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EDITED RESEARCH REPORT

Module on fish and shellfish hygiene for postgraduate students in Veterinary Public Health, School of Public Health, University of Ghana, Legon

BY

Ottah Atikpo, M.A. and Awumbila, B.

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VETERINARY PUBLIC HEALTH

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Course Title : Veterinary Public Health

Course credit : 3 credits

Course Code : GSPH 618

Module 2 : Fish and Shellfish Hygiene

Module Introduction

Goodmorning, and welcome to module 2 of the Veterinary Public Health Course which attracts 3 credits.

In this module, topics we are going to discuss under Fish and Shellfish Hygiene are arranged under six units, of which each unit would be equivalent to 60 minutes lecture time :

Unit 1 : Introduction and review of fish hygiene in Ghana.

Unit 2 : Fish harvesting, storage and distribution.

Unit 3 : a. Fish spoilage, fisheries and inspection.
b. Fish borne diseases.

Unit 4 : Fish preservation.

Unit 5 : a. Microbial safety of fishery products.
b. Fish and fish products as sources of food poisoning in man.

Unit 6 : a. Practical assessment of fish spoilage by physical and chemical tests.
b. Field trip.

Course goals

The goals for this course under Module 2 are to study fish and shellfish hygiene as relates to spoilage so as to be effectively able to investigate and control foodborne disease outbreak as relates to food poisoning in man caused by microorganisms, especially when such products are not properly handled and adequately processed from the harvesting of fish through processing, distribution to consumption.

On completion of this course, you will be in a better position to easily recognize fish and shellfish products that may cause public health hazards and appreciate the safety values attributed to the need for inspection of fish and its products, proper storage, preservation and handling of such products during distribution in order not to contaminate them.

Also, to be able to carry out internationally recognized physical and chemical standard tests on fish at the shores and on boats as well as at the sale depots for the purpose of finding out the level of freshness, in addition to investigating the causative organisms implicated in cases of food poisoning.

At the end of the course, you will be assessed by your level of participation in group discussions, written reports of field trips (i.e. visits to fish processing plants, fish harbour and fish marketing centers), classroom presentations, case studies and finally a written examination on the topics studied as outlined above.

Unit 1: Introduction and Review of Fish Hygiene in Ghana

Aim: To familiarize students with fish hygiene practices in Ghana and codes of practice in fisheries management.

Learning objectives:

On completion of this module, you will be able to:

1. review the trend of fisheries in Ghana.

2. familiarize with some definitions and principles involved in quality and quality assurance in the fish processing industry.

3. educate personnel in fisheries on good fish hygienic practices.

4. acquire a foundation on which to build on in order to develop as professionals in the application of good inspectors of fish, shellfish and their products.

Content :

The first Unit will include:

1. A brief introduction into legislation relating to fish hygiene i.e. the handling and processing of fish.

2. Codes of practice (which are normally not enforceable by law) which processors must follow, in order to maintain uniform standards based on good practice during handling and processing of fish.

3. Discussion on codes of practice for fish in developed countries via-a-vis what prevails in developing countries as Ghana i.e. :

    - Codex Alimentarius Commission Joint FAO/WHO Food Standards Programme.

The international food standards organization is the Codex Alimentarius Commission set up in 1962 under joint auspices of two bodies of the United Nations namely, Food and Agricultural Organization (FAO) and World Health Organization (WHO); and therefore membership of Codex is opened to member countries of FAO and WHO.

The purpose of Codex is the development of food standards for all principal foods (i.e. raw, semi-processed and processed) aimed at protecting both the consumer and manufacturer in the food trade, ultimately with the scope to facilitate international trade in terms of food hygiene, food additives, pesticide residues, contaminants, labeling and presentation, as well as methods employed in sampling and analysis.

- FAO Codes of Practice for fishery products

In addition to the Codex Standards for fishery products, a set of Codes of FAO Fisheries Circular 318FAO Department of Fisheries is referred to as FAO Codes of Practice that provide technical guidance to manufacturers of products that meet the standards set by Codex Alimentarius.

The FAO Codes of practice are specific for particular fish types e.g.
For i. Fresh fish (FAO Fisheries Circular 318)
ii. Frozen fish (FAO Fisheries Circular 145)
iii. Smoked fish (FAO Fisheries Circular 321)
iv. Canned fishery products (FAO Fisheries Circular 315)
v. Salted fish (FAO Fisheries Circular 336)

- OECD/IIR Draft Code of Practice for Frozen fish, 1969. (OECD – Organization for Economic Cooperation and Development, Paris; IIR – International Institute for Refrigeration, Paris).

- Recommendations for the Processing and Handling of Frozen Foods, IIR, 3rd edition.

In majority of developed countries, there are a well established codes of practice and guidelines for their fishermen, processors, retailers and people involved in the handling and processing of fish. However, developing countries do not have adequate legislation covering fish inspection and quality control.

In Ghana, on a large scale the implementation of fish hygiene has been questionable due to traditional/cultural norms.
Mostly emphasis has always been laid on hygiene and quality control programmes of fish for export purposes solely for the international markets, whereas the need for such legislation to cover control of fishery products to promote and enforce good manufacturing practices and effective quality control procedures in most fish processing plants for domestic consumption is limiting.

However, the national or local food laws address public health problems associated with the consumption of fish and these are enforced by a team of inspectors from Public Health institutions and the Ministry of Food and Agriculture (MOFA) in the country which are responsible for hygiene surveillance at fish landing sites, especially the Tema fish harbour where large landings are recorded.

Inspection of fishery products has been more pronounced for finished products meant for export than for raw fish at the shores and market centers; hence sometimes fish products like smoked fish have been rejected on the international market simply because the raw materials used may have been substandard thus resulting in poor quality finished products which on the surface may look attractive.

In Ghana, fish processors operate small and medium scale enterprises which are labour intensive, are laxed in enforcing quality control measures, do not practice adequate hygienic and sanitary control procedures and thus produce substandard finished products that have short shelf-life and little appeal to the consumer.

Fish markets often lack adequate facilities to ensure basic cleanliness and sanitation. Taps for clean running water are not located in the fish sheds. Fish landing places too are not properly provided with such facilities.

Ice is lacking for proper use on fish. Where available the ice is exposed to the weather and dust settles on it and becomes a source of contamination to the fish. Also the ice melts due to exposure so that it is wasted and not efficiently utilized.

Transportation, storage and distribution facilities for fish and its products follow the same pattern i.e. unsanitary practices of fish handling, preservation, storage, processing and distribution resulting in contamination and decomposition.

The processing and grading of fish and shellfish (Fig. 1a and 1b) products at fish landing areas and on sandy beaches, in sheds and markets contribute to further degradation of the quality of the raw material before it is even further abused during extended processing (Fig. 2a and 2b).

Insufficient education and training of workers and processors in the fish industry is also a contributory factor. Education in time factor during fish work must be enforced in order that the fish is processed fast enough to avoid a situation where deterioration sets in.

Few companies have well established and efficient quality control (QC) systems, and such companies are forced to comply as a result of the trade link and expectations with their importing partners in the developed country.

Few industries however have a well designed quality control system, which may not be well understood or may be poorly executed.

Terms to familiarize with before embarking on specific aspects of a quality assurance programme for fish inspection include :

1. **Quality :**

This is the totality of the features and characteristics of a product that bear on its ability to satisfy a given need.
Quality is the degree of excellence. In fish products, and is determined by factors like

Fig.1a. Crabs packaged for distribution and sale.

Fig.1b. Shrimps held out to attract consumers.

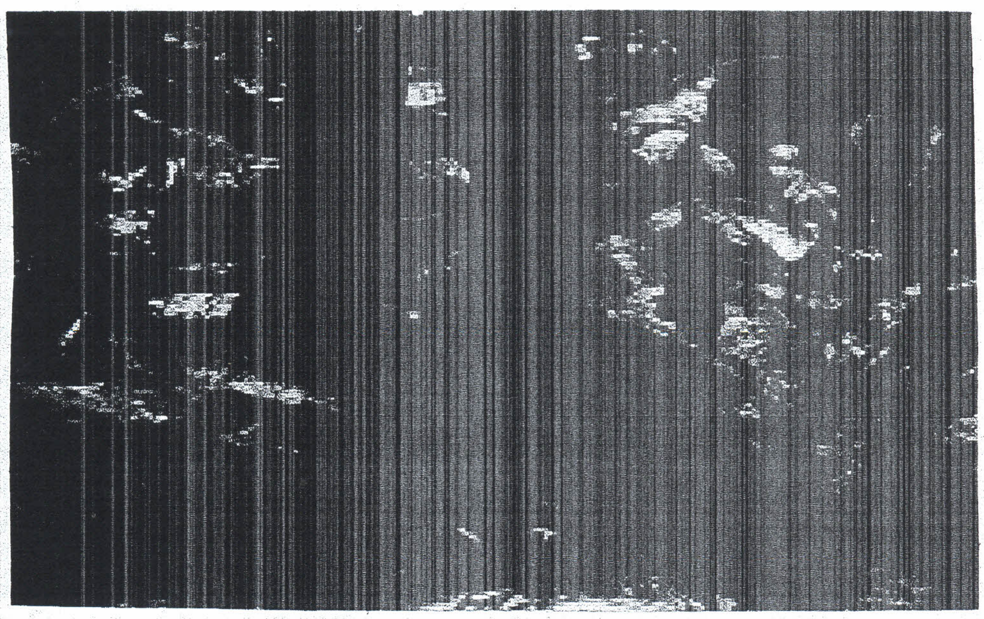

Fig.2a. Contamination of fish by man.

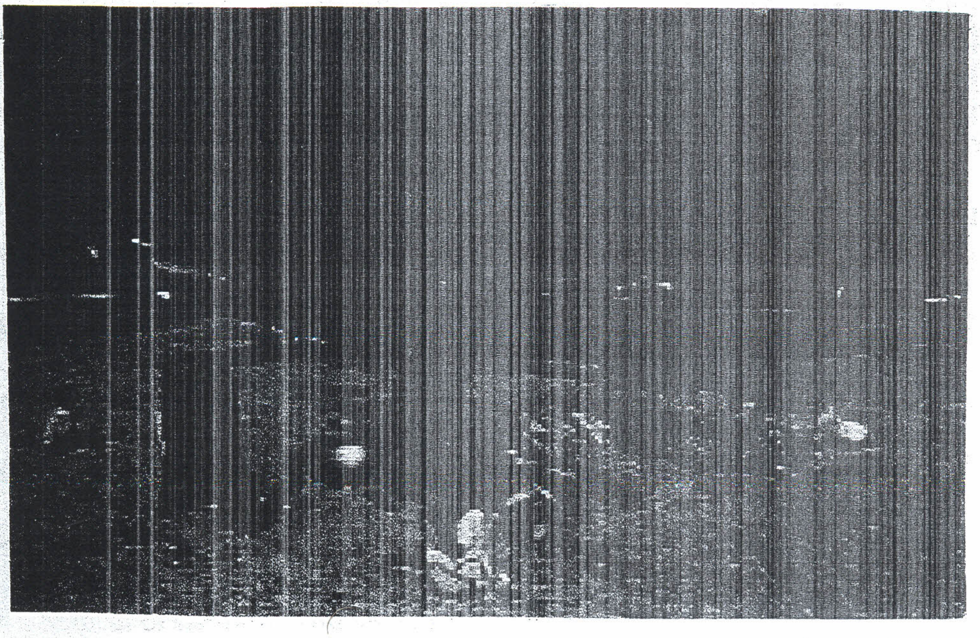

Fig.2b. Fish contaminated by dumping unprotected on sea sand.

freshness, storage deterioration, size of fish, freedom from blemishes, wholesomeness, packaging, species, ease of preparation, appearance, odour, flavour, texture, size, presence or absence of bones, blood, filth, absence of specific microorganisms, condition, packaging and composition.

2. Quality assurance :
Quality assurance in the fish industry is a combination of technological knowledge and managerial skills in a company to achieve and maintain high quality in the products of the company. It is all the activities and functions concerned with the attainment of quality from both the technical and management point of view that cover policy, administration, management and the technology of quality control.
As such, it is not only individuals in the Quality control department who make contribution to the quality assurance programme but others as well in the company indirectly linked to the provision of quality.
Consequently, personnel involved in food hygiene and sanitation must be adequately trained to maintain documentation on quality of fish entering the process and quality of end product must leaving the production line.

The need for quality assurance to be an integral part of company policy and management, and therefore a need for a systematic approach to quality assurance systems (i.e. ISO 9000 to 9004) must be encouraged and applied in the fish processing industry.

3. Quality control :
This is the maintenance of quality at a level that satisfies the customer and that is economical to the producer or seller, i.e. the operation techniques and activities that sustain the product quality to specified requirements, and the use of such techniques and activities. It is an active process that monitors and sometimes when necessary modifies the production systems in order to consistently achieve and maintain the required quality. Monitoring therefore will involve the measurement of properties of products (raw fish) entering and passing through the process to ensure that their qualities are appropriate for achieving the required quality of the end product.
Quality control therefore includes control over hygiene and sanitation of the plant and processes in order to achieve a wholesome product.

Documents required for a quality assurance programme are:

- Product specifications
- Process specifications
- Codes of practice
- Manual of sampling and testing, and
- Protocols for quality control.

Product specifications are written description of what the customer wants. A company must therefore as a matter of course prepare a specification of quality for the product or product types it makes, which may be prepared solely or independently by the company or prepared in consultation with the customer. The company may also have in-house specifications for intermediate products taken from the processing line.

Specifications should also be prepared for materials entering into the process apart from the fish itself i.e. ingredients like additives, and for non-food items like packaging materials, cleaning materials and sanitizers.

It is on the basis of these specifications that incoming materials are liable to inspections and testing, and that tenders for supply are either rejected, or the price renegotiated accordingly.

A specification therefore consists of a definition of the product, and any permitted sub-class like size or species, a list of factors which govern the quality, quality grades, factors that are present or absent, or definition of the product as a value on a scale, with the specification indicating how batches of the product should be sampled and how some factors should be measured.

Process specifications are written description of how the product is to be made, mainly for the benefit of the producer, and involves the essential requirements for stages in the manufacture of a product that are critical for its quality, particularly its safety. It involves specification of the nature and quality of raw materials to be used, how processing steps should be carried out, and hygienic and sanitary requirements for the product.

In general, process specifications must follow 'Good Manufacturing Practices' (GMPs) for the manufacture of the particular product.
Process specifications should be drawn up by the company as part of its quality assurance programme, or with the customer's specifications.

Codes of Practice are codes of Good Manufacturing Practice, which describe the procedures to be followed, and the precautions to be taken in handling and storage of material in order to ensure a safe and high quality product. They are specific for a type of product e.g. chilled fish, frozen fish, shrimps etc.

Manual of Sampling and testing describes general procedures for the taking and storage of samples, procedures for testing in the Quality Control (QC) laboratory or on the processing line. This manual is intended for QC staff.

Protocols for quality control involves procedures for the quality control of a product from reception of raw materials to dispatch from the factory. The protocol should be based on an appraisal of the critical points in the process where quality can be lost or the product rendered unsafe for consumption.

The protocol states the quality of raw materials entering the process, how the materials are to be sampled and tested, and the criteria for approving them, or not, for the process; stages where the process will be examined, measurements to be made, if any, and the action to be taken if the process or the products at that stage are not within the specified limits; descriptions of sampling and testing of the end product, including its packaging, and any final checks just prior to dispatch; limits of temperature and time of storage or any other storage conditions will be specified.

Analysis of a process for Critical Control Points

To analyze a process for Critical Control Point, a formalized system to ensure microbiological safety and quality, and to reduce food poisoning risks and prevent spoilage is used. This system is called the Hazard Analysis Critical Control Point (HACCP). It identifies the hazards associated with the production of food and their severity, identification of critical points in the process where hazards can be controlled, specification of the procedures for exercising control over hazard, and establishment of procedure for monitoring the effectiveness of the control procedures. For fish HACCP is important due to the perishable nature of fish.

HACCP is being encouraged in Ghana in large-scale fish processing factories such as Divine Seafoods and Pioneer Food Cannery, the two of which will form the basis of practical demonstration of quality control systems and good application of hygiene in the fish industry during the field trip (Unit 6).

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Unit 2: Fish harvesting, storage and distribution

Introduction:

Welcome to Unit 2 of the Veterinary Public Health Course. This unit will look into the harvesting of fish from the wild, how it is stored and finally distributed. It will give an insight to these aspects as relates to fisheries in Ghana in particular and also on the international scene.

It would be expected that on completion of this unit you would be able to differentiate between good fish handling practices and unhygienic ways of handling fish that invariably add to the introduction of spoilage microorganisms.

At the end of this unit, you will be expected to describe the different fish harvesting methods that pertains in Ghana and describe improvements in the storage of the different types of fish (i.e. fresh and processed).

Aim: To understand ways of harvesting, storing and distributing fish in Ghana.

Learning objectives are to:

1. study the artisanal ways of fish harvesting.
2. appreciate the limitations in storage facilities at the fish marketing centres with a view to proposing ways of improvement in infrastructure.
3. discuss in groups the distribution patterns of fish in Ghana as compared to other countries.
4. participate in other case studies related to the subject.

Content :

The second unit will look into ways in which fish is harvested from the wild, stored in the different ways and finally distributed to the various markets.

The contents will include:

i. Explanation of the different types of fresh and processed fish and their storage regime.
ii. Transportation and distribution pattern of the various forms of fish and shellfish.
iii. Reference to the Codes of Practice (Unit 1) as pertains to handling of the fish during storage and distribution.

Fish harvesting

The flesh of healthy fish at the time of harvest is sterile. However, bacteria that are introduced on the fish in many ways add up to spoilage.
These include

a. bacteria which are resident microflora of the fish environment which are also found on the fish skin and in the slime surrounding the fish.

b. microflora from the mud on the sea floor acquired by the fish when being dragged during trawling.

c. bacteria from the intestines (released in the form of excreta squeezed out of the fish) through pressure of the net on the fish.

d. bacteria from the surfaces of the fishing vessel.

e. bacteria from the hands of the handlers during handling and gutting of the fish.

f. bacteria from the knives during processing.

e. bacteria from the containers in which the fish are packed.

As a result, fish spoils very quickly immediately after harvesting and therefore must be frozen immediately or kept on ice to avoid resident bacteria organisms already on the fish from multiplying.
However, the storage life of wet fish on ice is limited and varies according to the fish type. For example cod and haddock stay for only about 15 days in ice while herrings stays for only up to 6 days.

Fish storage

Different types of fish harvested from the wild require different forms of storage and distribution regimes. As for example chilled wet fish, smoked fish and frozen fish products do not require the same temperature of storage.
Chilled fresh fish which contains no frozen water, requires storage at or near 0°C; whereas frozen fish which has already been stored at a temperature of -30°C may require a retail storage at -18°C or lower.

To have good quality fish, the fish must be quick frozen for storage otherwise there will be changes in gloss, colour, texture and flavour characteristics of the fish.
A rise in temperature also aids in deterioration of fish especially in tropical countries where temperatures are normally high.

Factors that limit fish storage life include changes in protein, fat, colour and dehydration.

The FAO Code of Practice for Frozen Fish recommends that frozen fish products should be stored at temperatures appropriate for the species, type of product and intended time of storage.
The recommended storage temperature for all fishery products in the UK and adopted in Europe is -30°C. At this temperature, spoilage by bacterial action is completely arrested so that there is a reduction in the rate of other undesirable changes in the fish.

The International Institute of Refrigeration recommends a storage temperature of -18°C for lean fish such as cod and haddock and -24°C for fatty species such as herring and mackerel. The code also recommends that for lean fish intended to be kept in cold storage for over a year, the storage temperature should be -30°C (Table 1).

Table 1. Practical storage lives (PSL) of fish products

		Storage life in months	
Products	-18°C	-24°C	-30°C
Fatty fish (glazed)	5	9	>12
Lean fish (fillet)	9	12	24
Flatfish	10	18	>24
Shrimp (cooked/peeled)	5	9	12

Storage of dried smoked fish as pertains in developing countries as opposed to cold smoking of fish in developed countries involves the use of perforated cane baskets. The fish is stacked in layers in the baskets previously lined with brown paper. If stacked excessively, the lower layers of fish are mashed during distribution and thus reducing consumer appeal for the product.

Salted dried fish and fermented fish are also similarly packaged for storage as in the case of dried smoked fish.

Changes in protein:

During freezing and cold storage, fish proteins change permanently. During this time, temperature is a crucial factor and affects the speed of protein denaturation in fish. Serious changes occur rapidly at temperatures such as $-2^{O}C$ that are not very far below freezing point. At even temperatures as low as $-10^{O}C$ to $-15\ ^{O}C$, changes in protein quality are so rapid and pronounced that an initially good quality product can deteriorate within a few weeks. However, to ensure that the rate of deterioration as a result of protein denaturation is reduced, storage of fish must be maintained at as low a temperature as possible. When the fish is not stored at the required temperature, the flesh will be seen to sag and break with substantial loss of fluid referred to as fish drip. When such fish is cooked, it becomes dry, fibrous and tough. In extreme cases, the fish muscle falls apart.

Changes in fat:

The rate of spoilage that result in changes in fish fat is also dependent on temperature and results in oxidation of the fat leading to rancidity and production of objectionable flavours and odours. The enzymes present in fish muscle, especially in dark muscle, speed up reactions between fish lipid and oxygen to produce rancidity. These rancid flavours range from cod liver oil to paint-like, while the odour becomes objectionable. At higher temperatures such as -10 ^{O}C, the autoxidation changes occur more rapidly. Therefore storage of fish at lower temperatures would effectively slow down the rate of fat deterioration. Also preventing exposure of the fish to oxygen can reduce the rate of oxidation.
Nevertheless, fish with very high fat content may spoil quickly even during cold storage. This condition may be salvaged to some extent either by wrapping the fish in plastic bags and sealed under vacuum; or glazing the fish to prevent moisture and oxygen.
Glazing is carried out after freezing either by brushing or spraying chilled water onto the surface of the fish or by dipping it in cold water.

Changes in colour:

Consumers invariably assess fish quality by appearance due to colour change in the fish. These changes in the fish flesh appear more pronounced at higher temperatures. Hence, storage of fish at lower temperatures reduces changes in appearance of the fish from its original acceptable colour; and therefore makes the fish more appealing to the consumer. The changes in colour occur particularly in fatty fish, which become yellow as rancidity builds up. In lean fish however, the appearance ranges from brown to grey with prolonged frozen storage.

Time of freezing fish is also very crucial to colour changes. Fish flesh frozen slowly has a colour similar to that of unfrozen fish but a fish frozen extremely slowly, in days rather than hours, takes on a dark glassy look.

Changes in dehydration:

Dehydration of fish occurs even when it is under cold storage. The surface of a badly dehydrated product appears very dry, wrinkled and dull. It also looks spongy and opaque instead of the normally bright, firm and elastic product expected of the fish in the fresh state. With time, the inner tissues of the fish also dry due to loss of moisture thus

resulting in a very light and fibrous tissue. These effects may be visible on the surface of the fish and is known as 'freezer burn'. As a consequence of the drying effect on the fish, denaturation of the protein and oxidation of the fat occurs.
Reducing the storage temperature of the fish has been found to slow down the spoilage of fish due to protein denaturation, fat changes and dehydration.

Fish display and distribution

Distribution of fish involves handling of the fish whether fresh or processed. Generally, all fish types must be quickly handled to avoid excessive exposure to contamination and spoilage factors.

Excessive handling of fresh fish especially must be avoided, so that they should be loaded from the source of harvest into refrigerated vans and sent to the cold storage rooms until ready to be sold or processed. In cold storage, frozen fish must be handled quickly and carefully to avoid thawing.
In the instance where the fresh fish may be processed immediately, short storage in ice flakes may be adequate.

Cold stores play a vital role in the distribution of fish. They can be used as production stores, bulk stores, distribution stores or retail stores.
A production cold store can store frozen raw materials, semi-finished and finished products.
Bulk stores provide similar services as production cold stores except that they are often located at some distance from the actual processing industries and are normally much larger than the production stores and in addition provide longer storage time for the fish.
Distribution stores are normally located in urban areas and they generally receive fish products from both the production and/or bulk stores in large quantities where the products are stored for short periods of from one to eight weeks, then further assembled in smaller sizes/quantities and sold to retail stores.
Fish display procedures in retail shops is thus an important ring in a chain of factors affecting the quality of captured fish from the moment it is fished till it reaches the customer.

High percentage of ill-practiced display procedures lead to unhealthy fish products on display in the Ghanaian fish markets. Such are fish shops/sheds which do not possess covered refrigerated slabs for fish display. Rather fish in high piles are covered with crushed ice instead of flaked ice only at the uppermost layer. When there is no ice between the layers of fish to ensure adequate amount of ice flakes and thus cooling effect around each fish, a temperature difference of up to 7°C may build up between the top and bottom layers. Hence ice is needed in large quantities between and around a layer of fish and should be uniformly distributed throughout the fish pile.
Hazardous amounts of the Gram-positive *Clostridium botulinum* can build up in fish piles that are left to warm up and this indicates sign of flesh poisoning. Where spores of this

bacterium exists in the flesh, heating the fish at 85°C for 15 minutes inactivates the toxin of this bacterium, while heating at 115°C for 12 minutes destroys the spores completely.

For the transportation of frozen fish during distribution, enclosed vehicles should be used or at least a cover provided to protect the fish from direct sunlight for short distances. However for long journeys, an insulated vehicle will be required to maintain the air space at a temperature of approx -20°C depending on the initial temperature of the fish, whether the vehicle is full or partly loaded, the size of the load, the insulation quality and thickness, the degree of air ingress and the local climatic conditions.

Fresh harvested fish from the sea or freshwater source are normally carried by women to the marketing centres or processing sites in large bowls (Fig. 3a and 3b) on the head and exposed to the heat of sunlight or dust. This causes the fish to be contaminated as a rise in temperature of the fish may provide an environment conducive to growth and proliferation of food poisoning microorganisms that may cause diseases.

Case studies:

1. In a market survey on fish storage and distribution practices, it was realized that fish retailers practiced bad display procedures leading to unhealthy fish products. About 92% of the retail shops were seen not to possess covered refrigerated slabs for fish display, but used crushed ice to cover the top surfaces of large piles of fish to keep it cool. A fish inspection officer during his rounds decided to measure the temperature of the fish pile at the top, middle and bottom. He observed a temperature difference of up to 7°C between the top and bottom piles.
In addition, the officer realized that about 80% of the examined retail shops purchase fish in so large quantities that they are not able to freeze all of it even after the day's sales. The extra fish is kept in the shop overnight till the next day. This caused a measurable amount of spoilage with some in severe condition of deterioration. Discuss all the possible factors and types of implicated bacteria organisms that may be involved in the spoilage.

2. A particular spoilage bacterium isolated from case study 1 above caused flesh poisoning in 58% of the fish. However in about 34% of the fish examined only the spores of the bacterium were present in the flesh. When the fish was heated at 85°C for 15 minutes, the toxin produced was inactivated. Then when heated at 115°C for 12 min, there was total destruction of the spores.
What bacterium is this?
Discuss the possible health implications if such fish were to be consumed.

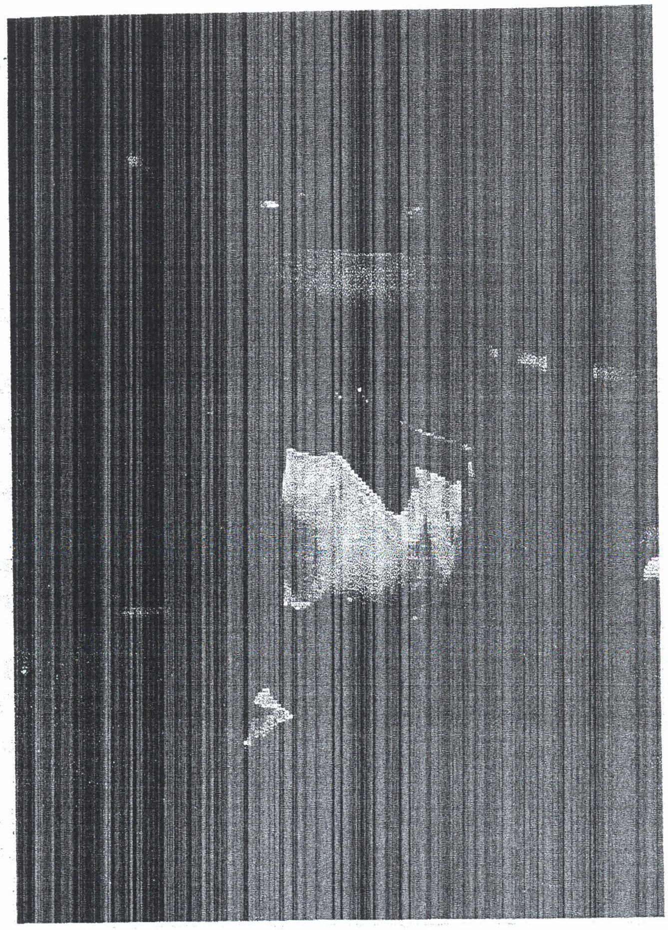

Fig.3a. Retailer with a head load of fish exposed to dust and heat.

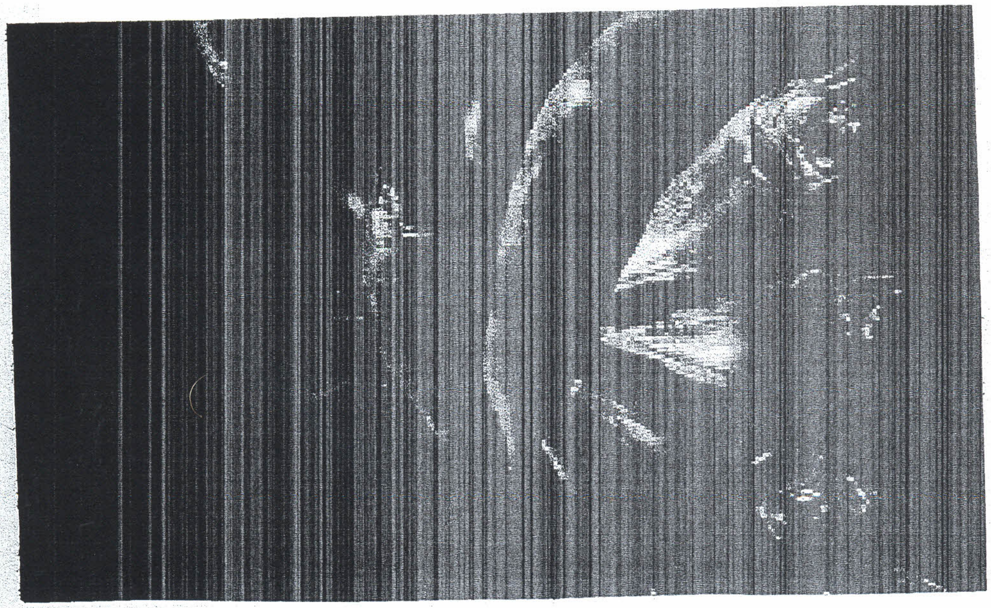

Fig.3b. Fish displayed for sale without ice in a bowl.

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Unit 3 :

Unit 3 will be divided into two sections namely,
3a. Fish spoilage, fisheries and inspection and
3b. Fish borne diseases.

3a. FISH SPOILAGE, FISHERIES AND INSPECTION

Spoilage of fish

Spoilage of fish begins as soon as it dies. The spoilage pattern of fresh fish is complex, meaning that a number of interrelated systems occur with some being suppressed by others. These include the degradation of protein resulting in the formation of various products like hypoxanthine and trimethylamine, as well as the development of oxidative rancidity and the action of microorganisms.

The effect of enzymes on fish spoilage

During the reduction of food to simple substances in the gut and flesh of live fish, certain enzymes act as catalysts to these chemical reactions. These enzymes remain active after death and cause self-digestion, thus affecting the flavour, texture and appearance of the fish.
Consequently, the action of the enzymes causes stiffening of the muscle referred to as rigor mortis. As self-digestion proceeds, softening of the flesh advances rapidly especially in small fatty fish which is full of feed since the gut enzymes are active then. In sardines and herrings, "belly burst" resulting from a weakening of the belly wall due to self-digestion can occur in only a few hours after catch.
The rate of self-digestion, which is dependent on temperature, can only be retarded but not terminated by chilling the fish to just above the freezing point. However, heating can stop enzyme action. Salting, frying, drying and marinating are other methods that can also control enzyme action to some extent.

The effect of microorganisms on fish spoilage

Microbiological action is the main and fastest cause of spoilage. Since the growth rate is highly dependent on temperature, the fish must be cooled as soon as possible after catching with the application of good hygienic conditions.

Microorganisms that are found in the surface slime, on the gills and in the intestines of fresh fish contribute greatly to the spoilage of fish. Other sources of microorganisms that contribute to fish spoilage are from the hands of handlers, from the cooling medium, dust and the environment generally. The muscle of fish is however sterile at the time of catch.

After catch or harvesting the fish from the wild, the bacteria penetrate the skin at a number of points to reach the muscle. These access routes are through the gills and into the blood vessels, through the lining of the belly cavity and eventually through the skin to reach the muscle where they grow and multiply rapidly, producing disagreeable odours and flavours.
Fish that is very fresh is seldom the cause of food poisoning since the bacterial growth tends to make the muscles or flesh unpalatable before any toxins develop.

Certain types of microorganisms dominate during spoilage; since among the different microorganisms, each type has its particular conditions for optimum growth. These conditions however depend on the initial infection, the properties of the food material, the temperature and other factors. So that when the fish is cooled to around 0^0C, some of the bacteria groups responsible for spoilage cease to grow and the rate of spoilage reduces.

Microbiological activity is also greatly affected by the ambient prevailing conditions such as the available oxygen and the amount of moisture.

In melting ice the rate of fish spoilage is to some extent dependent on the rate of melting so that if there is enough quantity of ice to ensure the desired fish temperature of 0^0C, a higher melting rate can give slightly better results than a lower melting rate.
Where the fish is in direct contact with surfaces such as wood, metal or other fish instead of ice, foul odours can arise due to the action of certain anaerobic bacteria, which thrive in the absence of oxygen.

Activity of microorganisms result in chemical alteration of some constituents some of which disappear completely thus resulting in changing the sensory characteristics such as the odour and flavour of the fish. When these substances (known as extractives) are changed by the effect of the microbiological activity, the protein of the muscles later changes considerably. These extractives are found in varying amounts depending on the species of fish. As for instance, whereas cod and haddock contain only traces of amino-acid histamine, herring and mackerel contain large amounts. Also, although urea is completely absent in cod, large quantities are found in skate, dogfish and shark. Further, Trimethylamine oxide that is found in all salt-water fish (Fig. 4a and 4b) is usually absent from fresh water fish species. Hence, to assess the quality of salt-water fish, chemical determination of Trimethylamine (TMA) resulting from the breakdown of Trimethylamine oxide can be used. Similarly in some species such as sharks, the determination of ammonia that is formed during the breakdown of urea is indicative of the quality status of the fish.

Chemical denaturation of proteins

Chemical denaturation of proteins appears normally late in the deterioration process, as

Healthy saltwater fish harvested for storage.

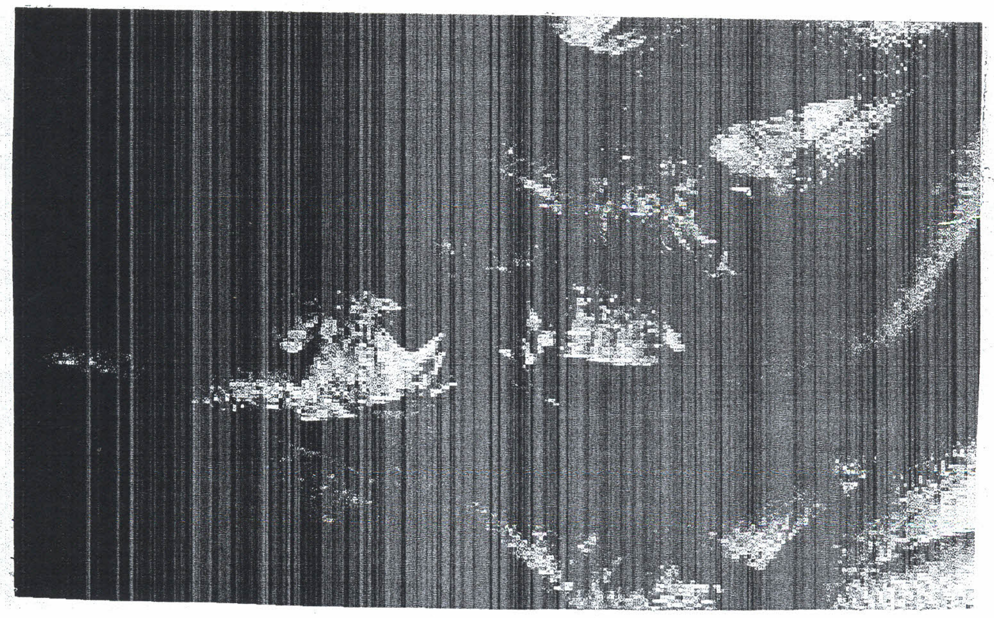

Fig. 4a.

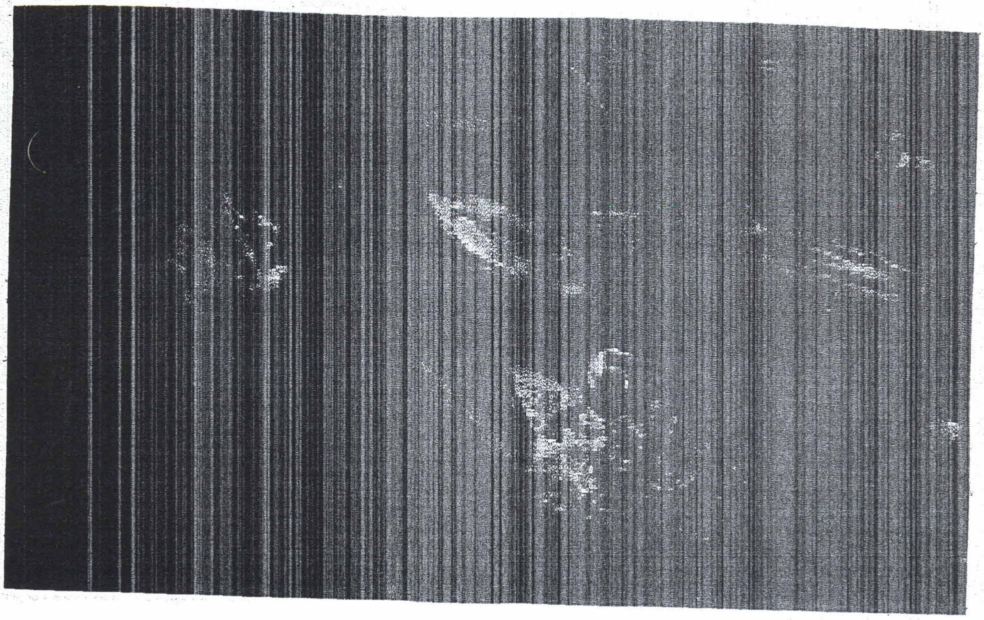

Fig. 4b.

does oxidation of fat when compared with microbial spoilage. In fresh fish however, variability occurs in the development of oxidative rancidity with some fish species undergoing oxidative rancidity with ease due to the large proportion of the highly unsaturated fats contained in their structure.
Fatty species like mackerel and herring have a high lipid content mainly in the form of phospholipids and lipoprotein in the muscle proteins, while cod and haddock do not contain high amounts of lipids. In addition, while variations in susceptibility of fish to rancidity have been found to be seasonal, within a single fish itself there is a difference in the ease with which different portions undergo rancidity.

Fisheries and Inspection of fish

To prevent bacterial contamination and spoilage of fish, it is essential to adopt a high standard of hygiene during handling and processing since the main processing problem is the quality of the fish that forms the raw material.

Inspection of fish is a quality assurance operation which is followed by most developed countries, although in most developing countries strict adherence to specific programmes for fish and fishery products are lacking in many fish processing industries.

In Africa, countries with well established fish inspection and QC programmes are few namely Côte d'Ivoire, Mozambique and Egypt although most of the programmes are directed only to cover the control of fishery products for the international market for the purpose of attracting foreign exchange.

In Ghana, a national system of food control has been developed and generally involves more than one Ministry or Department responsible for carrying out fish inspection and quality control activities. The official inspection agencies include the Fisheries department of the Ministry of Food and Agriculture (MOFA), the Ghana Standards Board (GSB) and the Food Research Institute of the Council for Scientific and Industrial Research (CSIR), which collaborate their activities in the inspection of fish and fishery products, including laboratory analysis of fish products with the sole inspection agency, the GSB being responsible for the issuance of export certificates.

There are food laws controlling conditions under which fish products may be stored, manufactured or sold in Ghana. Such laws are enforced for fish intended for the export market where a special form of official inspection is carried out on such products before they are allowed to leave the shores of Ghana. There is however no adequate legislation covering fish inspection and quality control. Laws and regulations have been issued without proper coordination of the work of the different governmental agencies involved thus leading to overlapping of duties and contradictions in the enforcement.

The majority of establishments in the fish-processing sector for the domestic market is small-scale and labour intensive with no mechanized efficient and modern operations. They overlook proper hygiene and quality control procedures and do not enforce standards hence the production of sub-standard fish products with very short shelf life with very little appeal to consumers. Fish markets lacking adequate facilities for ensuring basic standards of cleanliness and sanitation further enhance this problem. Furthermore, transport, storage and distribution facilities follow the same pattern.

Quality Control and the Industries:

Large and medium-scale fish industrial establishments like Pioneer Fish Cannery and Divine Sea Foods have a well designed quality control system which is adhered to in the processing of fish where emphasis is not limited to the quality control laboratory within the factory, but also to unit operation from catch, reception of fish at the factory door, through processing to storage and distribution. This is necessary in order to retard spoilage, avoid contamination, and release a product to the consumer that meets the required GMP (Good Manufacturing Practices) and specifications. This is possible where there is a strong technical and financial link between the factory and the importing company.

However, due to the low understanding of quality standards by the fishermen and the wrong approach to fishing, the quality of raw material is often a serious problem since the fish is poorly handled, preserved or stored on-board the boat; in addition to the application of wrong techniques of unloading, handling and transportation practices of the fish from the shore.
Such pre-processing operations carried out under poor technical, hygienic and sanitary conditions are the main cause of contamination and decomposition of the fish.
At the artisanal level fish landed are sometimes deposited on the sand at the beach, where sometimes the fish is degutted, washed with seawater and carried exposed in large bowls to sheds or homes where the fish is left without further protection before processing.

Over the last three decades, many developing countries that could not meet the requirements of fish importing countries of strict standards for fish had faced substantial rejections of fish consignments and severe economic losses.

Under the auspices of the Codex Alimentarius Commission, the Codex Committee on Fish and Fishery Products has developed so far 10 codes of Practice and 14 standards for fish and fishery products. Both documents aim to protect the health of consumers and to ensure fair practices in the trade of fish and fishery products at national and international levels.

The Codex Alimentarius Commission

This Commission is an intergovernmental body comprising of about 135 member countries established in 1963. Its aim is to protect the consumer against fraud and health hazard in the manufacture and trade of food, and to ensure fair and smooth national and international trade in food. Its programme covers the composition, labeling, additives,

contaminants, pesticide residues, hygiene, sampling and analysis of foods. The Commission has three groups of 27 subsidiary bodies; the General Subject matters include general principles of the Codex Alimentarius, food additives, pesticide residues, residues of veterinary drugs, in food hygiene, meat hygiene, food labeling and methods of analysis and sampling. The Commodity Committees elaborate standards for various food commodities including fish and fishery products.
The Codex Alimentarius also elaborates codes of recommended Practice to serve as advisory guidelines for use by member Governments as they deem fit. So far, over 35 Codes of Practice have been established covering a wide range of foods including fish and fishery products.

The Codex Committee on Fish and Fishery Products

It started its activities in 1966 with the participation of 15 Member countries and now risen to 31. This Committee has developed so far 10 Codes of Practice and 14 standards.

The Codes of Practice on Fish and Fishery Products

The following recommended codes of practice developed are:
Code of Practice for Fresh Fish, 1974
Code of Practice for Canned Fish, 1976
Code of Practice for Frozen Fish, 1978
Code of Practice for Shrimp or prawns, 1978
Code of Practice for Molluscan Shellfish, 1987
Code of Practice for Lobsters, 1978
Code of Practice for Smoked Fish, 1979
Code of Practice for Salted Fish, 1979
Code of Practice for Minced Fish prepared by Mechanical Separation, 1983
Code of Practice for Crabs, 1983 and
Code of Practice for Quick Frozen Breaded Fish Portions (under elaboration)

The Codes of Practice contain essential technological and hygienic requirements for the production of high quality fishery products based on long established and widely recognized good manufacturing and commercial practices. They are revised periodically as research, experience and facilities suggest new improved techniques or practices in the fisheries production and trade.

The Codes comprise information useful to those engaged in harvesting, processing, handling (at sea and on shore), storing, distribution and marketing of fish and fish products. They are not rigid rules but guidelines to be applied depending on the local conditions with regards to the complex technological problems that may be unique to a specific geographical area. It is therefore imperative that personnel involved in public health enforcement be conversant with these codes in order to effectively advice on such issues.

Each Code of practice ends with general specifications for the end product and calls for the adoption of appropriate methods of sampling and examination to determine compliance.
The following requirements are in general the essence of all end product specifications:

- The end product should be free from microorganisms in amounts harmful to humans, free from parasites harmful to humans, and should not contain any substances originating from microorganisms in amounts that may represent a hazard to health;

- The end product should be free from chemical contaminants in amounts that may represent a hazard to health;

- The product, to the extent possible by good manufacturing practice, should be free from other objectionable matter and also parasites not harmful to humans; and

- The end product should comply with any requirements set forth by the Codex Alimentarius Commission on pesticide residues and food additives as contained in Codex lists of maximum limits for pesticide residues, or Codex commodity standards, or should comply with the requirements on pesticide residues and food additives of the country in which the product will be sold.

CODEX STANDARDS FOR FISH AND FISHERY PRODUCTS

The following are the standards developed so far by 1976 by the Codex Committee on Fish and fishery Products and reissued in 1981:

Canned Pacific Salmon (Codex Standard 3)
Quick Frozen Gutted Pacific Salmon (Codex Standard 36),
Canned Shrimps or Prawns (Codex Standard 37),
Cod and haddock Quick Frozen Fillets (Codex Standard 50)
Ocean Perch Quick Frozen Fillets (Codex Standard 51)
Canned Tuna and Bonito in Water or Oil (Codex Standard 70),
Quick Frozen Fillets of Flat Fish (Codex Standard 91),
Canned Crab Meat (Codex Standard 90)
Quick Frozen Shrimps or Prawns (Codex Standard 92)
Quick Frozen Fillets of Hake (Codex Standard 93)
Canned Sardines and Sardine-Type Products (Codex Standard 94)
Quick Frozen Lobsters (Codex Standard 95)
Canned mackerel and Jack Mackerel (Codex Standard 119)
Quick Frozen Blocks of Fish Fillets and Minced Fish Fillets
Quick Frozen Fish Sticks (fish fingers) and fish portions
Dried Salted fish of the Gadidae Fish Family.

Implementation of these standards are however subject to their final acceptance by Member Governments before they are enforced.

Influence of temperature on fish spoilage

Fish begins to spoil immediately after death. This is reflected in gradual developments of undesirable flavours, softening of the flesh and substantial losses of protein and fat in the form of fluid. Lowering the temperature of the fish can minimize this, and the process can be completely arrested by further lowering the temperature.

The freezing process alone is not a method of preservation. It is merely the means of preparing the fish for storage at a suitably low temperature. In order to produce a good product, freezing must be accomplished quickly. A freezer requires to be specially designed for this purpose and thus freezing is a separate process from low temperature storage.

Fish that die undergo rigor mortis. Rigor mortis (over a period of hours of the death of the fish) can have a bearing on handling and processing. In some species the reaction can be strong, especially if the fish has not been chilled. This renders the fish muscles under strain to tend to contract resulting in some tissue breakage. In the case where the fish had been previously handled roughly, the flesh breaks terribly and falls apart. If the muscles are cut before or during rigor, they will contract and in this way fillets from such fish can shrink and acquire a somewhat rubbery texture.
In many species, however, rigor mortis is not strong enough to be of much significance.

3b. FISH BORNE DISEASES

Botulism food poisoning

Clostridium botulinum is a large anaerobic bacillus that forms subterminal endospores which are widespread in nature and found where organic compounds are present, including soils, aquatic sediments, decaying vegetation, in the intestinal tract of animals, and on the gills of fish. During optimum conditions these spores develop into the vegetative state, growing rapidly to produce lethal toxins. These spores have been found to be resistant to heat, drying, salting and freezing.

There are seven toxigenic types of *Clostridium botulinum,* and each produces an immonologically distinct form of botulinum toxin which are designated A, B, C1, D, E, F, and G.
In contrast to the others, the non-proteolytic types of *C. botulinum* namely types A, E and F can grow and produce toxins at temperatures as low as 3.3^{0}C; but they do not attack complex proteins and their growth in food cannot be detected by off-odours and off-flavours.

Clostridium botulinum type E is associated with fish and fish products and has a wide temperature range for toxin production ($3.3^0C - 45^0C$) at less than 5% salt content and at pH of more than 5.3. It can grow and produce toxins in both vacuum packed and unpacked foods. It is however destroyed by heating above 82^0C for 30 minutes. In contrast *Clostridium botulinum* types A, B require heating above 120^0C for over 15 minutes to be destroyed.

C. botulinum spores are relatively heat resistant and may survive the sterilizing process of improper canning procedures in the case of canned fish. The anaerobic environment produced by the canning process may further encourage the outgrowth and proliferation of spores, especially where the pH is greater than 4.5.

Pathogenesis of Botulism

The intoxication caused during food borne botulism results when the preformed clostridial toxin is ingested with partially cooked or uncooked food in which the spores have germinated and the organism has grown. The toxin is then absorbed by the upper part of the gastrointestinal tract in the duodenum and jejunum from where it into the blood stream thus reaching the peripheral neuromuscular synapses. The toxin then binds to the presynaptic stimulatory terminals and blocks the release of the neurotransmitter acetylcholine that is required for a nerve to simulate the muscle.

Clinical symptoms

Botulism symptoms begin 18-36 hours after the toxin has been ingested resulting in an illness characterized by weakness, dizziness, dryness of the mouth, nausea and vomiting, followed by symmetric cranial nerve paralysis. The later condition is manifested by blurred vision, inability to swallow, difficulty in speech, descending weakness of skeletal muscles and respiratory paralysis, leading to death.

Prevention

The most important prevention is proper food handling and preparation especially in the case of such perishable commodities as fish. However, individuals who have ingested food with botulism toxin should be treated immediately with antiserum.
The spores of *C. botulinum* can survive boiling (100^0C at 1 atm.) for more than 1 hour although they are killed by autoclaving. Since the toxin is heat-labile, boiling or intense heating of contaminated food will inactivate the toxin.
In the case of canned fish, containers that are found to be bulging may contain gas produced by *C. botulinum* and should be discarded.

Table 2. Botulinogenic properties of fish products (after Huss 1981).

Fish Product	Factors adding to botulism hazard	Factors reducing botulism hazard	Safety of product based on:	Classification
Fresh and frozen	Vacuum packing	Traditional chill storage Putrefaction before toxin is produced	Cooking before being eaten	No risk
Pasteurized	Prolonged storage life Toxin produced before putrefaction Vacuum packing Poor hygiene	Chill storage (< 3°C) Synergistic aerobic flora eliminated	Cooking before being eaten Chill storage	No risk if cooked High risk if not cooked
Cold-smoked	Same as above Not cooked before being eaten No tradition for chill storage	Chill storage Salting (NaCl concentration >3%) High redox-potential in unspoiled products	Chill storage Process control (Raw material, salting when applicable)	High risk
Fermented	Fermentation may be slow High temperature during fermentation. Not cooked before being eaten	Salting (NaCl concentration >3% in brine) Chill storage Low pH	Process control Chill storage	High risk
Semi-preserved	Not cooked before being eaten	Application of salt, acid etc. Chill storage	Process control	Low risk
Fully preserved	Not cooked before being eaten Packed in closed cans	Autoclaving	Process control (Autoclaving, closing of cans)	Low risk

Cholera (*Vibrio cholerae* and V. *parahaemolyticus*)

Vibrios are typically marine organisms and are one of the most common organisms in surface waters (both marine and freshwater habitats) and are in association with aquatic animals including fish. For optimal growth, most species require 2-3% NaCl.

V. cholerae and *V. parahaemolyticus* are two notable pathogens of humans. They both cause diarrhea in humans but in ways that are entirely different. *V. parahaemolyticus* is an invasive organisms affecting primarily the colon, while *V. cholerae* is noninvasive, affecting the small intestines through secretion of an enterotoxin.

V. cholerae is transmitted to humans by water and food and produces cholera toxin whose action on the mucosal epithelium is responsible for the characteristic severe and rapidly fatal diarrheal disease frequently referred to as epidemic cholera. In severe cases, within an hour of the onset of the symptoms, a person may become hypotensive and may die within 2-3 hours if no treatment is provided. Commonly, the progress of the disease advances from the first liquid stool to shock in 4-12 hours, with death following in 18 hours to several days.

V. cholerae Serogroup 01 caused sporadic disease between 1973 and 1991 in the United States and were associated with the consumption of either raw shellfish, improperly cooked or recontaminated fish. In the tropics, such sporadic cases of the disease are normally fatal since good sanitation at the beaches is lacking as fish and shellfish are harvested from faecally polluted coastal waters followed by unhygienic handling and processing regimes.

Pathogenesis of *V. cholerae* toxin

Clinical manifestation of cholera begins with sudden onset of massive diarrhea where the patient may lose gallons of protein-free fluid and associated electrolytes, bicarbonates and ions within a day or two. The cause of this situation is as a result of the activity of the cholera enterotoxin that activates the adenylate cyclase enzyme in cells of the intestinal mucosa, converting them into pumps which extract water and electrolytes (Na^+, K^+, Cl^-, and $HC0^{3-}$) from blood and tissues and pump it into the lumen of the intestine. The loss of fluid then leads to dehydration, anuria, acidosis and eventual shock.
The watery diarrhea (called "rice-water stool"), which is manifested by this disease, is found speckled with flakes of mucus and epithelial cells in addition to large numbers of vibrio organisms. When the disease remains untreated, high mortality rates (50-60%) frequently results as the loss of potassium ions may result in cardiac complications and circulatory failure.

Prevention and treatment

Fish and fishery products must be handled properly during processing and the water used

in the factories must be portable.
Treatment of cholera involves the rapid intravenous replacement of the lost fluid and ions. In addition, administration of isotonic maintenance solution should continue until the diarrhea ceases. If glucose is added to the maintenance solution, then it may be administered orally, thereby eliminating the need for sterility and intravenous administration.

Staphylococcus aureus

Staphylococci are spherical bacteria that occur in the nose and skin of normal humans. *Staphylococcus aureus* normally colonizes mainly the nasal passages, but may be found regularly in most other anatomical locales.

Pathogenesis of *S. aureus*

S. aureus causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes and furunculosis; more serious infections like pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections as well as deep-seated infections such as osteomyelitis and endocarditis. Furthermore, it causes infection of wounds. For the purpose of this unit (unit3) is the importance of the ability of *S. aureus* to cause food poisoning by releasing enterotoxins in food leading to the cause of toxic shock syndrome by the release of superantigens into the blood stream.
Staphylococcus aureus has a very high level of salt tolerance and can grow and produce enterotoxin at 0.9 Aw, 17% salt content, 35^0C and at a pH level between 4.8 and 5.5.

S. aureus expresses many potential virulence factors, namely surface proteins that promote colonization of host tissues invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase) surface factors that inhibit phagocytic engulfment (capsule, Protein A) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production) immunological disguises (Protein A, coagulase, clotting factor) memebrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin) exotoxins that damage host tissues or otherwise provoke symptoms of disease like SEA-G (six antigenic types A, B, C, D, E and G), TSST (toxic shock syndrome toxin) and ET (Exfoliatin toxin) inherent and acquired resistance to antimicrobial agents.

Escherichia coli (E. coli)

E. coli is a facultative anaerobic rod that lives in the intestinal tract of animals and are normal colonists of the human gastrointestinal tract, but may be associated with diseases of humans. *E. coli* is a consistent inhabitant of the human intestinal tract, and it is the predominant facultative organism in the human gastrointestinal tract and thus it is used as an indicator of feacal pollution and water contamination. Consequently, it is assumed that wherever *E. coli* is found, fecal contamination by intestinal parasites of humans may be the possible cause.

E. coli can grow in the presence or absence of oxygen. Under anaerobic conditions it will grow by means of fermentation, producing characteristic "mixed acids and gas" as end products. However, it can also grow by means of anaerobic respiration, since it is able to utilize NO_3, NO_2 or fumarate as final acceptors for respiratory electron transport processes. In part, this adapts E. coli to its intestinal (anaerobic) and its extraintestinal (aerobic or anaerobic) habitats.

Pathogenesis of *E. coli*

E. coli is responsible for three types of infections in humans namely urinary tract infections (UTI), neonatal meningitis, and gastroenteritis (intestinal diseases), depending on a specific array of pathogenic (virulence) determinants such as adhesins, invasins, chemotaxis/motility, toxins, antiphagocytic surface properties, genetic attributes, defence against serum bactericidal reaction and also against immune responses.

For the purpose of this unit however, the ability of *E. coli* to cause gastroenteritis is paramount as regards the consumption of contaminated fish.
Five classes (virotypes) of *E. coli* that cause diarrheal diseases and which falls within a serological subgroup and manifests distinct features in pathogenesis are :

i. enterotoxigenic *E. coli* (ETEC)
ii. enteroinvasive *E. coli* (EIEC)
iii. enteropathogenic *E. coli* (EPEC)
iv. enteroaggregative *E. coli* (EaggEC) and
v. enterohemorrhagic *E. coli* (EHEC)

Enterotoxigenic *E. coli* (ETEC)

ETEC causes cholera-like diarrhea syndrome especially in underdeveloped countries where poor sanitation prevails as a result of ingestion of contaminated food and water which have been colonized by the organism with the elaboration of enterotoxins.
The enterotoxins produced by ETEC include the heat-labile (LT) toxin and/or the heat-stable (ST) toxin.
Symptoms of ETEC infections include diarrhea without fever.

Enteroinvasive *E. coli* (EIEC)

EIEC are invasive, thus penetrate and multiply within epithelial cells of the colon causing widespread cell destruction with a clinical syndrome identical to Shigella dysentery which includes a dysentery-like diarrhea with fever. There is no production of LT or ST toxin, and unlike *Shigella*, they do not produce the shiga toxin.

Enteropathogenic *E. coli* (EPEC)

This induces a watery diarrhea similar to ETEC, but do not possess the same colonization factors and do not produce ST or LT toxins, but produce an enterotoxin similar to that of *Shigella*. EPEC strains are moderately-invasive, but not as invasive as *Shigella*, and cause an inflammatory response unlike the mode of action of ETEC or EaggEC.
Some types of EPEC, which are an important cause of diarrhea in Africa, are referred to as Enteradherent *E. coli* (EAEC).

Enteroaggregative *E. coli* (EaggEC)

These strains have the distinguishing ability to attach to tissue culture cells in an aggregative manner, causing persistent diarrhea in young children. Similar to the mode of action of ETEC strains, they adhere to the intestinal mucosa and cause non-bloody diarrhea without invading or causing inflammation by the production of a distinctive heat-labile plasmic-encoded EnteroAggregative ST toxin (EAST). In addition, they produce hemolysin.

Enterohemorrhagic *E. coli* (EHEC)

The *E. coli* strain (serotype 0157:H7) causes EHEC diseases characterized by a diarrhea syndrome with copious bloody discharge with no fever and can also cause hemolytic uremic syndrome (HUS) which is fatal due to its toxicity on the kidneys.
The toxin produced by EHEC strains produce is a type that is identical to the Shiga toxin, but phage encoded and with its production being enhanced by iron deficiency.

Salmonella

Salmonella causes food poisoning. Critical points in hot smoking of fish is time and temperature to avoid spoilage by microorganisms; where a minimum internal temperature of 65^0C is required to eliminate *Salmonella* as well as *Staphylococcus* species which cause food poisoning.
Salmonella is an enteric bacterium that is also regarded as a human intestinal pathogen.

Case Study :
A hospital in Town A reported illness among families that had partaken in a traditional feast made of uneviscerated salted fermented fish. The illness was characterized by nausea and vomiting, followed by symmetric cranial nerve paralysis. Many of these patients were admitted into hospital and placed on mechanical respiration. Several of them died later.
If you were the public health officer in that town, what in your opinion do you think may have caused this illness?
How would you diagnose the causative agent?
What control measures will you put in place to avoid such calamities in future?

Put down possible answers to the questions above and compare your answers to the following :

Possible causes :
The fish may have been purchased from a single sale outlet where contamination may have already occurred.
Clostridium botulinum type E toxin will be suspect and therefore *Clostridium botulinum* organisms will be expected to be isolated from the food.
Clinical specimens of vomit and also fish specimens from the shop will be collected and analyzed.
This is to remind you that most outbreaks of botulism associated with fishery products are invariably due to type E. At particular risk are especially fish products that are eaten without further cooking.

Have you also thought of *Staphylococcus aureus* bacterial toxins in food poisoning outbreaks? Do you not think that this organism may also have caused illness and death as in the scenario described above?

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Unit 4 : Fish Preservation

One of the important issues affecting fish preservation is the large biological variations existing from one region of the world to another and from one species of fish to another. This combined with the fact that catching methods and consumption habits vary, has a considerable influence on the handling and preservation of the product. Therefore some fish biology and factors influencing the quality are essential (refer Unit 1 and Unit 3). The composition of fish must therefore be appreciated.

Composition of fish

The muscles of fish form the edible part and are also attributed to most of the fish weight. The major components of fish muscle are however made up of water, fat and protein.

The protein content ranges between 15-20%, whereas the fat content varies widely from species to species and from season to season. It can however be as low as 0.5% in lean starved fatty fish and can reach over 20% in some fatty species. In lean fish, the bulk of the fat is stored in the liver and not in the muscle.

Fish however has water as the main constituent, with considerable variations between 60-80% but typically 80% in lean fish and 70% in fatty fish.

Carbohydrates, minerals, vitamins and some water extractable components are other minor substances present in fish.

Ways of preserving fish in Ghana

Fish preservation in Ghana involves icing (Fig. 5a and 5b) or freezing of fish, salting and/or drying, smoking, fermentation and frying.

Icing or freezing of fish

Freezing requires the removal of heat; and fish from which heat is removed falls in temperature.
There are three distinct stages through which fish undergoes freezing.
The first stage of cooling is characterized by a fairly rapid reduction in temperature to just below the freezing point of water (0 ^{O}C). During the second stage when more heat is removed from the fish, there is a period described as 'thermal arrest' where the bulk of the water is turned to ice due to a reduction in temperature of the environment by a few degrees. The third stage in the freezing of fish involves rapid reduction in temperature with removal of a comparatively small amount of heat where about 55% of the water is turned to ice and most of the remaining water freezes.
As the water freezes out as pure ice crystals, the remaining unfrozen water is bound in increasing concentration with salt and other compounds contained in the fish. By the time the fish temperature is reduced to -5 ^{O}C, about 70% of the water is frozen. Even at temperatures as low as -30 ^{O}C, a proportion of the water in the fish muscle is found in the unfrozen state.
There are two ways of freezing fish i.e. slow freezing and quick-freezing.

Quick freezing causes sudden cooling of the fish and therefore may cause disruption and tearing of the fish muscle tissue. In addition, since the water expands in the fish as a result of freezing, it causes the cell wall to burst under the pressure.
Quick frozen fish results in the formation of small ice crystals in the fish and may not cause an appreciable damage to the cell wall and therefore when the fish is thawed not much fluid loss will be experienced.
Since the temperature just below 0 ^{O}C is the critical zone for spoilage by protein denaturation, quick freezing had been described to refer to a state where the temperature of all the fish should have been reduced from 0 ^{O}C to 5 ^{O}C in up to a period of 2 hours and not more. After which the fish temperature should further be reduced such that its average temperature at the end of the freezing process is equivalent to the recommended storage temperature of -30 ^{O}C.

Slow freezing on the other hand results in the formation of large crystals which damage the fish cell wall so that when the fish thaws, copious amount of water is lost from the fish referred and this condition is referred to as 'drip loss'.
Slow freezing as compared to quick freezing results in a poor quality product as a result of protein denaturation due to reduction in temperature; in addition to also the presence of the concentration of enzymes and other compounds. The rate of denaturation is also increased due to the presence of a high concentration of compounds in the unfrozen portion; although the unbound water exists frozen as pure ice crystals.

Iced fish

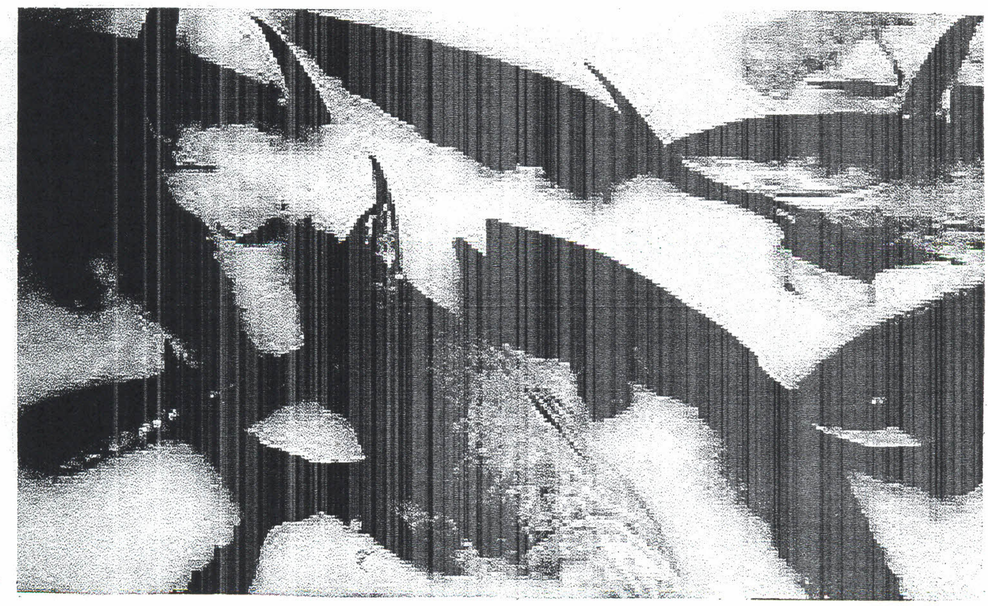

Fig. 5a.

Fig. 5b.

These two factors, which determine the rate of denaturation, act in opposition to each other as temperature is reduced and the temperature of maximum activity is between –1 and -2 ^{O}C. Thus the main factor accounting for the difference in quality between slow and quick freezing lies in the fact that whereas in slow freezing a longer time is spent in the zone of maximum activity, the reverse is true for quick freezing.

Controversy in the explanation in slow and quick-freezing can also be explained by the fact that the walls of fish muscle cells are elastic enough to accommodate the larger ice crystals without excessive damage. More so, since most of the water in the fish muscle is bound to the protein in the form of a gel, little fluid would be lost from the fish.

Table3. Practical storage life for fish (from IIR Guide to Refrigerated Storage)

Fish type	Storage life (months)		
	-18 ^{o}C	-25 ^{o}C	-30^{o}C
Fatty fish, sardines, salmon, ocean perch	4	8	12
Lean fish, cod, haddock	8	18	24
Flat fish, flounder, plaice, sole	9	18	24
Lobster, crabs	6	12	15
Shrimp	6	12	12

IIR – International Institute for Refrigeration, Paris.

Salting

Salt reduces the growth of bacteria, gives the fish its palatability and preserves the fish as well. Some organisms can however tolerate high salt levels in the fish while there are some whose growth is stimulated by the presence of salt.
When the salt used in the preservation of the fish is polluted, the fish in turn becomes contaminated.

For smoked fish, the use of both brine and dry salt for fish preservation purposes are practised. Brine is normally used for eviscerated mackerel while dry salting is used for whole herrings, taking into consideration the salting time/ambient temperature relationship. For example, 21-22% brine (w/v) and a brine/fish ratio of 2 to 1 can be used for 16-18h. While for dry salting, the application of a salt/fish ratio of 1 to 4 for 2-3 days depending on the ambient temperature is used. In certain countries after salting, the smoked fish is rinsed with portable water to prevent salt crystallization on the skin of the smoked fish before draining to remove excess water and then drying in a kiln.

There are 3 basic ways of salting fish i.e. dry salting, pickling, and brining – with many variants of the basic methods, and sometimes combinations of them.

For **dry-salting**, a mixture of large and small grain salt sizes give the best results. The salt must however be clean and devoid of contaminants such as dust, sand, debris or chemical impurities. Such impurities are Calcium and Magnesium chlorides and sulphates; Sodium sulphate and carbonate; and also traces of Copper and Iron.
Dry salting can be applied to either whole or filleted fish that are then stacked in layers interspersed with dry salt at a ratio of 1 part of salt to 2-3 parts of fish. The liquor that is formed is allowed to drain off and typically; the fish is stacked in high layers to enable the pressure caused by stacking to express the liquor. To effect an even liquor extraction from the fish, the stack is from time to time rearranged to enable fish at the bottom to be moved up to replace those at the top; and vice versa. Depending on the thickness of the fish, the fish is left to cure for periods up to 3 weeks at which time the water and salt contents will be about 55% and 20% respectively.

Pickling involves arranging the fish in the same way as for the dry-salting method and at a ratio of 1 part salt to about 3 parts fish. In this method, the liquor is not allowed to drain out so that the fish is immersed in it. Weights are added to weigh down the fish and exclude air from the container thus preventing oxidation of lipids and spoilage by the growth of aerobic bacteria. Pickled fish have been found to have better storage properties than dry salted fish.

Brining of fish entails the use of 80-100% saturated brine in the ratio of 1 part fish to 1 part brine.

Drying

Drying of fish is another preservative method that extends the storage life, especially if this is done in combination with salting. The traditional way of drying involves spreading the fish on racks to dry in the sun (Fig. 6a and 6b) for various times depending on the thickness of the fish. This method helps to reduce the water content in the fish to less than 45%. Salted fish (Fig. 7a and 7b) with 20% and 35% water content after drying can have a storage life of 15 and 6 weeks respectively.

In dry-salted fish, halophilic bacteria which may cause pink discoloration are the spoilage organisms particularly in the tropics due to ambient prevailing temperatures. Other spoilage organisms may cause unpleasant putrid odours causing the flesh to become brown and soft. Mould growth may also spoil the fish. Deterioration of such salted fish may also be caused by oxidation of lipids, manifested by rancid flavour formation, especially in the case of fatty fish.

Excessive handling may also result in contamination of the fish either from the hands or from dust, pests and animals.

Solar or mechanical dryers used for drying fish drastically reduce some contaminants such as dust and flies since they enclose the fish and serve as barriers to insects and animals.

Smoking

Smoking is the predominant method of traditional preservation of fish in Ghana.

Fish can either be smoked untreated; or smoked after a combination of salting and/or drying. Smoking can either be carried out in an oven or over an open fire (Fig. 8a and 8b).

Prior to smoking, fish must be dried fast to a certain degree. If too much humidity builds up during the drying process, and if processing takes too long, the product will fall apart as moisture in the fish will turn to steam that will solubilize the connective tissues. To prevent this, the protein must be set or denatured with low temperature drying before applying higher temperatures. When drying is however too fast, it will result in overdrying of the product surface, forming an exterior crust, which will prevent moisture from escaping from the inside of the product. This results in a long processing time and a wet soft product interior. In addition, with the development of a hard crust, smoke penetration and adhesion will be reduced with a resultant long processing time affecting the quality due to deterioration of protein. It is therefore advisable to dry the fish prior to smoking for 30min at temperatures not more than 30 ^{O}C.

The purpose of smoking is to impart desired flavour, colour and also to preserve the fish through the combined action of the chemical composition of the smoke as a bactericidal and antioxidant agent, the salt content, the heat and the drying effect.

Among the different components of smoke, phenols contribute essentially to the typical flavour of smoked fish, carbonyls contribute to the colouring while formaldehydes, phenols and cresol act as bactericidal, bacteriostatic and antioxidant agents. The residual effect of smoke in fish is greater against bacteria than moulds.

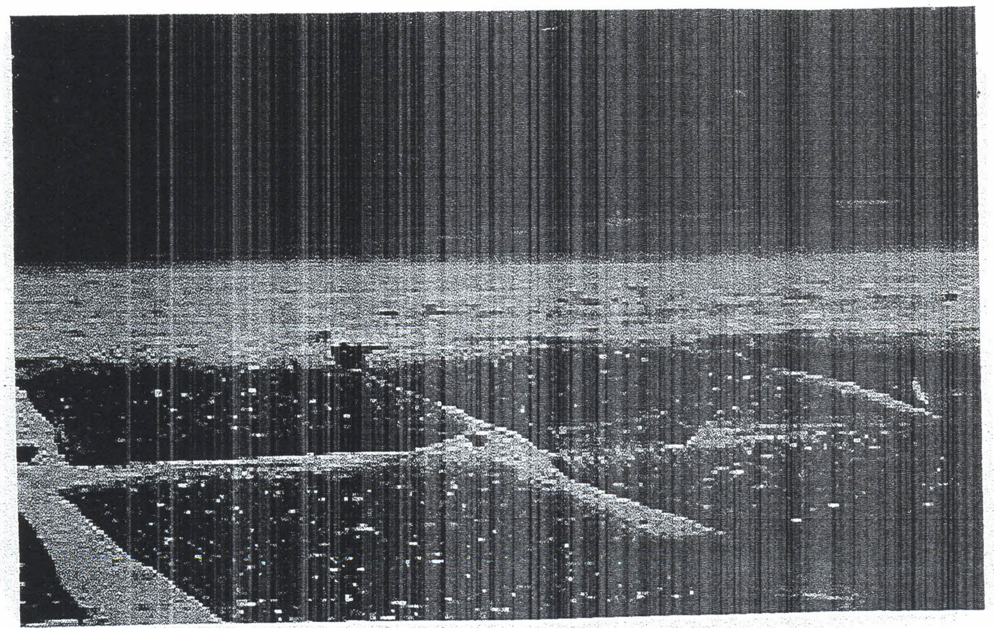

Fig. 6a. Sun drying of fish on mats at the seashore.

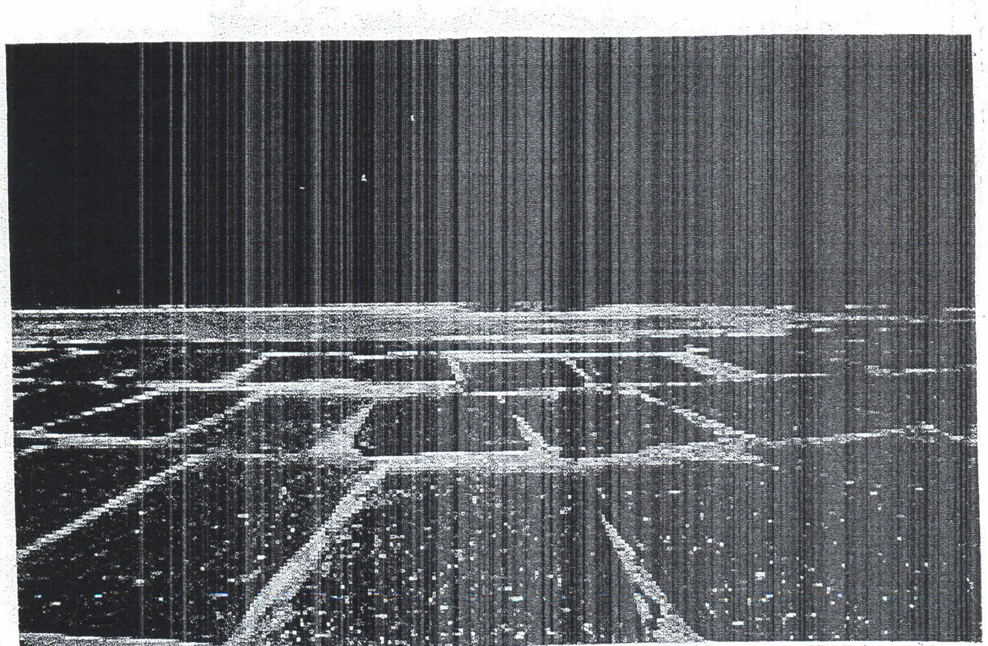

Fig. 6b. Sun drying of fish near a lagoon.

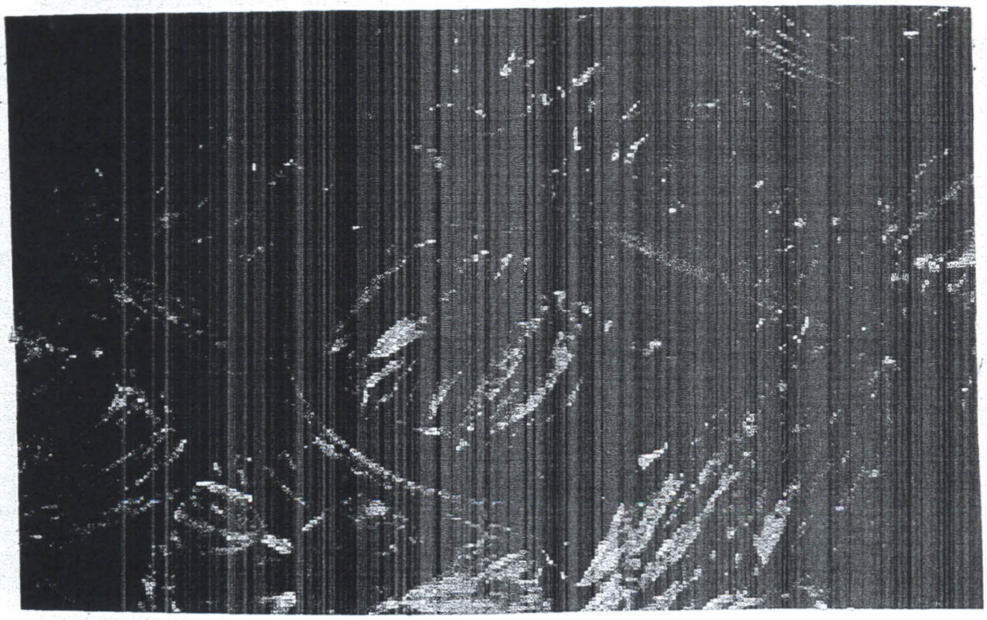

Fig. 7a. Salted dried fish packed in baskets.

Fig. 7b. Degutted, split and salted dried fish.

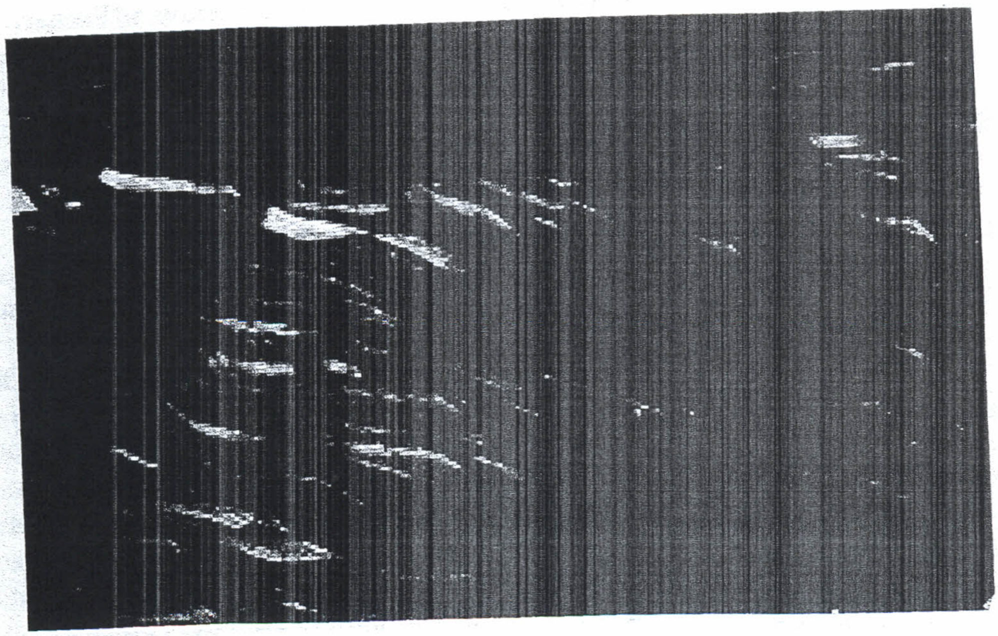

Fig. 8a. Oven smoked fish.

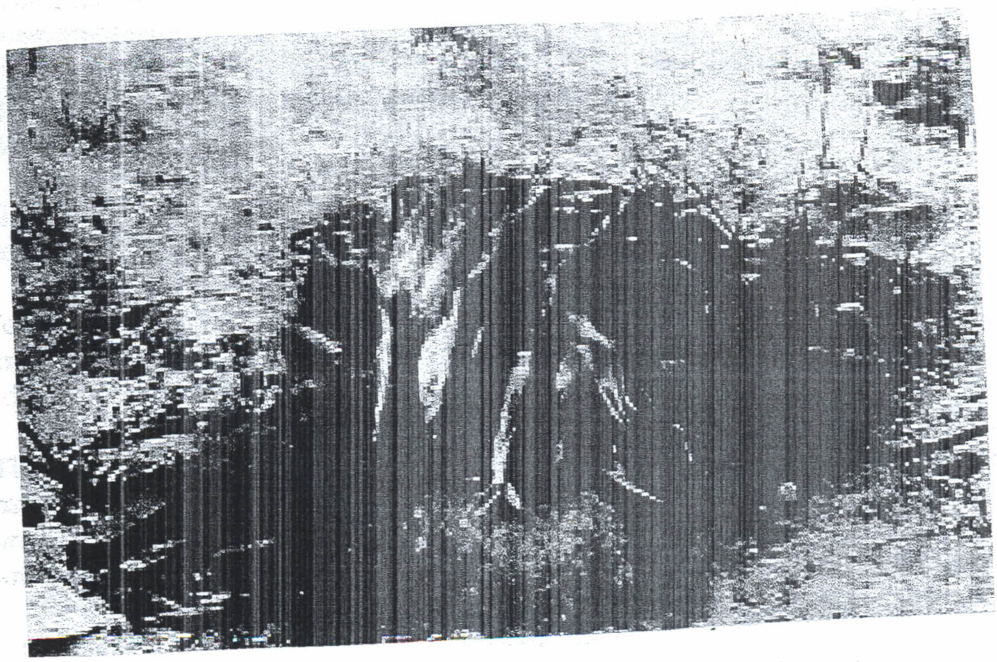

Fig. 8b. Smoking fish over an open fire.

Some smoke components are carcinogenic, especially the aromatic hydrocarbons such as benzypyrene. The World Health Organization (WHO) has therefore specified a maximum content of 1mg benzypyrene per one kg of flesh. Smoke that is almost free of benzypyrene can be obtained when the temperature of burning wood used for the smoking process does not exceed a temperature of 425 ^{O}C.
Time and temperature of smoking are the two most important factors which define the type of smoking and also constitute the initial important critical point.

There are two types of smoking processes namely, cold and hot smoking; although in Ghana only hot smoking is practised.

In hot smoking, a minimum internal temperature of 65 ^{O}C is required to eliminate *Salmonella* and *Staphylococcus aureus* which cause food poisoning. *Clostridium botulinum* type E is destroyed by heating above 82 ^{O}C for 30min. *C. botulinum* types A.B require heating above 120 ^{O}C over 15 min to be destroyed.
The non-proteolytic types of *C. botulinum* (A, E, F) can however grow and produce toxins at temperatures as low as 3.3 ^{O}C. They do not attack complex proteins and their growth in food cannot be detected by off-odours and off-flavours. The *C. botulinum* spores are resistant to heat, drying, salting and freezing.
During smoking, heat from the fire causes the moisture content of the tissues to be reduced, consequently leading to a reduction in the activity of spoilage bacteria. Some of the free water also migrates from inside the fish tissues to the skin surface from where it eventually evaporates.

For prevention of contamination of smoked fish products so as to have safe products, the following precautions must be taken in the fish processing plant:

- periodic medical examination of all personnel; preventing those affected with pathogenc or infections diseases from entering the processing area.

- all persons suffering from common cold or having hand injuries must be prevented from handling the product to prevent contamination.

- For the prevention of cross-contamination or recontamination after smoking, it is very important that the finished product be protected from bacteria from the raw fish or contaminated working areas.

- The finished product must be stored separately from the raw fish. Separate personnel must be used to handle finished products. In the case where the same set of personnel are to be used for both operations, care must be taken to thoroughly wash their hands and keep to strict clean personal hygiene.

- After each use, all equipment should be thoroughly washed by soap and rinsed with chlorinated water. If necessary, caustic soda solution must be used for cleaning especially the smoking cabinets, trays, trolleys, the smoking hall and the tanks or receptacles. These are then thoroughly rinsed with water.

- The floors and drains must also be washed with chlorine, which is an efficient disinfectant as well as a deodorizer.

Fermentation

In Ghana, fermentation of fish involves the use of left over fish after sales and therefore may not be good quality fish. Major species normally fermented are *Lates*, *Synodontis*, *Citharinus* or fatty species.
Fermentation involves heavily salting the fish and these products are normally solely used for flavouring soups and stews. The resultant product is soft and has strong stinking flavour with moisture content of between 48 and 65%.

The process of fermenting fish involves heavily salting the fish and packing them in layers interspersed with a lot of rock salt in fermentation vats. Weights are then placed on the fish to keep it below the level of liquid expressed from its tissues. The fish is then allowed to mature in the vats for weeks and up to several months.

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Unit 5: This unit comprises of the following:

a. **Microbial safety of fishery products.**
b. **Fish and fish products as sources of food poisoning in man.**

Microbial safety of fishery products

Previously, inspection and microbiological testing of end products have been the standard procedures to ensure safe foods; but now the Hazard Analysis Critical Control Point (HACCP) is a useful method to anticipate and prevent potential safety problems of food including fish products.
Implementation of HACCP in a total quality management system is a continuous activity requiring the involvement and commitment of management and staff alike. In HACCP system, food borne diseases (pathogenic microorganisms and the toxins they may produce) may be viewed as a top food safety priority. Such microbiological hazards may consist of pathogenic bacteria, viruses, parasites as well as biotoxins.
Depending on the catchment area some of the potential foodborne pathogens may occur naturally in the environment (eg. certain vibrios, *Cl. botulin*um type E, nematodes) and therefore total eradication from the raw material is not possible. Other pathogens (eg. *Shigella*, toxigenic *E. coli*,, hepatitis virus) are associated with faecal pollution and their presence will depend on the water quality. Thus most raw seafood should be considered potentially dangerous and processing for safety using the HACCP as a tool thus becomes the essential objective.

In order to conduct the hazard analysis itself and the selection of critical control points, it is necessary to know the disease agents associated with seafood and their behaviour during processing, distribution and cooking.

In assessing the safety of fish, certain other parameters such as the total volatile basic nitrogen (TVBN) and histamine levels are also important factors to consider.
However, TVB-N cannot be taken as a quality attribute in heat-treated fish product such as canned fish (e.g. sardine). It is only a valid attribute for the quality of fresh/frozen fish. For fish intended for fresh consumption, it is recommended that the safe value be up to 30mg/100g, while in fish intended for canning (and not a canned fish product) should not exceed 25mg/100g.

The Histamine content on the other hand may be used as an index of spoilage in scombroid fish. Scombroid fish poisoning (SFP) results from ingestion of fish containing high levels of free histamine. Initially, the disease was associated with the consumption of scombroid fish such as tuna, mackerel, bonito and saury. The disease in most cases is mild, of short duration (some hours) and self-resolving.
The Food and Drug Administration (FDA) considers a level of 20mg of histamine/100g flesh as an indication of spoilage (Defect Action Level), and a Hazard Action Level (HAL) of 50mg Histamine/100g flesh. Histamine is heat-stable.

Wholesomeness of fish

The wholesomeness of a product (that is, its safety) is generally assessed through different indices other than its sensory characteristics. Sensory methods alone are inappropriate for deciding on wholesomeness, though appropriate for evaluating the factors that determine fitness for human consumption (particularly spoilage factors).

Fish freshness is therefore most accurately evaluated by sensory methods inspite of the considerable effort that has gone into microbiological, chemical and physical indicators of fish quality.

In the case of canned and preserved fish products, sanitation and good manufacturing practices are particularly important as the products are of low acid nature and thus could be ideal media for highly toxic pathogens if not processed and handled properly.

The following general provisions included in each of the Codex standards state that

1. All products, when tested by appropriate methods of sampling and analysis should be free from pathogenic microorganisms capable of development under normal conditions of storage and shall not contain any substances originating from microorganisms in amounts which may represent a hazard to health.

2. Canned and similar products with an equilibrium pH above 4.6 shall have received a processing treatment sufficient to destroy all spores of *Cl. botulinum*, unless growth of surviving spores is permanently prevented by product characteristics other than pH.

3. In the case of frozen products, quick-freezing shall not be regarded as complete unless and until the temperature has reached -18°C at the thermal centre after thermal stabilization.

5b. Fish and fish products as sources of food poisoning in man.

Fish and its products as sources of food poisoning in man have been dealt with in Unit 3b (Fishborne diseases).
Refer also to the case study in unit 3 on food poisoning.

In this unit therefore, mention will be made of other fishborne diseases which can be caused by Protozoa, Flatworms (Platyhelminths), Roundworms (Nematodes), Crustacea, Fungi and bacteria. In addition, poisoning caused by naturally toxic species of fish will be highlighted.

Protozoa

Protozoa are often one-celled microscopic organisms that are mostly free living. Few of these cause diseases in man and animals. Protozoal infections in fish occur in the flesh causing severe localised or generalised softening. Some of these organisms are found to cause lesions on the skin and therefore regarded as important in fish farming activities. An example of this is *Chloromyscium thyrsites* found in certain fishes where the flesh is softened by proteolytic enzymes produced by the parasite. This is manifested by the fish flesh appearing like toothpaste. In certain fishes however, the flesh is seen dotted with pits of ulcerated spots full of white pustular material.

Flatworms

Flatworms or Platyhelminthes are free living and live in a host as parasites at certain stages of their life cycle. These include flukes, trematodes, tapeworms and cestodes. Fish is therefore the intermediate host of these parasites.
Flatworms (e.g. adult fish tapeworm) in fish may be found in the gut of fish and can only be exposed to man through consumption of whole fish whose gut has not been removed. This therefore does not pose much problem to man if properly handled and managed during processing of the fish. The larval tapeworms however may be found in the flesh of the fish.

The trematode larva of *Cryptocotyle lingua* exists in dark coloured cyst on or just under the skin of herring, mackerel and other related species and may be transfered to man when not properly cooked before consumption.

Two important diseases in man caused by eating raw or partially cooked fish with cysts of flatworm are caused by the lung fluke and the adult broad tapeworm; namely *Paragonimus* sp. and *Diphyllobothrium latum* respectively.
The intermediate host of the lung fluke (*Paragonimus*) are water snails and crustacea (crayfish and freshwater crab). On consumption of these (especially when improperly cooked), the adult fluke then migrates and lives in the lung of man.

The adult broad tapeworm of man (*Diphyllobothrium latum*) on the other hand is found in human intestines where it is transferred by eating improperly cooked fish. The intermediate hosts of this worm are many fishes such as Salmon or perch.

Roundworms

Roundworms or Nematodes are found in the gut and viscera of fish. As cysts they exist in the flesh of fish. Some of these parasites cause discolouration of the flesh such as the cod worm *Porrocaecum* (*Terranova*) decipiens whose larval form is brownish in colour and forms brown cysts in the flesh. The adult parasites however occur in the gut of seals.
Although fish nematode diseases are rare in man, Anisakis larvae which has its host as herring and squid causes serious inflammation of the stomach or gut wall of man if the fish is eaten uncooked. However, prolonged freezing and cold storage of the fish easily kill these nematodes. Hence it is recommended that fish before being eaten raw must be frozen at –20 °C for at least 24 hours.

Crustacea

Most parasitic forms of crustacea belong to subgroup copepods. As for example, the sea louse (*Lepeophtheirus* sp.) found on salmon is a free-living crustacean. Another type of crustacean, which occurs in the flesh of redfish, is *Sphyrion lumpi. Sebastes marinus* is also a crustacean parasite of importance that may constitute a health hazard to man.

Fungi and bacteria

Fungal infections may cause problems in tropical fish. A fungus (*Ichthyosporidium hoferi*) causes softening of fish flesh and has a sickly smell. When the fish is smoked, it is seen in the flesh of the smoked fish as white spots.

Bacteria infections which present itself as lesions, nodules and pustular areas in fish have been discussed extensively in Unit 3.

Biotoxic fish

Marine biotoxins are responsible for a large number of cases of foodborne diseases. Control of these type of biotoxins is difficult and the disease cannot be entirely prevented, since they are non-protein in nature and extremely stable. This indicates that cooking, smoking, salting and drying, which are normally used for food processing, do not destroy these toxins. In addition, the physical characteristics such as the appearance of the fish and changes in the colour of the skin, gills and eyes cannot be indicators to show the toxicity in fish and shellfish.

These marine biotoxins include tetrodotoxin, ciguatera (ciguatoxin), PSP-paralytic shellfish poison, DSP-diarrhoeic, NSP-neurotoxic and ASP-amnesic toxins.

Tetrodotoxin

Tetrodotoxin (TTX) is found in mostly the ovaries, liver and intestines of Puffer fish (*Tetraodontidae*), although some percentage of the toxin may exist in the testes, skin and muscle as well.
Examples of such fish species are *Fugu niphobles*, *F.chrysops*, *Lagocephalus laevigatus inermis*, *Ostracion immaculatum*, *Aracana aculeata* and other poisonous fishes called castor oil fishes like *Ruvettus pretiosus* and *Lepidocybium flavobrunneum* which cause pronounced purgative effect.
The lethal dose of TTX for man is estimated at 10,000 mouse units (MU), which is equivalent to 2mg of TTX.
Mortality rate due to Puffer fish poisoning is over 50% and it causes neurological symptoms 10-45 min after ingestion. These are manifested as tingling sensation in the lips and extremities, followed by paralysis and eventually death as a result of respiratory arrest and cardiovascular collapse within 6 hours.

Ciguatoxin

Ciguatera poisoning is caused by eating the flesh of carnivorous fish and shellfish inhabiting shallow waters in or near tropical and subtropical coral reefs.
Fish in these reefs acquire their toxicity from feeding on microscopic marine planktonic algae or toxic dinoflagellates. The principal sources are the benthic phytoplankton namely, *Gambierdiscus toxicus* and *Osteopsis lenticularis*.

Over 400 fish species inhabiting tropical or warm waters have been found to cause this type of food poisoning by elaboration of ciguatoxin, which has a comparatively long duration in man. This toxin is mostly found in the gut, liver and muscle tissue.

Symptoms are rarely fatal and include nausea, diarrhoea, vomiting, and severe neurological effects such as tingling sensations in the extremities. The neurological dysfunction includes reversal of the sensations of hot and cold, referred to as dry ice sensation.

Paralytic Shellfish Poisoning (PSP)

Two groups of PSP toxins have been identified and they are saxitoxins (STX) and gonyautotoxins GTX).

The largest number of human death from PSP occurs from the consumption of mollusc bivalves, such as mussels and clams. A large number of crabs have also been implicated to cause human death by their ability to accumulate large amounts of saxitoxins. The sources of saxitoxins are certain dinoflagellates and freshwater blue-green algae on which these crabs feed.

Symptoms of PSP are manifested within 1-2 hours of ingestion of the food. This takes the form of tingling, extreme numbness, muscle weakness and respiratory paralysis leading to death. STX has a weak hypotensive effect and may lead to death through cardiovascular collapse.

In countries where large production of bivalves is made commercially, marketing is prohibited when the toxicity, which is monitored regularly, exceeds the quarantine level of 80µg/100g or 4 MU/g.

Diarrhetic Shellfish Poisoning (DSP)

DSP is divided into three chemical structural classes, namely okadaic acid, penteno toxins and yessotoxins. Of these 3 groups, 7 toxins have been identified, all of which have been found to be lipophilic compounds. The most widely distributed DSP toxin however is okadaic acid (a polyether compound) and its derivatives and was first isolated from a sponge (*Halichondria okadai*).
Toxins of DSP are normally accumulated in the digestive glands of shellfish and bivalves which feed on dinoflagellates of the genera *Dinophysis* and *Prorocentrum*. Such dinoflagellates include *Dinophysis fortii*, *D.acuminata* and *D. acuta.*

Symptoms of DSP occur within 30min to a few hours after consumption of the shellfish. These symptoms manifests itself as gastrointestinal disorders such as diarrhoea, vomiting and abdominal pain. The victims however recover within 3-4 days and no fatality has been recorded so far.

The tolerance level is 0-60µg/100g.

Neurotoxic Shellfish Poisoning (NSP)

The sole known source of this toxin is a photo-synthetic dinoflagellate (*Ptychodiscus brevis*) which causes 'red tide' bloom found in the Gulf coast and off the coast of Florida.
The dinoflagellates as well as the toxin (brevetoxin) are highly lethal to fish, although no fatalities have been recorded on humans. The symptoms of this kind of poisoning include respiratory irritation.
With NSP, the tolerance level considered unsafe is any detectable level/100g.

Amnesic Shellfish Poisoning (ASP)

The intoxication in the case of ASP is caused by domoic acid, an amino acid produced by the diatom *Nitschia pungens*.
Fatality has been recorded for this toxin after consumption of blue mussels.

The symptoms range from slight nausea and vomiting to loss of equilibrium and central neural functions such as confusion and memory loss. In the case of ASP, the brain damage appears irreversible.
The tolerance level is 20ppm domoic acid.

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Unit 6 : This unit comprises of the following :

a. **Practical assessment of fish spoilage by physical and chemical tests.**
b. **Field trip.**

6a. Practical assessment of fish spoilage by physical and chemical tests.

Introduction

In all the countries in the World, FAO/WHO fish standards are used to ensure fair and uniform practices in the fish trade, to protect the health of the consumer and to promote the quality of the fish.

The TVBN (Total Volatile Basic Nitrogen) is widely used as freshness/spoilage indicator for wet or chilled fish.
Total volatile bases are defined as all volatile amines and ammonia produced due to bacterial and enzymatic action, and can be used as an index of fish spoilage (for fresh fish).

For quick frozen fish, other tests include determination of the composition of the fish, dimensions, weight, absence of defects, (e.g. freezer burn, discolouration, bones, parasites and foreign matter) are essential for the determination of the quality of the final product.

Quality of fish is however based on a combination of microbiological, chemical, biochemical and physical interactive changes.

1. Physical tests

Characteristics used in quality assessment of fish are the appearance and colour of the skin, eyes, outer slime, gills, peritoneum (gutted fish) as well as the odour of the gills. For fresh harvested fish, the skin will look clean and bright with full bloom, while if left to stand unpreserved for some hours, the skin becomes dull, gritty and unattractive with no bloom.

The slime of newly caught fish is transparent. The gills are also bright red and have strong seaweedy odour. The eyes look convex with black pupil and a translucent cornea. These are the characteristics used to describe very fresh and uncontaminated fish. As much as possible, fish meant for consumption and processing must be handled carefully in order to maintain close to these attributes described.

Eurofish Report, September 27, 1978

Table.4.

Description of the characteristics belonging to different freshness grades

(Modification of the EEC scheme, based on proposals by Torry Research Station)

Elements	Characteristics			
	E	A	B	C
Skin	bright, shining, iridescent or opalescent, no bleaching, full bloom, clean	Waxy, slight loss of bloom, very slight bleaching, slight dullness	dull, some bleaching, loss of bloom	dull, gritty, marked bleaching and shrinkage, no bloom
Outer slime	transparent or water white	milky	yellowish-grey, some clotting, brownish	yellow-brown, very clotted and thick
Gills	bright red, mucus translucent	pink, mucus slightly opaque (dark red for saithe)	grey and bleached, mucus opaque and thick (brown for saithe)	brown or bleached, mucus yellowish grey and clotted
Eyes	convex, black pupil, translucent cornea	plane, slightly opaque pupil, slightly opalescent cornea	slightly concave, grey pupil, opaque cornea	completely sunken, grey pupil, opaque dis-coloured cornea
Peritoneum (gutted fish)	glossy, brilliant, difficult to tear from flesh	slightly dull, difficult to tear from flesh	gritty, fairly easy to tear from flesh	gritty, easily torn from flesh
Gill odour	fresh, strong seaweedy, shellfishy (fresh oil, metallic, freshcut grass, peppery for plaice)	no odour, neutral odour trace musty, mousy, milky, caprylic, garlic or peppery (oily, sea-weedy, aromatic citric for plaice)	definite musty, mousy, milky, caprylic, garlic or peppery, malty, beery, lactic or slightly sour (oily, definite musty, slightly rancid, painty for plaice)	acetic, butyric, fruity, turnipy, amines, sulphide, faecal (muddy, grassy, rancid for plaice)

tes: The descriptive terms are meant to be guides and not all the characteristics described will necessarily occur in every fish. For two important species, ie plaice and saith, important deviations from the general characteristics are mentioned. With fat fish like herring and mackerel deviations may occur as well.

2. Chemical tests

The following chemical indices are used to assess the spoilage of fish and fish products:

i. Total Volatile Bases (TVBN):

These are defined as all volatile amines and ammonia produced due to bacterial and enzymatic action, and can be used as an index of fish spoilage (for fresh fish).

It involves making the fish or an extract of the fish alkaline and allowing the bases to volatile by distillation which is collected for measurement by titration. There are variations of this method. A disadvantage of the methods is blank values, which are caused by deamination of nitrogenous part of the fish during the alkaline volatization step. In order to minimize those blank values, several modification have been introduced which yield varying results.

TVBN cannot however be used as a reliable index of quality in canned products or in heat treated fish since heat treatment causes a substantial increase in the total volatile bases.

ii. Trimethylamine (TMA):

Trimethylamine (TMA) is the reduction product of TMAO (Trimethylamine Oxide). Amounts of TMA and TVB-N present in the fish can also be used as indices of fish spoilage, and thus an indication of fish freshness.

Bacteria growing on the surface of fish tissue produce volatile amines. One of such volatile base is trimethylamine (TMA), a reduction product of the component trimethylamine oxide. TMA has been used as an indicator of general fish spoilage. While the fish is still in *rigor*, dimethylamine begins to form and after *rigor* trimethylamine is formed.

Other volatile amines produced include ammonia and small amounts of monomethylamine, in addition to dimethylamine (DMA).

Although not universally accepted, the TMA determination has become one of the established procedures for determining fish quality. It has been proposed that TMA levels between 5 and 10mg/100g tissue should be considered the maximum allowable levels in international trading. TMA is however not useful in determining quality deterioration since TMA values are dependent upon the storage temperature of the fish and varies accordingly.

Dimethylamine (DMA) can be used as a basis for quality assessment of frozen fish. DMA has been shown to be produced autolytically at sub-zero temperatures.

While DMA has been shown for cod, haddock, husk, hake and Alaskan Pollock, no DMA has been found in similar studies of frozen lobsters, scallops or shrimps after extended storage at $-5^{0}C$.

Chemical tests for dimethylamine are most valuable in the early stage of spoilage and trimethylamine is most sensitive as an indicator in the later stages of spoilage.

iii. HISTAMINE :

This is formed as a decomposition product in fish and the highest levels are often found in scombroid-type fish. With the consumption of such fish with high levels of Histamine violent allergic reactions are caused in sensitive persons.

The development of histamine is species specific and is produced at different rates in different conditions. As a result, it cannot be relied upon as a quality indicator in all species of fish.

iv. Thiobarbituric acid (TBA) :

TBA has been used to assess the development of oxidative rancidity. Oxidation of fat-containing foods leads to the formation of malondialdehyde or derivatives of this compound. The reaction of malondialdehyde with TBA is an effective means of measuring the extent of auto-oxidation, but unreliable as an index of fish freshness.

v. pH

Measurement of the hydrogen ion concentration (pH) is not considered as a reliable indicator of fish quality, although it is included in some standards.

6b. Field Trip

Field trips will be organized and undertaken to fish landing sites along the Accra/Tema coastline including James Town and Osu beaches as well as the Tema fishing harbour market where interaction with the fishermen and fishmongers will be made.
Small, medium and large-scale fish processing plants will be visited in order to observe at first hand the various fish processing methods and techniques with a view to highlighting most of the quality control systems, and prevention of hazards as discussed in the different units of the module. This will include fish processing plants such as Divine SeaFoods and Pioneer Food Cannery companies at Tema.

Visits will also be made to processing plants of the Ghana Association of Foodstuff Exporters (GAFEA) in order to observe the way fish is handled and packaged for export.

Summary

The amounts of all the above chemical substances formed often depend upon fish species, bacteria present and time/temperature relationship.

The limits of the chemical quality parameters therefore vary depending on the fish type. A high level of some of these chemical indicators is often a normal quality characteristic in certain processed and fermented fish products; e.g. TVB-N thus cannot be used as a reliable index of quality in canned products or in heat-treated fish since heat treatment causes a substantial increase in the total volatile bases.

Also the development of histamine is species specific and is produced at different rates in different conditions. As a result, it cannot be relied upon as quality indicator in all species.

However, since in fresh fish the concentration of these chemicals increases with time as a result of their deterioration, chemical analysis is used to support sensory testing and physical appearance in assessing whether a sample is fit for human consumption or not.

A number of different tests can be used for estimating the degree of spoilage in fish. These include total bacterial numbers, total volatile bases, TMA, total volatile reducing substances, sensory analyses, refractive index of the eye fluid, electrical parameters of the fish flesh, volatile acids, volatile ammonia and total volatile nitrogen.

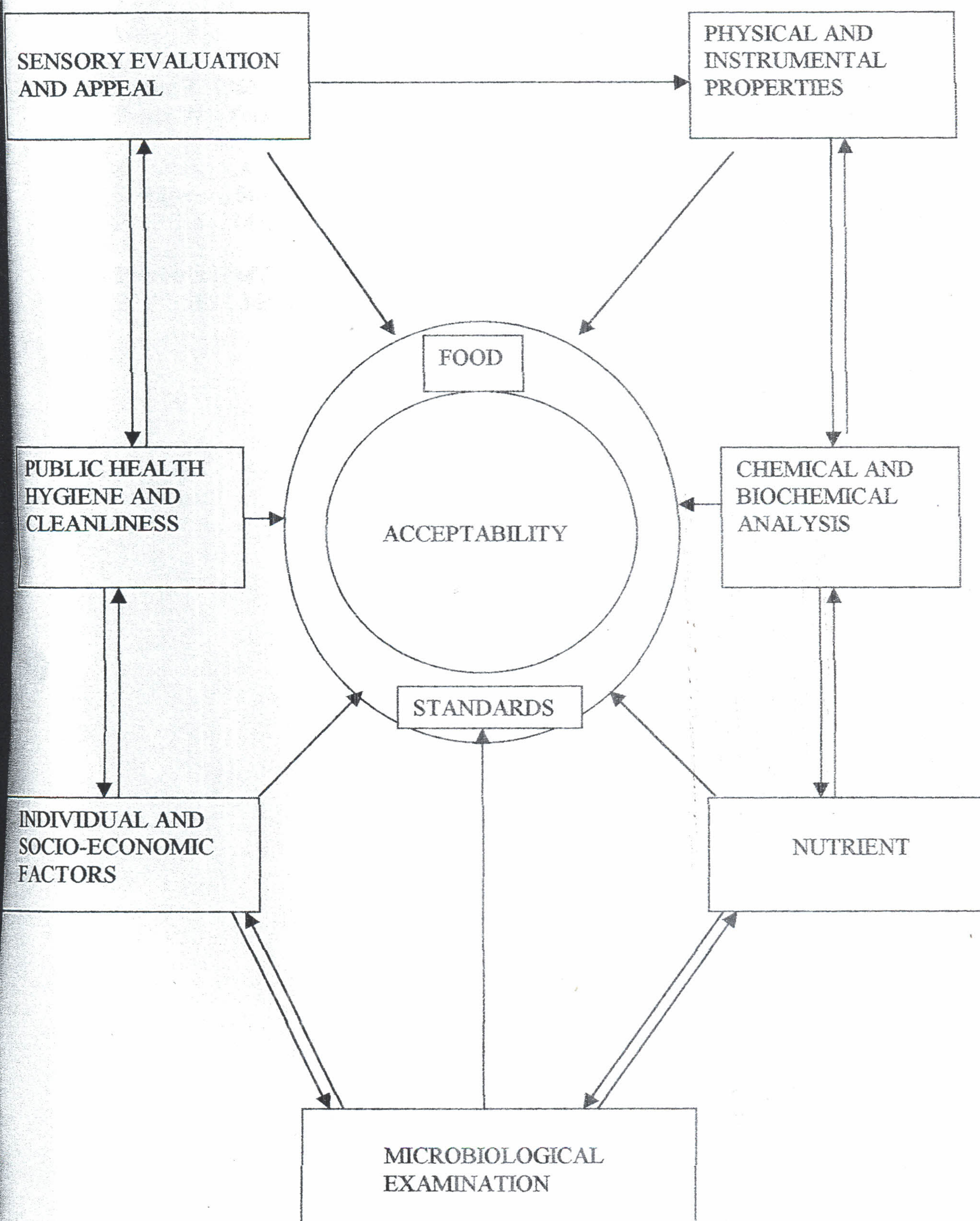
The safety or wholesomeness of fish standard should therefore be viewed as a combination of physical, chemical, biochemical and microbiological indices.

The microbiological indices as described in earlier units include investigation into the total viable bacterial count, the mould count as well as levels or numbers of the following bacteria organisms in the fish: *Salmonella* spp., *S. aureus* and its enterotoxin, *Vibrio parahaemolyticus,* Coliforms and *E. coli*, and *Cl. botulinum*

The specification states the limits of acceptability in terms of the presence and levels of these organisms in the fish and/or its products for its use to assess the wholesomeness or safety of the fish.

In addition chemical analysis for histamine content and heavy metals (particularly mercury) are also used to assess the safety of fish products.

Fig. 9. Some factors determining quality in food
(Harper, 1983)



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