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Formaldehyde content of selected fish from the wet markets of Kathmandu valley

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Abstract

In Nepal, the supply of fish inside the country does not meet the domestic need and is imported from neighboring countries mainly from India. Therefore, fishes are stored in ice for a long time before they arrive to the local market in Nepal. Thus, there is a high chance of treating the fish with formaldehyde solution (formalin) to preserve the shelf life. An attempt was taken to assess the fish quality by investigating the concentration of formaldehyde and pH in selected fish available in Kathmandu city. From three different local markets, six species of fishes were collected and quantitative determination of formaldehyde was performed using UV-Vis spectrophotometer. The study indicates the mean range of formaldehyde from 0.393±0.004 µg g^{-1} to 2.328±0.304 µg g^{-1}. Of the species analyzed Magur contained the highest concentration of formaldehyde (2.328±0.304 µg g^{-1}). The pH was found to be in the range of 6.

Introduction

Fish and seafood are a good source of protein and thus are a vital part of a healthy diet (Ashie *et al*., 1996). Importantly, fat, free amino acids and water contained within the fish are susceptible to fast spoilage during post mortem processes (Fernandes and Venkatraman, 1993; Ismail, 2005). Therefore, keeping fish fresh in ice for more than one week is difficult and also depends upon the species chosen. The issue becomes more important to a country like Nepal where the fish supply within the country does not meet the domestic need and it must be imported from other countries, principally India. Lately, many reports in public media have focused to the problem of wholesalers and vendors treating fish with formaldehyde solution (formalin) to preserve shelf life in order to reduce their cost and increase revenues at the price of public health.

Formaldehyde is a member of aldehyde family which occurs in the gaseous form whereas the liquid form is formalin made up of 37% formaldehyde by weight. Formaldehyde is widely used in chemical industries and also as a disinfectant and preservative. Importantly, International Agency for Research on Cancer (IARC) has classified formaldehyde as a Group I carcinogen to humans (IARC, 2004). Those people dealing with formalin over a long period of time are likely to be afflicted with health issues e.g. blindness, asthma and lung cancer (Hossain, 2011). According to the United States Environmental Protection Agency (EPA), maximum daily dose reference for formaldehyde is 0.2 µg g^{-1} body weight per day (Wang *et al*., 2007).

Formaldehyde has been observed at different concentrations in many food items such as fruits, vegetables, meat, fish etc. which forms as a result of normal metabolism in plants and animals. In addition, there is a mechanism of formation of formaldehyde during the ageing and deterioration of fish flesh. However, the resulting formaldehyde also converts to other chemical compounds; therefore high levels of formaldehyde do not accumulate in the fish tissue (Tsuda *et al.*, 1988). Bianchi et al. have reported the concentration of formaldehyde in fresh cod fish from 6.4±1.2 to 21.8±2.8 µg g^{-1} (Bianchi *et al*., 2007).

In Nepal, there is widespread discussion on formalin adulteration in fish but it has not been estimated quantitatively. The purpose of this report is to assess the fish quality by investigating the concentration of formaldehyde and pH in selected fish (commonly available) stored in Kathmandu valley. This report brings the information on the colorimetric determination of formaldehyde measured by UV-Vis spectrophotometer in fish samples. Thus the determined concentration of formaldehyde and pH will be indicators to assess the quality and freshness of the frozen fish.

Materials and Methods

Sample preparation

Fish samples were purchased from three different wet markets in Kathmandu, namely Lagankhel

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(M1), Khichapokhari (M2) and Kalimati (M3). The selection of fish species was based on higher demand of consumers as told by the vendors. The selected species were Mrigal carp (*Cirrhinus* sp.), Magur *(Clarias batrachus)*, Spiny eel *(Macrognathus pancalus)*, Gold fish *(Carassius auratus auratus)*, Rohu *(Labeo rohita)* and River catfish *(Eutropiichthys vacha)*. Each fish sample was kept in a plastic bag and stored in ice after buying from the market. Flesh was separated from the skin and gut using knife. Around 500 g of samples were packed in plastic boxes and stored at -20°C until further analysis. The experimental protocol was followed in accordance with that established earlier (Noordiana *et al*., 2011).

Chemicals

All chemicals were obtained from commercial sources and used without further purification. Formaldehyde, acetyl acetone and trichloroacetic acid (TCA) were obtained from Rankem, Loba Chemie and Fisher Scientific respectively. A 0.1 M NaOH and 0.1 M HCl were used to adjust the pH of the distillate to be in the range of about 7. Nash's reagent (Nash, 1953) was prepared by mixing ammonium acetate (15 g), acetyl acetone (0.3 ml), acetic acid (0.2 ml) and the volume was brought to 100 ml by adding water. Nash's reagent is light sensitive and was kept in a dark-glass bottle covered with aluminum foil. 6% w/w TCA was prepared in water. A 100 ppm standard formaldehyde stock solution was prepared.

Formaldehyde determination

The fish samples were thawed and cut into small pieces. Finely chopped fish (30 g) was added to 6% w/w TCA (60 ml). An ultrasonic water bath was used for the homogenization of the mixture. The mixture was filtered through a Whatman No. 1 filter paper, the filtrate (5 ml) was collected and pH of the filtrate was adjusted to around 7.0 with NaOH or HCl. The extract was then stored in a deep freezer for 30 min. Finally, Nash's reagent (2 ml) was added to the already prepared extract and heated in a water bath at 60°C for 30 min. The absorbance at 415 nm was measured immediately by UV-Vis spectrophotometer (Thermo Electron Corporation, Genesys 10 UV).

Calibration curve

By diluting 10 ppm stock solution of formaldehyde; 0.005, 0.1, 0.5, 1 and 5 ppm concentration of formaldehyde was prepared as mentioned here. To each of the flasks containing required volume of formaldehyde solution, 2 ml Nash's reagent was added and heated in water bath at 60°C for 30 min. Then the volume was brought to 100 ml by adding water in order to make desired concentration. The absorbance of each standard concentration of formaldehyde was determined by measuring the absorbance at 415 nm using the same UV-Vis spectrophotometer. A calibration curve was then plotted from obtained data.

pH determination

Fish flesh (10 g) was weighed and homogenized thoroughly with 100 ml water for 20 min. The pH of the supernatant was recorded using a pH meter.

Results and Discussion

The correlation between concentration and absorbance of the standard formaldehyde solutions was established. As shown in Figure 1, there was a linear relationship between the concentration of the formaldehyde and the absorbance with R^2 value of 0.968. The equation of the line from the calibration curve is y= 0.398x where y is the absorbance; x is the concentration of formaldehyde in ppm and 0.398 is the slope of the line.

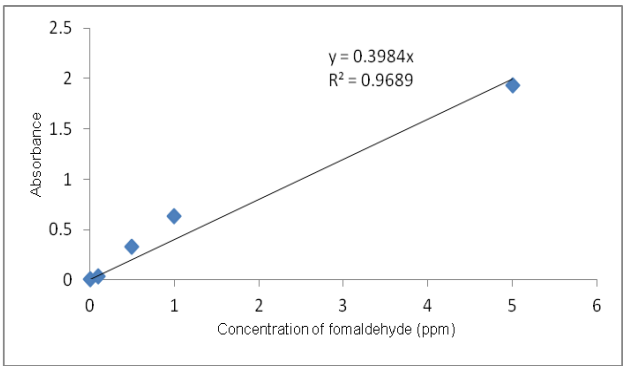

Figure 1. Calibration curve of standard formaldehyde solution

The concentration of formaldehyde in the fish samples collected from three different locations was then calculated from the absorbance measured and calculating the corresponding concentration from the calibration curve. The actual formaldehyde concentration of the solutions of fish extracts were measured using the Nash test followed by colorimetric analysis in the UV-Vis spectrophotometer. The formaldehyde content of the most frequently bought frozen fish in Kathmandu valley are summarized in Table 1. The concentration obtained from UV-Vis spectrophotometer was in ppm unit which was later converted to µg g^{-1} of fish and then tabulated. The highest amount of formaldehyde was in Magur (2.328±0.304 µg g^{-1}) while the lowest amount was in Gold fish (0.393±0.004 µg g^{-1}). The concentration of formaldehyde in all species of all locations is

Table 1. Formaldehyde content in selected fish from different wet markets

Fish Type	Amount of Formaldehyde (μg g^{-1})			
	M1	M2	M3	Mean
Mrigal carp	0.436	0.756	0.696	0.629±0.170
Magur	2.582	1.991	2.411	2.328±0.304
Spiny eel	1.676	1.323	0.795	1.265±0.443
Gold fish	NA	0.390	0.395	0.393±0.004
Rohu	0.619	0.542	0.331	0.497±0.149
River catfish	0.521	NA	0.605	0.563±0.059

NA: not available; mean values are expressed as ± standard deviations

relatively low with general mean value at around 1 μg g^{-1}. In a similar study performed by Bianchi *et al.* (2007), the authors did not investigate further when the formaldehyde was < 1 μg g^{-1} because the low concentration levels are not considered hazardous to health. Different types of fruits, meat and also fish contain certain level of formaldehyde as a metabolite. Bianchi *et al.* (2007) have observed >5 μg g^{-1} as the formaldehyde content in fresh fish samples. Similarly, Noordiana *et al.* (2011) have observed 0.38 to 15.75 μg g^{-1} formaldehyde in different fish species. Our current results of formaldehyde concentration in stored fish match with their findings and it can be assumed that there was no deliberate formalin contamination to the fish. Interestingly, formaldehyde is produced and the concentration increases to 134% after 6 days even when stored at 0°C (Bianchi *et al*., 2007). Therefore, there is a high chance of formaldehyde content increasing when stored for a long time period while transporting from neighboring countries to Nepal. At the same time, cooking decreases the concentration of this volatile analyte because of evaporation (Bianchi *et al*., 2007). In conclusion, the current study revealed the low concentration of formaldehyde in fish and these values are far lower than the limit amount suggested by the Italian Ministry of Health which is 60 μg g^{-1} and 10 μg g^{-1} for fish belonging to the Gadidae family and crustaceans respectively (MINSAM-telegram). Therefore, there is minimal risk to the consumer of fish in Kathmandu with respect to formalin adulteration.

pH also gives a valuable information to determine the quality of freshness. In the current study, pH of the fish species was found to be in the range of 6.0 to 6.5. Typical pH of live fish muscle is approximately 7.0 (Noordiana *et al*., 2011). Slightly lower pH could be a result of the conversion of glycogen in the muscle to lactic acid whereas higher pH indicates storage period and spoilage of the fish (Kyrana and Lougovois, 2002).

Conclusion

The quality of stored fish collected from three different local markets in Kathmandu valley was assessed on the basis of formaldehyde content and pH. Formaldehyde was quantitatively determined by using the Nash test in conjunction with spectrophotometric analysis. The formaldehyde content in all the analyzed fish species was in the range of 0.393±0.004 μg g^{-1} to 2.328±0.304 μg g^{-1} which is indeed low similar to that of fresh fish. Additionally frying or boiling the fish while cooking can reduce the concentration further. The pH of the fish was also in the normal range (around 6). As a general conclusion, it can be mentioned that all the analyzed fish samples reveals low formaldehyde concentration and thus can be considered safe for consumption.

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