

Chapter 5

Orientation to hazards - Chemical -I (Antibiotic residues, Additives & Heavy metals)

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Introduction:

Global population is depending upon seafood as a healthy diet choice because of its richness in high value proteins, health beneficial vitamins, minerals and poly unsaturated fatty acids. Fish is also a primary protein source in most parts of the world. Even though fish supplies many health benefits, seafood can be compromised by different chemical contaminants which are harmful to consumers. Fishes are harvested from waters that are contaminated by varying amounts of industrial chemicals, heavy metals, pesticides and antibiotics. These contaminants may accumulate in fish at levels that can cause human health problems (e.g. carcinogenic and mutagenic effects). Food can become contaminated at any point during production, distribution and preparation. Everyone along the production chain, from producer to consumer, has a role to ensure the safety of seafood.

The number of chemical contaminants is increasing day by day, hence threats associated with chemical contamination of seafood is also increasing. Environmental contaminants mainly include ubiquitous pollutants such as heavy metals and dioxins. Even though they are naturally present in the environment their level can be increased due to anthropogenic influences. Contaminants can also come as toxins produced by fungi (Eg. aflatoxins) and algae (Eg. ciguatera toxin). The different chemical contaminants in seafood can also include food additives that are intentionally added like preservatives, colour retention agents etc. The contaminants can also generate during processing or cooking which include acrylamide and heterocyclic amines. Residue of agricultural chemicals resulting from previous application of pesticides, and veterinary drugs during production and storage of food crops and animals, have been considered as human health hazards. But these types of contaminants have a great potential in control by proper conditions of usage and their presence. Also some natural components of food can also act as contaminant like allergic substances and phyto haemagglutinin.

Basically the chemical contaminants are classified into three main groups such as:

(i) Naturally occurring – allergens, Mycotoxins, Scomberotoxin (Histamine), Ciguatera poison, Puffer fish poison, Shellfish toxins (PSP, DSP, NSP, ASP)

(ii) Unintentionally or incidentally added chemicals – Pesticides, Fungicides, Fertilizers, Toxic compounds, Toxic metals

(iii) Intentionally added chemicals and food additives - Food preservatives, Food additives, Vitamins, Minerals, Antibiotics used in aquaculture, Sulfites used in shrimp to prevent melanosis, Nitrites as preservatives, Colouring agents, Detergents

Biotoxins

Marine biotoxins are responsible for many seafood borne diseases. It includes both shellfish toxins and ichthyotoxins (fish toxins). Shellfish toxins include Paralytic shellfish toxins, Diarrhetic

shellfish toxins, Azaspiracid shellfish toxins, Neurotoxic shellfish toxin and Amnesic shellfish toxins. Ichthyotoxins include Ciguatera toxin and Tetrodotoxin. Fish poisoning is caused by consuming fish containing poisonous tissues and shellfish poisoning results from ingestion of shellfish that have accumulated toxins from the plankton they have consumed.

(i) Tetrodotoxin (Puffer fish poison): It is the most lethal of all fish poisons. Toxin production is due to the activity of symbiotic bacteria. Toxin will be accumulated in liver, ovaries and intestine as a defence mechanism. But the muscle is free of toxin. It is also called as Tetradon poisoning or Fugu poisoning. It is 275 times more toxic than cyanide. On an average a dose of 1-2mg of purified tetrodotoxin can be lethal to humans.

(ii) Ciguatera - Ciguatera is a clinical syndrome caused by eating the flesh of toxic fish caught in tropical reef and island waters. Most common fish poisoning and the fish becomes toxic due to feeding of toxic algae – dinoflagellates, *Gambierdiscus toxicus*. Red snapper (*Lutjanus bohar*), Grouper (*Variola louti*) and Moray eel are recorded as ciguateric. More than 400 species have been implicated in ciguatera poisoning.

(iii) Paralytic shell fish poisoning (PSP) – This is associated with dinoflagellate blooms (*Alexandrium catenella*, *Gonyaulax tamerensis*). Heat stable saxitoxin will be accumulated in mussels, clams, oysters, scallops etc. grown in algal bloom areas. Greater number of human deaths is reported due to consumption of contaminated shellfish. The current regulatory level for fresh bivalve molluscs in most countries is 80 µg/100 g.

(iv) Diarrhetic shellfish poisoning (DSP) - Dinoflagellate *Dinophysis fortis* is the algae which produces okadaic acid, the causative of DSP. Primary symptom is acute diarrhoea. Regulatory level in fresh bivalve molluscs in most countries is 0-60 µg /100 g.

Mouse bioassay and analysis by HPLC are the important methods for monitoring biotoxins. Reliable sampling plans are required for effective monitoring.

Heavy metals

Heavy metals are toxic metals and above a normal level can affect the quality, safety and marketability of seafood. They are “Cumulative poisons” which can irreversibly accumulate in the body. They have atomic weight higher than 40.04 and specific density > 5g/cm. The main threats are Arsenic, Cadmium, Mercury and Lead. These metals have no beneficial effects in human and they have no homeostasis mechanism. These contaminants are highly dependent upon geographic location, species and fish size, feeding pattern, solubility of chemical and their persistence in the environment.

Lead is mostly deposited in bones and not in soft tissues. But, from food safety point of view lead accumulation in edible parts is important. Compared to fish lead content is higher in shellfishes as it is getting accumulated in hepatopancreas. The organic form of lead, tetra alkyl lead is mostly found in fish. In fishes Cd is mostly deposited in kidney and liver and in muscles the level is quite low. In invertebrates like Cephalopods it can go as high as 30 ppm in digestive glands. Hence the digestive gland must be removed immediately after catch. Both Cd and Pb are carcinogenic in nature. Mercury is one of the most toxic heavy metal in the environment. Among metal contaminants methyl mercury has elicited the most concern among consumers. It is toxic to the nervous system especially the

developing brain. Arsenic is a widely distributed metalloid and major contaminant in case of ground water. IARC has classified inorganic arsenic as a human carcinogen.

The most widely used techniques for detection and quantification of heavy metals are Atomic Absorption Spectrometry, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Determination of heavy metals in seafood

Principle

Plasma is a stream of highly ionized gas containing an equal number of electrons and positive ions. Plasma is electrically conductive. It is affected by a magnetic field. When plasma energy is given to an analysis sample from outside, the component elements (atoms) is excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays and the content of each element is determined based on the rays intensity.

To generate plasma, first argon gas is supplied to torch coil, and high frequency electric current is supplied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the centre of torch tube. The steps leading to the emission are desolvation, vaporization, atomization and ionization.

Sample digestion

Sample should be homogenous, representative of bulk, free of suspended particles and free flowing. Samples are digested in a microwave digestion unit. Take 0.25 to 0.5 g of sample to pre cleaned digestion vessel. Add 8 ml nitric acid and slowly add 2 ml H₂O₂ to it. Keep it for 10 minutes. Close the vessel and keep in microwave digestion chamber for digestion. After digestion the samples are made up to 100 ml. digested sample is introduced to ICP-OES for analysis.

Hydride generation kit is used for analysing elements like Hg, As, Bi and Se.

Sample analysis – Inductively Coupled Plasma (ICP) Spectrometer ICAP 6300 Duo view

The detector is solid state CID detector, which can simultaneously analyse a sample for multiple elements. ICAP 6300 has a high performance optical system. The design has been optimized to offer resolution over the entire spectrum from 166 nm to 847 nm enabling access to all wavelengths and minimizing spectral interference.

Histamine in fish

Though all types of biogenic amines can be formed in fish, the most toxic amine detected in fish is histamine. Histamine poisoning is the most common form of toxicity caused by ingestion of fish and is generally due to the ingestion of foods containing unusually high levels of histamine. The commonly implicated incidents of histamine poisoning are associated with the fish families Scombridae and

Scomberesocidae. It is also known as Scombroid poisoning. Histamine is a powerful biologically active chemical present in the mast cells and basophils in larger amounts. Histamine poisoning is often manifested by a wide variety of symptoms. Major symptoms affecting the cutaneous system include rashes, urticaria, edema and localized inflammation etc. gastrointestinal effects include nausea, vomiting, diarrhoea and abdominal cramps. Also include symptoms like hypotension, headache, palpitation, tingling and flushing. Severe suffocation and respiratory distress have been reported in severe cases of histamine poisoning. The onset of histamine poisoning can extend from 10 minutes to 1 hour following consumption of contaminated fish and can last from 12 hour to a few days. Histamine concentration required to produce poisoning varies with respect to the susceptibility of each individual. In case of susceptible individuals concentration between 5 and 10 mg/100g can cause symptoms. Many foods contain small amounts of histamine which can be tolerated easily.

As per USFDA guideline the toxicity and defect action level established are 50 mg/100g and 5 mg/100g respectively. According to EU regulation No 2073/2005 mean value all samples (nine) must not exceed 10 mg/100g, two samples may be > 10 mg/100g but < 20 mg/100g and no sample may exceed 20 mg/ 100g. According to USFDA guideline for the control of histamine production a core temperature of 4.4 °C or less should be achieved and maintained throughout handling, processing and distribution of susceptible species.

A wide variety of procedure for the determination of histamine and biogenic amines is available. Include both semi quantitative and quantitative methods. Methods based on colorimetry, fluorometry and enzyme-linked immunosorbent assay (ELISA) are available. Mostly biogenic amines including histamine is analysed by High Performance Liquid Chromatography (HPLC) methods with pre and post column derivatisation and UV-visible or fluorescence detection. LC with tandem mass spectrometry (MS/MS) can also be a useful approach for an unequivocal confirmation of the studied analytes.

Antibiotics

Illegal use of antibiotics for veterinary purposes has become a matter of public concern. Antibiotics are used in aquaculture as prophylactics, as growth promoters and for treatment of diseases. They are usually administered in feeds and most commercial shrimp feeds contain antibiotics. The feeding of antibiotics as growth promoters is associated with decrease in animal gut mass, increased intestinal absorption of nutrients and energy sparing. But inappropriate and frequently abusive, use of antibiotics can affect human health. The two major concerns are the presence of antimicrobial residues in edible tissues and the emergence of antimicrobial resistance, which represents a huge threat to public health worldwide.

The greatest potential risk to public health associated with antimicrobial use in aquaculture is the development of a reservoir of transferable resistance genes in bacteria of aquatic environments. The antibiotics lose their efficacy over time because of the emergence and dissemination of resistance among bacterial pathogens.

EU implemented “zero tolerance policy” regarding antibiotic residue. Using LCMSMS method EU laboratories are equipped to detect traces of prohibited carcinogenic antibiotics like chloramphenicol up to 0.3 ppb and nitrofurans up to 1 ppb levels. Many of the antibiotics are listed as prohibited substance in fish and fishery products. In India the tolerance limit has been set only for the following antibiotics

| Antibiotic | MRL (ppm) |
|-------------------|------------------|
| Tetracycline | 0.1 |
| Oxytetracycline | 0.1 |
| Trimethoprim | 0.05 |
| Oxolinic Acid | 0.3 |

The monitoring of antimicrobial residues in fish tissues requires sensitive and selective analytical methodologies to verify the accomplishment of the legal framework and reach the desirable high standards of quality and food safety. The methods can be microbiological, immunochemical or physico chemical. European council directive 96/23/EC, 1996 gives direction on measures of monitoring residues in live and animal products. It specifies spectrometric detection, GC, HPLC, ELISA and LC-MS/MS methods.

Food additives

Food additives means substances that normally are not used independently as food or its ingredient and which, after being added to the food during its production, processing packaging, transportation or storage, remain included in the food, even in changed state. In simpler terms, food additives are the substances which are added to food by the manufacturers to facilitate processing or to improve appearance, texture, flavour and keeping quality. Functions of food additives are

- To maintain product consistency – Eg: emulsifiers, stabilizers, thickeners etc
- To improve nutritional quality – Eg: vitamins, minerals
- To improve product safety and quality – Eg: preservatives, antioxidants
- To aid in process or preparations – Eg: leavening agents
- To enhance sensory characteristics of the product

Classification of food additives

Food additives are classified based on their function in food, i.e. the purpose for which the additive has been incorporated in the food.

- ⇒ antioxidants
- ⇒ preservatives
- ⇒ food colours
- ⇒ food flavours
- ⇒ emulsifiers and stabilizers
- ⇒ anti-caking agents
- ⇒ sequestrants
- ⇒ acid, bases and buffers
- ⇒ anti-foaming agents
- ⇒ sweeteners
- ⇒ enzymes, and
- ⇒ leavening agents.

⇒ Preservatives

Preservatives are substances which when added to food, retard, inhibit or arrest the activity of microorganisms such as fermentation, acidification and decomposition of foods.

In India, the preservatives have been grouped into two classes – Class I and Class II preservatives.

Class I preservatives are

Common salt 2. Sugar 3. Dextrose 4. Glucose 5. Spices 6. Vinegar or acetic acid 7. Honey 8. Edible vegetable oils

Class II preservatives are

Benzoic acid including salts thereof 2. Sulphurous acid including salts thereof 3. Nitrates or Nitrites and/or Sodium and Potassium in respect of foods like ham, Pickled meat 4. Sorbic acid and its sodium, 5. Potassium and calcium salts 6. Propionates of Calcium or sodium, 7. Sodium, Potassium and Calcium salts of Lactic acid. 8. Nisin 9. Methyl or Propyl parahydroxy Benzoates 10. Sodium Diacetate.

Sulphur dioxide, bisulphites and sulphites –

Some of the commonly using additives are detailed here.

Sulphites, as a source of Sulphur dioxide - preservation of a number of food items viz. jam, jelly, marmalade, fruit, fruit pulp and juices, syrups and sherbets, alcoholic beverages, confectionery, dry fruits and meat products. Control enzymatic and non-enzymatic browning reactions

Nitrates and nitrites – effective against bacteria like *Clostridium botulinum* and *Staphylococcus aureus*

Benzoic acid and salts - protect foods against yeasts and moulds. Benzoic acid is used in squashes, fruit syrups, cordials, juices, jams, marmalades, preserves, sweetened ready-to-serve beverages, pickles, chutneys, sauces, tomato puree and paste and fat spread

Sorbic acid and its salts - These are effective against moulds, yeasts and many bacteria. widely used for bakery and confectionery products like cakes, fillings for chocolates and various types of cheese, cheese spreads and fat spread, paneer and ready-to-eat preserved chapatis

Antioxidants

Antioxidant means a substance which when added to food retards or prevents oxidative deterioration of food. According to the FSS (Food Product Standard and Food Additive) Regulation, 2011 this does not include substances like sugar, cereal, oils, flours, herbs and spices. Under the regulation, no antioxidant other than lecithin, ascorbic acid and tocopherol shall be added to any food. However, the following antioxidants, not exceeding in concentration mentioned against each, may be added to edible oils and fats except ghee and butter, namely

1. Ethyl gallate or mixture thereof 0.01 per cent
2. Propyl gallate or mixture thereof 0.01 per cent
3. Octyl gallate or mixture thereof 0.01 per cent

4. Dodecyl gallate 0.05 per cent
5. Ascorbyl palmitate 0.02 per cent
6. Butylated hydroxyanisole (BHA) 0.02 per cent
7. Citric acid Limited by Good Manufacturing Produces (GMP)
8. Tartaric acid Limited by Good Manufacturing Produces (GMP)
9. Gallic acid 0.01 per cent
10. Resin Guiace 0.01 per cent
11. Tertiary butyl hydro quinone (TBHQ) 0.02 per cent

Food Colors

Many of the food processing operations like drying, canning, roasting, frying etc. lead to loss of the attractive natural colour of foods. Colour additives are also used in foods to correct natural variations in food colour. Food colors are used to

- ✚ to enhance colours that occur naturally but at levels weaker than those usually associated with a given food.
- ✚ to provide a colourful identity to foods that would otherwise be virtually colourless.
- ✚ to protect nutrients such as vitamins and flavours that may be affected by sunlight.
- ✚ to provide an appealing variety of foods to consumers.
- ✚ to compensate for natural or seasonal variations in food, raw material or the effects of processing and storage to meet consumer expectations.

The colouring matter in foods can be broadly classified into two groups – natural and synthetic colours. Beta-carotene, Beta-apo-8' carotenal, Methylene ester of Beta-apo-8' carotenoic acid, Ethylester of Beta-apo-8' carotenoic acid, Canthaxanthin, Chlorophyll, Riboflavin (Lactoflavin), Caramel, Annatto, Saffron, and Curcumin or turmeric natural colouring matter which are permitted with indication in label. Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, Sunset yellow FCF, Indigo Carmine, Brilliant blue FCF and Fast green FCF are synthetic food colors permitted for use in food.