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Effects of irradiation on formaldehyde concentration and nutritional changes of formalin treated fish, *Pampus chinensis*

M.M. Hasan[1,*], M.Z. Alam[2], M.H. Kabir[3] and S.A. Mony[3]
[1]Department of Fisheries, University of Dhaka, Dhaka 1000, Bangladesh
[2]Food Processing and Preservation Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commission, Savar, Dhaka, Bangladesh
[3]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
*Corresponding author. Email: msdl.hasan@gmail.com

Abstract
Formaldehyde is a very reactive compound capable of interacting with many functional groups of proteins including intermolecular and intramolecular cross-links of the molecules. The formation of cross-linking bonds may induce conformational change in proteins that favor further interaction of functional and hydrophobic groups. Formaldehyde which has been using illegally as a chemical preservative by some fish traders in our country. A study was carried out to determine the effects of irradiation (1.5 KGy) on formaldehyde concentration and nutritional (protein and lipid) changes of formalin (37% formaldehyde) treated fish (fresh) samples and found that the concentration of formaldehyde both in treated samples (0.37% formalin and 0.37% formalin with 1.5 KGy irradiation) were 37.0 μg/gm and 36.75 μg/gm. On the other hand, the amount of protein and lipid in treated samples before radiation (14.56% and 3.49%) and after radiation (14.15% and 3.25%). That means, radiation has no effect on the change of protein, lipid and formaldehyde.

Key words: Formaldehyde, Irradiation, *Pampus chinensis*

Introduction

Fish is the principle source of supply of protein food for the people of Bangladesh. Traditionally, the people consume fish of fresh water or near shore brackish water origins. Marine fish production has been started and increased considerably only in the recent part. Now, many of marine fish species become popular for consumption to our people than that of fresh water fishes. Marine fish is an excellent source of protein. About 80% of animal protein comes from fish. The protein content of fish on an average, 20%. Marine fish lipids are rich in fatty acid and also contain some glycogen. Fisheries sector plays an important role in the economic maturity of Bangladesh, contributing about 5.5% of the countries GDP during 2000-01 fiscal cycles (Chowdhury 2001). Formalin is a colorless strong-smelling chemical substance usually used in industry of textiles, plastics, papers, paint, constructions and well known to preserve

human corpse. It is water solution of formaldehyde (37% or 50%) which may contain up to 15% methanol as a stabilizer. The breakdown products of formaldehyde in air include formic acid and carbon monoxide that can cause irritation to the eyes, nose and respiratory tract, causing sneezing, sore throat, larynx constriction, bronchitis and pneumonia. Multiple exposures can lead to asthma. It can also affect the skin, causing dermatitis or allergic reaction. Small amount of formaldehyde develops in marine fish but its presence in freshwater fish is unexpected. Formaldehyde develops postmortem in marine fish and crustaceans, from the enzymatic reduction of trimethylamine oxide to formaldehyde and dimethylamine (Sotelo *et al.* 1995). While formaldehyde may be formed during the ageing and deterioration of fish flesh, high levels do not accumulation in the fish tissues, due to subsequent conversion of the formaldehyde formed to other chemical compounds (Tsuda *et al.* 1988).

In the aquatic environment data on the aquatic toxicity of formaldehyde are numerous. Various scientists such as Chou and Que Hee (1992) worked on the toxicity of formaldehyde for freshwater algae, microorganisms, invertebrates, fish as well as marine algae. They found the sensitivity of different organisms varies widely, however, the most sensitive aquatic effects identified were observed for marine algae. Sen (2002) reported the acceptable range of formaldehyde in fish muscle for human consumption is 1 mg/Kg for freshwater fish and 1-5 mg/Kg for marine fish. With a culture of malpractice seeping into every sector and level it is hardly surprising that it has reached the most important of our basic needs food. Many dishonest fish traders use formalin as a chemical that is mainly used with imported fish and it makes the fish stiff and keeps them looking fresh for a longer duration. So, the study was carried out to find out the effects of irradiation on formaldehyde concentration and nutritional changes of formalin treated fresh fish (*Pampus chinensis*) samples.

Materials and methods

All investigations were carried out in the laboratory of Food Processing & Preservation Division, Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh. There the research was initiated through the collection of specimen fish. Specimen fish, Chinese pomfret, (*Pampus chinensis*) commonly known in Bangladesh as 'Rup Chanda' was selected in this study. Fresh pomfret used in this experiment were collected from the Malibag Bazar, Dhaka. Usually, collections were made early in the morning and after collection; samples were taken in a polythene bag with ice and immediately brought to the laboratory of Food Processing and Preservations Division, IFRB, AERE, Savar, Dhaka, Bangladesh. Fishes were divided into the following sampler- Sample A: Only formalin (0.37%) was treated for 20 minutes. Sample B: Both formalin (0.37%) and irradiation (1.5 KGy) were treated here. Panoramic Co-60 (1.5 KGy) source supplied by the Atomic Energy of Canada Ltd. Formaldehyde concentration of the fish tissue was measured by using perchloric acid ($HClO_4$) extraction and the Nash reagent, was developed by Castell and Smith (1973).

Perchloric acid (10%) was taken in a one liter volumetric flask and weighed 85.7 gm of 70% reagent grade perchloric. It was dissolved in a small amount of distilled water and diluted to volume. Potassium hydroxide (30%) was dissolved in 30 gm of KOH along with 65 ml distilled water, cooled and diluted to 100ml. Standard buffer solution was commercially available at p^H 6 and 8. Nash reagents were combined in 2 ml acetyl acetone with 75-80 ml distilled water in a 125 ml Erlenmeyer flask. It was caped and shaken vigorously. Ammonium acetate (150 gm) was dissolved with 300 ml water in a 500 ml beaker. The above solutions combined and diluted to 500 ml. It was made fresh daily and stored in refrigerator. Formaldehyde (1M aqueous) was taken in a 100 ml volumetric flask, weighed 8.12 gm of 37% w/w formaldehyde solution and diluted to volume with distilled water.

A dilution of 1 ml of 1 M HCHO (Stock-1) was prepared to 100 ml with distilled water. Stock-2 was prepared by taking two ml of stock-1 and diluted to 100 ml with distilled water. Working sample was prepared by taking 0, 1, 2, 3 and 4 ml aliquots to give final formaldehyde concentration of 0, 0.2, 0.4, 0.6 and 0.8 μ moles. Standard sample was prepared by diluted all taken standards to 5 ml with distilled water and added to 5 ml Nash reagent and mixed well on vortex. Tubes were heated for ten minutes in a 60 degree centregrade water bath and cooled in cold water for 5 minutes. Absorbance read at 415 nm.

Table 1. Concentration of formaldehyde and absorbance at 415 nm for standard curve

No of observation	Volume of formaldehyde solution (ml)	Formaldehyde concentration in standard solution (μ moles)	Absorbance at 415 nm
1	0	0	00
2	1	0.2	0.138
3	2	0.4	0.289
4	3	0.6	0.434
5	4	0.8	0.576

The samples were blended in food processor. Fifty to hundred gm minced samples were taken. Fifty to hundred gm pre-weighed portions along with 10% $HClO_4$ (two times of sample wt) were blended for 2 minutes. It was required few minutes for setting. Fifty ml of extracted aliquots were collected by filtering. p^H meter was standardized at 6 and 8. Fifty ml of extracted aliquots neutralized using 30% KOH. The volume of KOH was required for keep the samples neutralizing. Neutralized samples were taken of (1 to 5 ml) in 18*150 ml test tube. The samples were diluted to 5 ml with distilled water. Then Nash reagent (5 ml) was added and mixed well on vortex. The tubes were heated for ten minutes in a 60 degree centre grade water bath. Then it cooled in cold water for 5 min. Then the absorbance read at 415 nm.Concentration of formaldehyde in collected samples calculated by using the following formulas-

1. Prepared standard curve
2. (Formaldehyde) concentration expressed in μ moles/ gm fish

$FA = F*M*V_1/V_3*W*(50+V_2)/50$.....(i)

Where, F= μ moles FA read from standard curve, M= moisture content of fish (%), V_1= volume (ml) of perchloric acid added for 1:2 extraction, V_2= volume (ml) of KOH used to neutralized the sample, V_3= volume (ml) of extract added to tube, W= weight of fish used in 1:2 extraction

3. Expressed in μg/gm

$FA = F*M*V_1/V_3*W*(50+V_2)/50*G$.....(ii)

Where, G= 30 i.e. gram molecular weight of formaldehyde

Results and discussion

The protein and lipid content in treated fish samples before radiation (14.56% and 3.49%) and after radiation (14.15% and 3.25%) (Table 2). The concentration of formaldehyde both in treated samples (0.37% formalin and 0.37% formalin with 1.5 KGy irradiation) were 37.0 μg/gm and 36.75 μg/gm (Table 3, Fig. 1). That means, radiation has no effect on the change of protein and lipid. The concentration of formaldehyde at different treatments has remained nearly same. That means, radiation has no effect on change of formaldehyde. There have been no systematic investigations of levels of formaldehyde in a range of foodstuffs as a basis for estimation of population exposure. Although formaldehyde is a natural component of a variety of foodstuffs, monitoring has generally been sporadic and source directed.

Table 2. Determination of Protein and Lipid of treated fish samples

Nutrients	Treatments							
	Dip in formalin (0.37%) for 20 min.				Both formalin (0.37%) and irradiation (1.5 KGy)			
				Mean				Mean
Protein (%)	15.15	13.57	14.16	14.56 (±0.23)	13.57	15.10	13.78	14.15(±0.12)
Lipid (%)	3.26	3.45	3.76	3.49 (±0.03)	2.80	3.15	3.90	3.25 (±0.08)

Table 3. Concentration of formaldehyde (HCHO) of treated samples

	Treatments							
	Dip in formalin (0.37%) for 20 minute				Both formalin (0.37%) and irradiation (1.5 KGy)			
Formaldehyde (μg/gm)	F_1	F_2	F_3	Mean	F_{T1}	F_{T2}	F_{T3}	Mean
	38.45	36.20	36.35	37.00 (±0.07)	37.25	35.50	36.06	36.75 (±0.12)

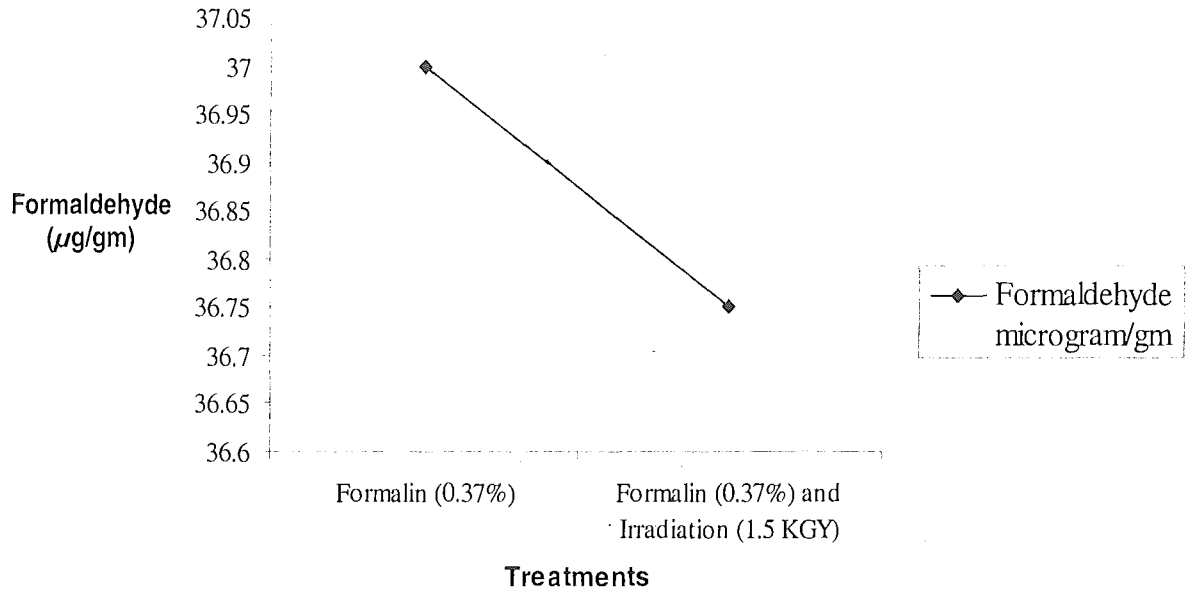

Fig. 2: Effect of treatments on the change of formaldehyde concentration in fish muscle

Lipids and DNA are particularly sensitive to ionizing radiation. Riebroy *et al.* (2007) were investigated the effects of irradiation at different doses (0, 2 and 6 KGy) on the microbiological, chemical and physical properties of Som-fug, a Thai fermented fish mince and they found that irradiation at high dose (6 KGy) might induce lipid and protein oxidation, though the growth of microorganisms was inhibited. Therefore, the irradiation at low dose (2 KGy) could be used to control the over fermentation of Som-fug up to 20 days at 4°C without adverse effects on quality and acceptability.

Crone *et al.* (1992) detected 2-alkyl-cyclobutone, a cyclic compound formed from fatty acids in irradiated but not cooked lipid containing foods. Yasuhara and Shibamoto (1995) suggested that fish containing the highest levels of formaldehyde (e.g., 10-20 mg/Kg) may not be considered palatable as a human food source. Again, in the few studies of the formaldehyde content of foods in Canada, the concentrations of formaldehyde were within the range <0.03-14 mg/Kg (Health Canada 2000). However, the proportion of formaldehyde in foods that is bioavailability is unknown. Available data suggest that the highest concentrations of formaldehyde naturally occurring in foods (i.e., up to 60 mg/Kg) and marine fish (Tsuda *et al.* 1988).

Study on the effects of radiation on the change of formaldehyde concentration in fresh fish was shown that there are no significant effects of radiation on the change of formaldehyde. But its presence in fishes is due to the use of formalin (as preservative) is evident without questions. For that it is recommended to avoid

the use of formalin in fish because it binds with protein of fish muscle and form a macromolecule, which is not digestible that means decline the nutritive value of fish.

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