{-1}$ for Gadidae and crustaceans respectively were proposed in 1985 by the Italian Ministry of Health (Bianchi et al., 2007). Formaldehyde poses no significant health risk to humans when consumed in little amounts but at higher doses, it is dangerous to health. According to Norliana *et al.* (2011), USEPA have fixed maximum daily reference dose (RfD) for formaldehyde as 0.2 µg/g body weight. Exposures increasingly higher than the recommended RfD, the potential for adverse health effects increases (Wang *et al.*, 2007). Toxic levels of formaldehyde class 2a carcinogen can induce a variety of illnesses ranging from localized skin/ respiratory tract irritation,

genotoxic effects to cancer (Bosetti *et al.*, 2008, IARC, 2004). In addition, there is evidence linking formaldehyde with nasopharyngeal cancer (Tang *et al.*, 2009). The International Agency for Research on Cancer (IARC) has classified formaldehyde (as well as formalin) as a Group 1 carcinogen (Hossain, 2011).

Fishes are one of the major sources of food and protein to human beings, but in recent times, this consumable fishes are being contaminated with formalin by some evil suppliers or traders who preserve their fishes with it. Connel (1995) have stated that freshness is a property of fish that has a considerable influence on its quality. As a result of this, many fish sellers tend to use preservatives to maintain the freshness of their fish stock for a longer period of time and one of such preservatives is formalin. Consequently, the fishes may become contaminated with high doses of preservatives or formalin. Although some fishes contain little amounts of formaldehyde in their fleshes naturally, higher doses in fish are due its addition as a preservative.

Fish and seafoods constitute an important food component for a large section of world population. The major problem is that the food manufacturers often add formalin in excess to extend the shelf life and freshness of fish which at the end becomes harmful to the health and general wellbeing of the consumers. It is also a thing of concern as people cannot really tell which fish is preserved with formalin or not. Hence this study is set out to determine formalin in three species of iced fish consumed in Calabar, Cross River State of Nigeria.

MATERIALS AND METHODS

The materials used include beakers, conical flasks, ultrasonic water bath, crusher, stirrer, reagent bottles, Aluminium foil and source of power /heat for apparatus, pH Meter and UV- Spectrometer. The reagents used were Ethylacetone, Trichloroacetic acid, Sodium hydroxide, Hydrochloric acid, Acetic acid and Ammonium acetate, all of analytical grade. The fish samples were Mackerel, Horse mackerel and Sadinella.

Sample Collection: A total of nine iced fish samples, three each of the Mackerel, Horse mackerel and Sadinella species were bought from three different sellers in Watt Market, the central market in Calabar and taken immediately to the Chemistry Department Laboratory of the University of Calabar in a plastic food flask loaded with ice blocks to maintain freshness of the samples. The samples were kept in the freezer and used within 2 days in the laboratory.

Sample Preparation: Each fish sample was thawed and cut into small pieces. 60 mL of 6% w/w Trichloroacetic acid was added to 30g of finely chopped fish and the mixture was homogenized in an ultrasonic water bath. The mixture was filtered using a Whatman No. 1 filter paper, 5 mL of the filtrate was collected and its pH adjusted to around 7.0 with NaOH or HCl (Joshi *et al.*, 2015). A solution of 4g NaOH in 1000 mL and 0.1 M HCl were used to adjust the pH of the extract to about 7.0. The almost neutral extract was then stored in a freezer for about 40 minutes.

Modified Nash's Reagent was prepared by mixing 15g Ammonium acetate, 1 mL Ethylacetone, 0.2 mL Acetic acid and the volume was brought to 100 mL by adding distilled water. The prepared

Nash's reagent was kept in a dark-glass bottle covered with Aluminium foil because it is light sensitive. Note: Nash's reagent used here is modified because Ethylacetone was used in place of the usual Acetylacetone. A standard stock solution of formaldehyde of 1.0 Molar concentration was also prepared and kept. 2 mL of the prepared Nash's reagent was then added to the earlier prepared sample extract and heated in a water bath at 60 ^{o}C for about 30 minutes. Finally the absorbance of formaldehyde by the extract was measured immediately at 415nm using a UV Spectrometer.

Sample Analysis: The samples were analyzed using a UV Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at a wavelength of 415nm. At first, a standard formaldehyde solution was analyzed in different fractions the readings recorded by UV Spectrophotometer and the concentrations were used to prepare a standard curve. The formaldehyde concentration of the different fish samples were compared and determine using this standard curve and the results tabulated.

RESULTS AND DISCUSSION

The results of the analysis are presented in Table 1 below.

Table 1: Formaldehyde concentration in mg/L of three (3) different iced fish species consumed in Calabar, Cross River State of Nigeria.

Iced Fish Species	Formaldehyde Conc. in mg/L
Mackerel	1.42 ± 0.02
Horse mackerel	1.83 ± 0.01
Sadinella	2.20 ± 0.02

Values reported in Mean± Standard deviation format, with N=3.

The Standard curve of Absorbance versus Molar Concentration of Formaldehyde is presented in Figure 1 below.

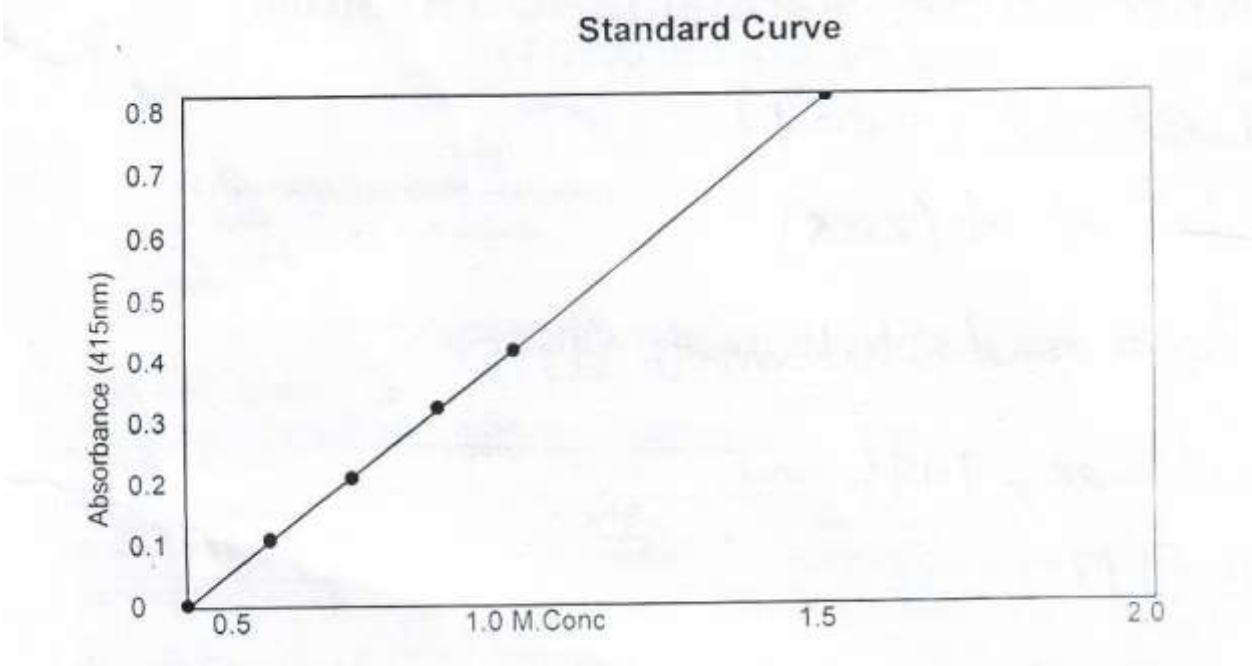

Figure 1: Standard Curve of formaldehyde concentration calculated on the basis of Absorbance versus Molar concentration.

Beer's Law is obeyed within the concentration range of (0.80-23.0) mg/L, with $Y = bx + m$ obtained by the method of least squares, where Y=Absorbance, b= slope, m= Intercept and x= Concentration in mg/L.

The results in Table 1 above revealed that the average concentration of formalin in the three species of iced fish were 1.42, 1.83 and 2.20 mg/L for Mackerel, Horse mackerel and Sadinella respectively. The source of this formaldehyde in fish may be as a result of the addition of certain amounts formalin to the fishes by suppliers or sellers to preserve and maintain the freshness of the fish or extend the shelf life of the fish till they are completely sold, to avoid spoilage and loss of money. On the other hand, the formaldehyde may be produced by the enzymatic reduction of trimethylamine oxide to equimolar amounts of formaldehyde and dimethylamine after postmortem like the case of some marine fish (Sotelo *et al.*, 1995). The amount of formaldehyde might accumulate over time during frozen storage after death.

These results also indicate that amount formaldehyde (Formalin) in the 3 common species of fish consumed in Calabar is still very low and within the permissible limits of World Health Organization (WHO). The WHO have estimated the range of the daily intake of formaldehyde in food to be between 1.5 to 14 mg/L, with the mean value of 7.75 mg/L for an average adult, with no fixed or set limits (WHO, 2001).According to the European Food Safety Authority (EFSA), the daily oral exposure to formaldehyde from the total diet should not exceed 100 mg per day (EFSA, 2014). The United States Environmental Protection Agency (USEPA) has fixed their limit at 2 mg/L (Zhang *et al.*, 2009). Based on these facts, the concentration of formaldehyde in the 3 species of iced fish is low and within the permissible limits for consumption. Even Sadinella whose

average concentration of formaldehyde is 2.20 mg/L and is at the USEPA limit of 2 mg/L may not be all that bad for consumption if it is properly washed and chilled for some time, the concentration of formaldehyde would be further reduced. Thus, the fishes are suitable for consumption and the amount of formaldehyde (Formalin) in them may not cause any harm to the consumers in the study area for now.

CONCLUSION

The findings of this study have shown that the amount of formaldehyde present in the 3 major species of iced fish consumed in Calabar is low and is within the permissible limits of WHO. Also, their consumption may not pose any health risk to the consumers or the inhabitants of the area at the time of this study. However, efforts have to be made by the relevant government regulatory agencies to monitor the fish quality with regards to formaldehyde contamination and other food contaminants in order to safeguard health of the people.

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