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Short Review

Formaldehyde in Seafood: A review

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Abstract

Seafood is a nutrient rich commodity accepted all over the world. Illegal addition of formalin (37% formaldehyde) to seafood for extending shelf life affects the health of consumers as it is identified as a potential carcinogen by International Agency for Research on Cancer (IARC). The occurrence of naturally varying levels of formaldehyde present in different food materials including seafood due to post-mortem enzymatic reaction makes the monitoring of illegally added formalin difficult. Understanding the natural levels of free formaldehyde along with proper regulation and monitoring of this harmful chemical is essential for safeguarding health of seafood consumers.

Keywords: Formaldehyde, seafood, carcinogen, background level, detection

Introduction

There is an increased global concern and need for food safety research as the contamination associated with food commodities is on the rise. It is extremely necessary to protect human health to ensure safety in case of food production, distribution, and preparation. Food adulteration, a significant problem in many parts of the world, can lead to many detrimental health effects including developmental defects, chronic diseases or death. The World Health Organization (WHO) works along with Food and Agriculture Organisation (FAO) to tackle the food safety issues based on risk analysis (WHO, 2002a). Microbiological and chemical hazards are the most significant sources of food borne diseases. Inadequate preservation facilities and the time lag due to transport from distant places considerably reduces fish quality by the time it reaches consumers (Haque & Mohsin, 2009). Lack of strict regulatory controls, inadequate cold storage facility, refrigeration, transport and increased demand from consumers has created the space for fraudulent practices for long term preservation.

Reports on marketing of seafood treated with toxic aldehydes like formaldehyde has raised safety concern among consumers (Yeasmin et al., 2010; Noordiana et al., 2011). Illegal use of this chemical for preservation is a widespread practice. Many unscrupulous traders are using this toxic chemical to increase shelf life with least concern to consumer safety. This compound will react with protein and subsequently affect muscle texture (Sotelo et al., 1995). Being antimicrobial in nature, formaldehyde has gained backdoor entry in many industries. But the greatest concern is in food industry, where it is inappropriately used during production of dried foods, vermicelli and chicken paws (Wang et al., 2007). Formaldehyde is classified as Group-I carcinogen to humans (IARC, 2004) and has been reported as one of the chemical mediators to cause programmed cell death called apoptosis. Application of this chemical by means of dipping or spraying on fish can result in residual content in the treated material leading to carcinogenicity (Zhang et al., 2009). This review overview of the toxicity of formaldehyde to humans, its natural formation in marine organisms and its various detection methods.

Formaldehyde as a chemical

Globally, formaldehyde is identified as one of the top 25 most widely produced chemical substances because of its high reactivity, colourless nature, purity, and low cost. Formaldehyde (CH_2O) is a highly flammable, colorless gas with pungent and

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irritating odour and is normally commercially sold as 30 – 50% (by weight) aqueous solutions with a Chemical Abstract Service (CAS) No of 50-00-0 (WHO, 2002b). Aqueous solutions of formaldehyde usually contain 7 to 8% methanol as the stabilizing agent in addition to various metabolic impurities. It is a highly reactive chemical which can undergo quick polymerization. It is also known as methanal, methylene oxide, oxymethylene, methyl aldehyde, oxomethane, and formic aldehyde (Norliana et al., 2009). The other trade names of formaldehyde include Formol, Fannoform, Lysoform, Morbicid acid and Superlysoform. It can decompose at a temperature above 150°C. It readily dissolves in water, alcohol and other polar solvents. In the case of aqueous solutions, it exists as methylene glycol, polyoxymethylene and hemiformals (WHO, 2002b).

Pure form of formaldehyde is not available commercially. Formalin, a solution of 37% formaldehyde gas dissolved in water is the most common form of solution. Formaldehyde is marketed as trioxane $[(CH_2O)_3]$ and paraformaldehyde, a polymer with 8–100 units of formaldehyde in solid form. The chemical characteristics of this compound, particularly its germicidal activity, make it a product of extensive industrial applicability. The physical and chemical characteristics are given in Table 1.

Industrial usage of formaldehyde

Commercially, formaldehyde is produced from methanol. It is a widely used chemical in the production of phenolic polymers such as urea formaldehyde resin, phenolic formaldehyde resin, pentaerithritol etc. In fertilizer production industries it is used as a preservative and disinfectant (WHO, 2002b). In hospitals and anatomical laboratories it is used as a fixative for biological specimens. It finds applications as a wood preservative, a disinfectant in agriculture and in paper pulp producing industries (WHO, 2002b) perfume and cosmetics industry etc. It is a chemical compound that is most widely used in hair products to alter the protein structure of the hair and provide smoothening effect.

In case of food industry it is used as a bacteriostatic agent in cheese preparation (IARC, 1995) in sugar industry (Wilbur et al., 1999), and as a food additive in caviar preparation in Scandinavian countries (WHO, 2002b). Formalin is used as a controller of external parasites in case of cultured fish. In hatcheries it is used as a fungal controller, especially to control aquatic fungi (*Saprolegniaceae*). Formalin is an approved aquaculture drug as per USFDA for the control of parasites and fungi in marine fish farming. A routine treatment concentration of formalin ranging from 15-250 mg L^{-1} for control of protozoan and trematodes on fish and shrimp and up to 2000 mg L^{-1} is used for fungal control on fish eggs (Jung et al., 2001). Its usage is not permitted for aquaculture in Australia, Europe and Japan due to its carcinogenic effect.

Toxic effects in humans

Formaldehyde is an essential metabolic intermediate formed in all cells during the metabolism of amino acids such as serine, glycine, methionine and choline. However, formaldehyde is a highly toxic substance having human carcinogenicity. Chronic inhalation can result in respiratory symptoms and

Table 1. Physical and chemical properties of formaldehyde

Property	Range of reported values
Relative molecular mass (Dalton)	30.03
Melting point (°C)	-118 to -92
Boiling point (°C, at 101.3 kPa)	-21 to -19
Vapour pressure (calculated) (Pa, at 25°C)	516 000
Water solubility (mg L-1, at 25°C)	400 000-550 000
Henry's law constant (Pa. m3 moL-1, at 25°C)	2.2×10^{-2}-3.4×10^{-2}
Log octanol/water partition coefficient (log Kow)	-0.75 to 0.35
Log organic carbon/water partition coefficient (log Koc)	0.70-1.57
Conversion factor	1 ppm = 1.2 mg m^{-3}

*WHO (2002b)

irritation of eyes, nose and throat (Zhang et al., 2008). Increased exposure of this chemical can increase the risk of cancers in pharynx, nasopharynx, and brain, as well as dermatitis and allergic reactions. Ingestion of 30 ml 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. These injuries are most pronounced in the pharyngeal mucosa, epiglottis and esophagus. Systemic effects include metabolic acidosis, Central Nervous System depression and coma, respiratory distress, renal failure and associated cancer and tumor development (Wooster et al., 2005).

During inhalation, formaldehyde can get deposited and absorbed in the upper respiratory tract. For a long period formaldehyde was considered as a potential human carcinogen (Group 2A) based on experimental studies and evidences. In June 2004 International Agency for Research on Cancer (IARC) reclassified it as a human carcinogen (Group 1) based on the evidence of nasopharyngeal cancer in humans (Bosetti et al., 2008). Those who are engaged in spraying or injecting formalin for long period of time will have health complications such as blindness, asthma and lung cancer (Hossain, 2011). The metabolism of formaldehyde in the body is rapid. The metabolic process is catalysed by glutathione-dependent formaldehyde dehydrogenase (alcohol dehydrogenase 5, ADH5) and S-formyl-glutathione hydrolase which results in the formation of formic acid. Formic acid can be incorporated through methyl group into nucleic acids and proteins and is either excreted out in urine or oxidized to form carbon dioxide and will be exhaled out. Formaldehyde of both exogenous and endogenous origin can enters the formaldehyde dehyderogenase metabolism and will be eliminated.

The added formaldehyde content in fish will decrease during storage due to loss along with ice melt water, but cannot be fully removed (Sanyal et al., 2017). A significant reduction in content of formaldehyde during ice storage of treated Indian mackerel is reported (Laly et al., 2017). Increase in TCA soluble proteins during storage of treated fishes was reported previously indicating the protein degradation effect of formaldehyde (Sanyal et al., 2017). A health risk assessment on formaldehyde contaminated fish consumption was reported by Siti et al. (2013). The short term health effects of formaldehyde exposure is well known, but the potential health effects due to lower levels of long term consumption is less known (Goon et al., 2014).

As per the United States Environmental Protection Agency (USEPA) the maximum daily dose reference (RfD) is 0.2 mg kg^{-1} body weight. European Food Safety Authority or EFSA (2014) recommends a oral exposure to formaldehyde as 100 mg formaldehyde per day, corresponding to 1.7 and 1.4 mg kg^{-1} bw per day for 60 kg and 70 kg respectively in humans. The estimated average dietary exposure is about 11 mg kg^{-1} food per person per day (Agence Francaise de Securite Sanitaire des Aliments (AFSSA), 2004). Values of 60 mg kg^{-1} for fishes under family of Gadidae and 10 mg kg^{-1} for crustaceans were proposed by Italian Ministry of Health in 1985 (Bianchi et al., 2007). As per Yasuhara & Shibamoto (1995), formaldehyde in the range of 10-20 mg kg^{-1} in fish cannot be considered as palatable for humans. In the Malaysian Food Regulations 1985, Regulation 148 and 159 (2006), only smoked fish and meat are permitted for formaldehyde absorption while processing and it should not exceed 5 mg kg^{-1}.

Background levels of formaldehyde in seafood

Endogenous formation of formaldehyde occurs in many food items. The occurrence of naturally high levels of formaldehyde in fish can complicate the accurate process of detection of illegally added one. Background levels of formaldehyde are highly variable based on developmental stages and environmental factors and it can range from 0.1-0.3 mg kg^{-1} in milk to over 200 mg kg^{-1} in some fish species (EFSA, 2014). Trimethylamine oxide is an important natural constituent in marine fish muscle which acts as the natural source of formaldehyde and is responsible for the fluctuating formaldehyde concentration in different fish species (Bianchi et al., 2005). The post-mortem formation of formaldehyde in fish is the result of the reduction of trimethylamine-N-oxide to formaldehyde and dimethylamine by trimethylamine-N-oxide demethylase (TMAOase) (Badii & Howell, 2002; Leelapongawattana et al., 2005) which increases with storage. Trimethylamine dehydrogenase (TMADH) from *Methylophilus methylotrophus* bacteria can catalyzes the oxidative demethylation of trimethylamine to dimethylamine and formaldehyde (Jang et al., 1999). TMADH is an iron-sulfur containing flavor protein. Due to subsequent conversion by reaction with proteins, nucleic acids, free amino acids, amines, nucleotides etc, high levels of formaldehyde do not accumulate in the meat (Tsuda et al., 1988). The formation of formaldehyde depends upon time and temperature of frozen storage and it can result in muscle toughening, lower acceptability and functionality.

Post-mortem formation of formaldehyde is reported in case of cod (*Gadus macrocephalus*), alaska pollock (*Theragra chalcogramma*), blue shrimp (*Penaeus stylirostris*) and pacific shrimp (*Pandalus jordani*) (Amano & Yamada, 1964; Flores & Crawford, 1973; Hose & Lightner, 1980). Formation of endogenous formaldehyde in the range of 0.1 – 31.8 mg kg^{-1} were reported in eel (*Anguilla japonica*) (Ueno et al., 1984), striped bass (*Morone saxatilis*) (Xu & Rogers, 1955), banana shrimp (*Peanaeus merguiensis*) (Yamagata & Low, 1995) and Nile tilapia (*Tilapia niloticus*) (Xu & Rogers, 1995). Increase of formaldehyde during frozen storage of whole lizard fish was reported by Benjakul (2004). The natural formation of formalin during post mortem is reported to be much lower in freshwater fish compared to marine fish (Jaman, 2013). While Sanyal et al. (2017) reported a formaldehyde content of 0.066 mg kg^{-1} on 14^{th} day of ice storage of *Cirrhinus mrigala.* The background levels of formaldehyde in seafood as per the available reports are given in Table 2.

Rehbein et al. (1987) distinguished formaldehyde bonding type in fishes as 'free', 'bound' and 'total'. The free form of formaldehyde is having toxicological importance (Bechmann, 1998). The bound form of formaldehyde can result in protein aggregation, insolubilization, changes in conformational and functional properties (Benjakul et al., 2004). Chanarat & Benjakul (2013) reconfirmed the increase in free formaldehyde in lizard fish during extended ice storage and further reported that large amount of formaldehyde get bound to proteins tightly and comparatively lower amount is present in free form. Jaafar et al. (2013) reported that formaldehyde content in all marine fish are influenced by various factors such as the amount of dark muscle, the amount of substrate, cofactors, temperature, storage time and the degree of combination of the flesh.

Chung & Chan (2009) reported the levels of TMAO, TMA, DMA and formaldehyde in 266 different fishes traded in Hong Kong, China and found their presence in three fish species of *Harpadon nehereus*, *Saurida elongata* and *Saurida tumbil*, belongs to the family of Synodontidae (Lizard fishes) and subfamily Harpadontinae. Joshi et al. (2015) reported formaldehyde content in selected fish from the wet markets of Kathmandu valley. Immaculate & Jamila (2018) reported formaldehyde content in immediately iced and delayed (for 7 h) iced fishes of commercial importance such as *Chirocentrus dorab*, *Arius jella*, *Trachinocephalus myops*, *Cephalopholis forosa*, *Sillago sihama, Lates calcarifer* and *Lutjanus malabaricus.* It was in the range of 0.001 - 0.32 mg kg^{-1} in iced fishes and is 10.64 - 18.75 mg kg^{-1} in un-iced.

Detection techniques

Different techniques are available for detection and quantification of formaldehyde in food materials. A

Table 2. Background levels of formaldehyde in seafood

Commodity	Formaldehyde (mg kg^{-1})	Methodology	References
Marine fish	2.38–2.95	Colorimetric	Siti et al. (2013)
Frozen cod	20	Colorimetric	Möhler and Denbsky (1970)
Shrimp	0.39–1.44	HPLC	Rehbein (1987), Radford and Dalsis (1982)
Crustaceans	1–60	Colorimetric	Cantoni, Milva, and Bazzani (1987)
Fish	1-98		WHO (2002b)
Dried squid	35.3	HPLC	Jianrong et al. (2007)
Squid	19.3/26.6	GC/HPLC	Tai-Sheng et al. (2013)
Fish/shellfish	0.33 – 16	HPLC	Wahed et al. (2016)
Fish	6.5 - 293		EFSA (2014)
Bombay duck (*Harpodon nehereus*)	4.7-45.3	Colorimetric	Ting et al., (2005)
Squid (muscle)	10.7-19.7	HPLC	Li et al., (2007)

spectrophotometric method proposed by Nash (1953) is the reaction between acetylacetone, ammonia and formaldehyde to form 3,5-diacetyl-1,4-dihydrotoludine is the basis of Hantzch reaction and is the principle of spectrophotometric reaction by Nash (1953). But this procedure needs long reaction time and cannot be simply adopted for an automatic analysis. Other colorimetric methods are based on 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazol (AHMT), pararosaniline, 3-methyl-2-benzothiazolonehydrazone (MBTH) and chromotropic acid etc., but the colourimetric methods in general are relatively slow and less sensitive (Indang et al., 2009).

Chromatographic methods such as High Performance Liquid Chromatography (HPLC) method having better selectivity, precision and accuracy was proposed by Li et al. (2007) for detection of formaldehyde in squid products. Bianchi et al., (2005) evaluated formaldehyde content in different fish products using a Solid Phase Micro Extraction-Gas chromatography Mass spectrometry (SPME-GC-MS) method based on fiber derivatisation with pentaflurobenzyl hydroxyl amine hydrochloride. Wahed et al. (2016) reported an in-house validated HPLC method for determination of formaldehyde in food and feed. Bhowmik et al. (2017) reported the formaldehyde content in wet marketed fish by HPLC method. Biosensors based on electrochemical transducer have the advantage of being economic and fast response. Biosensors for determining formaldehyde level have been developed based on cells (Korpan et al., 2000), or alcohol oxidase enzyme (Dzadevych et al., 2001) or formaldehyde dehydrogenase (Herschkovitz et al., 2000). New and efficient electronic nose technology is a promising method for seafood quality evaluation and formaldehyde level is reported (Zhang et al., 2009). An electrochemical biosensor for foramdehyde detection in fish is also reported (Aini et al., 2016).

Conclusion

Illegal addition of carcinogenic formaldehyde to food materials including seafood is matter of serious concern to public health. It can also be produced endogenously in range of food materials and in case of seafood the enzymatic action of trimethylamine-N-oxidase results the endogenous origin of formaldehyde. The free form of formaldehyde is of potential toxicological importance, whereas the binding of formaldehyde within the tissue protein affects the characteristics and textural qualities of food concerned. High levels of endogenous formaldehyde complicate the detection of illegally added one. So the background level of free formaldehyde in different varieties of seafood should be investigated, proper regulation and monitoring should be put in place to control the use of this harmful chemical in food applications.

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