UNIT 2 SPOILAGE INDICES

Structure

2.0 OBJECTIVES

After reading this unit, you will be able to:

- explain why do fish spoil?;
- identify the organoleptic parameters related to spoilage;
- discuss the chemical parameters related to spoilage; and
- analyze the microbiological parameters of spoilage.

2.1 INTRODUCTION

Fish is a highly perishable commodity. Spoilage of fish begins as soon as the fish dies. Soon after catch, if the fish is not immediately cooled, it spoils rapidly. However, spoilage rate can be reduced by following good handling practices and effective temperature control. The major changes occurring during spoilage are biochemical, microbial and autolytical changes. There are number of enzymes present in the body tissue and the intestinal tract of fish. Apart from this, the numerous bacteria present on the surface, gills and in the gut also release several enzymes bringing spoilage. As long as the fish is alive, no spoilage takes place in the fish muscle. When a fish dies, the biochemical changes responsible for anabolism stop. As a consequence of this process, catabolism starts bringing spoilage to fish flesh. The digestive enzymes still being active instead of acting on the food present inside the gut begin to digest tissue components of the fish body such as lipids, carbohydrates and proteins. This process is called “autolysis”. In addition to this, the native bacteria present in the fish also multiply very rapidly.

These bacteria also secrete a number of enzymes causing rapid break down of fish tissues. Another factor responsible for the deterioration in quality of fish results from oxidation of lipids. Apart from these changes, autolysis also causes loss of pigments, development of off-flavour and browning. The rapid colonization and multiplication of bacteria and the autolytic changes eventually complete the total break down of fish muscle. Thus, spoilage of fish is caused by the collective action of autolytic and microbial parameters. As a result of their action, simple chemical substances are produced and their presence helps us to find out the extent of spoilage of fish. The net result is the spoilage making the fish inedible for man.

2.2 ORGANOLEPTIC QUALITIES

You must realize that apart from health hazards, there are certain properties of the food, which are not liked by the consumer. Some of these characteristics can be evaluated by human sense organs, for raw as well as ready-to-eat products. They are generally referred to as organoleptic quality characteristics. Colour, odour, appearance, texture and taste are the most common organoleptic characteristics. By experience and training, one can easily evaluate qualitatively as well as quantitatively (score) any change in colour, appearance, texture, odour and taste. As fresh, raw and freshly cooked food/fish items have specifically characteristic organoleptic properties, any significant variation can be evaluated and the reason for change namely the extent of spoilage or decomposition can be quantified to decide on the suitability for human consumption. Organoleptic evaluation is widely used for deciding the quality of fish and shellfish. The organoleptic changes due to spoilage will also show certain chemical changes in the form of increasing levels of total volatile nitrogen (TVN) in fish meat, Trimethyl Amine Nitrogen (TMA-N) in marine fish, indole in protein rich foods, rancidity in fatty foods etc. Their presence above a certain level is found to reduce consumer acceptance and hence these indicators are called chemical quality parameters or quality defects.

Apart from the above chemical effects, growth and multiplication of bacteria also bring about certain changes which are perceptible by human sense organs. These changes can also be evaluated to determine the extent of spoilage. Such physical changes are called ‘organoleptic indices’. The important organoleptic indices of spoilage are as follows:

1) **Texture:** In case of fresh fish, the texture of fish meat on pressing with finger will be firm and elastic. In other words, the distortion created by finger pressing will be removed immediately and the pressed surface will come back to its original shape. On spoilage, with extend of spoilage, the texture will gradually change to soft and flabby with retention of finger impression or distortion of finger pressing.

2) **Eyes:** In case of fresh fish, the eye balls will be protruding and the eye lens will be transparent and pupil will be jet black. On spoilage, the eye balls will sink (Sunken eyes), the eye lens will become opaque and cloudy.

3) **Gills:** The gills of fresh fish will be bright red and free from mucous deposit. With spoilage the bright red colour turns brown and then gets bleached. The gills also get covered with thick mucous. This mucous covering also changes its thin transparent nature to thick and yellow in colour on spoilage.

4) **The appearance of anal opening:** The anal opening of fresh fish will be normal and constricted. On spoilage, it will become red and swollen.

5) **Fish surface:** The colour and surface of fish body also undergo changes with spoilage. In fresh fish, the body surface will show a characteristic colour with metallic sheen. The surface also will be covered with a thin and transparent layer of slime. On spoilage, the characteristic colour and metallic sheen will be lost and the surface will get covered with thick cloudy or yellow slime.

6) **Cross-section:** A critical observation of the cross-section of the fish is also found to give a clear indication about the extent of spoilage. In case of fresh fish, the tissue around backbone at the cross-section of fish will be bluish and transparent without reddish brown colour. On spoilage, the muscle will turn waxy and opaque with or without reddish brown discoloration.

A) Fresh Fish: Scoring key

The tolerance for organoleptic criteria (as applied to fish and shellfish) are shown in Table 2.1.

Table 2.1: Organoleptic Evaluations of Fish and Prawn

Fish (Raw)	
Characteristic	**Maximum Mark Assigned**
General Appearance	5
Flesh including belly flap	5
Odour	10
Texture	5
Flavour (Cooked)	10
Chewable texture (Cooked)	5

Parameters	Excellent	Very good to Good	Satisfactory to Poor
1) Appearance of: a) Eyes (2 marks)	Convex, Jet black pupil, Translucent cornea.	Flat, Grey pupil, slight opalescent cornea and lens.	Sunken eyes, Milky white pupil, Opaque cornea and lens.
b) Gills (2 marks)	Bright red and without slime.	Darkening of gills with mucus smear.	Gills dark brown or bleached with thick slime.
c) Skin/scale (1 mark)	Characteristic shining and thin transparent slime.	Outer slime, Viscous and milky. Bleaching/ dull colour of skin.	Viscous and opaque outer slime. Bleaching of the characteristic colour, loose scales.
2) Cross-section around back bone	Bluish translucent flesh. No reddening around backbone.	Muscle with waxy appearance. No reddening around backbone.	Opaque muscle. Light reddening around backbone.

3) Belly Flap	No discolouration of belly flap	Some discolouration along belly flaps	Yellowing of belly flaps* (* In case there is clear discolouration around backbone with severe discolouration and breakage of belly flaps, the sample shall be summarily rejected)
4) Odour (*Bring the fish close to the nose and try to get exact odour)*	Fresh sea weedy odour	Neutral odour like bready, malty, yeasty smell. (Slight fermented smell)	Smell of hydrogen sulphide/indole/ skatole etc. (foul smell)
5) Finger impressing	Firm and elastic. Scales tight. No retention of finger press impression.	Light softening but firm scales, Takes long time to regain shape after finger pressing.	Soft and flabby muscle and loose scales. Finger impression permanent.
6) Flavour	Characteristic fresh flavour, juiciness and sweetness	Faintly sweet and neutral taste, but juicy.	Some off-flavour. Reduction in juiciness and slight bitterness.
7) Texture on cooking and chewing	Firm and thick with no discoloration	Firm but woolly with slight yellowing.	Cheese / Soapy texture with yellow/ brown discoloration.

Grading based on total score:

Excellent	>35 - 40
Very good	>25 – <35
Good	>20 – <25
Satisfactory	>18 – <20
Poor (Unacceptable)	<18

B) Fresh Prawns: Scoring Key

Prawn (raw)	
Characteristic	**Maximum Mark Assigned**
Colour	10
Odour	10
Shell attachment	5
Texture	5
Colour (Cooked)	5
Texture (Cooked)	5
Flavour (Cooked)	10

Parameters	Excellent	Very Good to Good	Satisfactory to Poor
Colour *(Wash the prawn well and observe the colour of shells)*	Colour characteristic of the species Grayish green (tiger) Light brown to pink (Marine prawns) Pale white (Naran prawn) Bright pink (deep sea prawn)	Some bleaching of the characteristic colour.	Bleaching or dull reddish colour
Odour *(Bring the prawn close to the nose and feel the smell)*	Mild characteristic odour of the species (Sea weedy odour)	Bready/Mouldy/ yeasty odour (Slight fermented smell)	Slight Ammoniacal smell or decomposed smell (foul smell).
Shell Attachment *(Except at the time of moulting). Peel a portion of the shell and examine.*	Shell and meat firmly attached – Some meat attached to peeled shells.	Shell slightly loose, head slightly hanging,	Soft shell, which can be easily detached from meat and loose hanging head
Texture (*Press lightly the peeled prawn between fingers and feel the firmness)*	Meat firm and elastic.	Soft meat with slight grittiness	Soft meat and severe grittiness- meat flabby.
Cooked colour (*Cook the shell on prawn in 3% brine at 100^{o}C for 10 min)*	Shell and meat appear attractive pink with some deep pink spots.	Pale pink colour without brightness	Dull pink to bleached condition.
Texture (Cooked)	Juicy, succulent, tender but firm texture.	Loss of tenderness, slight firmness and less juiciness	Tough and fibrous texture. Loss of juiciness.
Flavour (Cooked)	Fresh, Sweet, characteristic flavour	Fresh but less sweet to bland flavour	Slight bitter taste or off flavour.

Grading based on total score:

Excellent	>40 - 50
Very good	>35 – <40
Good	>25 – <35
Satisfactory	>22 – <25
Poor (Unacceptable)	<22

? **Check Your Progress 1**

Note: a) Use the space given below for your answers.

b) Check your answers with those given at the end of the unit.

1) Name some of the most common organoleptic characters?

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2) What is the use of organoleptic evaluation?

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3) How can you judge spoilage of fish from the gills?

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2.3 CHEMICAL PARAMETERS

Spoilage of fish is caused by the cumulative action of autolytic and microbial parameters. As a result of their action, simple chemical substances are produced and their presence helps us to find out the extent of spoilage of fish. Let us attempt to learn something about these.

2.3.1 Autolytic Spoilage in Fish

The spoilage of fish can be defined as "irreversible changes occurring in post-mortem fish muscle making it unacceptable to consumers". Such changes are brought about as a result of careless handling and faulty pre-processing or storage. Spoilage can be broadly classified into two types; bacterial spoilage and autolytic spoilage. Bacterial spoilage results from growth and multiplication of microorganisms at the expense of muscle constituents. Even though bacterial growth is the major cause of spoilage of fish, it can be effectively controlled by proper processing methods.

The rate and extent of autolytic spoilage in fish are considerably less than bacterial spoilage, but at first, autolysis plays an important role in flavour development and the onset of bacterial spoilage. Absolutely fresh and healthy fish is impermeable to bacteria due to the intact skin. Further, the absence of simple and easily available nutrients in absolutely fresh fish makes it difficult for bacteria to grow and multiply. However, after the death of the fish, autolysis sets in, making the fish skin permeable to bacteria and at the same time releasing simple sugars, free amino acids, free fatty acids, etc. These nutrients provide a nutrient-rich medium for bacteria to grow and multiply.

In simple terms, autolysis is defined as the degradation of muscle and skin constituents by endogenous enzymes. Since the enzymes causing autolysis arise from within the fish muscle (endogenous), the prevention and control of autolysis is very difficult unless drastic treatments are used. However, a clear understanding of autolysis would be useful in devising suitable methods to effectively reduce spoilage, thereby preserving the delicate flavour.

2.3.2 Role of Enzymes in Autolysis

Live fish contain numerous enzyme systems required for the complex metabolic reactions taking place. These enzymes are distributed both in the intracellular (within the cell) and extracellular (outside the cell) compartments throughout the fish muscle. Their individual concentrations vary with the nature and function of the tissue. In live fish, all these enzyme systems are used in metabolic processes. Consequently, most of the enzymes occur as some sort of inactive precursors. In certain other cases, the enzymes are kept isolated from their substrates.

Once the fish is dead, the ability of its body to regulate the enzymes is lost. The absence of blood circulation, depletion (reduction) of oxygen, depletion of energy sources such as CTP (creatine triphosphate) and ATP (adenosine triphosphate), and the breakdown of the body's scavenging mechanism bring an end to all anabolic or biosynthetic processes. In effect, in post-mortem fish muscle, only the catabolic and degrading reactions are active. These changes lead to the accumulation of catabolic products.

2.3.3 Glycolysis and Decrease in pH

The major catabolic reactions occurring in post-mortem fish muscle relate to three groups of compounds, *viz.* carbohydrate, fat and protein. Even though the amount of carbohydrate in fish muscle is very low compared to protein and fat, its metabolism in live and post-mortem fish muscle is of paramount importance in deciding the extent of *rigor mortis* and subsequent autolytic spoilage in fish. Most fish are caught after vigorous struggles. During this period, the fish utilizes almost all its blood glucose and a substantial quantity of tissue and liver glycogen for deriving energy through the glycolytic pathway. Phosphorylase, the main enzyme responsible for initiating glycogenolysis, is distributed widely in almost all tissues in substantial quantities. The almost neutral pH optimum and the high activity of phosphorylase at ambient as well as near sub-zero temperatures considerably augment glycogenolysis/ glycolysis in fish muscle leading to accumulation of lactic acid which will be reconverted to glycogen if the fish is allowed to rest. However, this does not occur in a fish catch, as the fish struggle until they are dead. The fish are then usually frozen or chilled and stored pending utilization.

As a result of the glycolysis, a considerable amount of lactic acid accumulates in fish muscle. Lactic acid is neither neutralized nor removed from the site of formation resulting in reduction of the tissue pH.

2.3.4 Contribution of Lipolysis to Muscle pH

Another set of enzymes that are active during struggling as well as post-mortem are lipases. These enzymes are widely distributed in almost all fishes, especially the fatty fishes and the ones with red meat. Most lipases from fish and shell fish pancreas and muscle showed lipolysis in the pH range 6-10 and at all temperatures from –20 to 40°C.

The properties of lipases, as well as their environment in fish free of inhibitors are extremely favourable for lipid breakdown in fish muscle at ambient as well as low temperatures. The primary products of lipolysis are free fatty acids and glycerol. In dead fish, products of lipolysis also accumulate at the site of formation.

Of these products, free fatty acids, although weak, will make a significant contribution towards lowering the pH of the tissue.

The fatty acids of carbon chain (lower free fatty acids) formed as a result of lipolysis will also impart off-flavour to fish muscle and is known as hydrolytic rancidity. The higher carbon chain unsaturated fatty acids released will easily become vulnerable to oxidation by atmospheric oxygen, resulting in oxidative rancidity. During oxidation of fat, peroxide is formed. Therefore, peroxide value gives a measure of oxidative rancidity.

2.3.5 Acidic pH Activates many Autolytic Enzymes

The cell cytoplasm of fish muscle contains small circular organelles called lysosomes. Lysosomes contain number of hydrolytic enzyme systems capable of degrading almost all of the tissue components such as carbohydrate, fat, protein, nucleic acids, etc. The normal function of lysosomal enzymes in live fish or any other living organism is to digest dead cells for further processes of synthesis or oxidation for energy generation. This function has led to lysosomes also being called “suicide bags”, as the breakage of lysosomal membrane will result in self-digestion.

Fish-muscle lysosomes are rich in proteolytic enzymes, especially the cathepsins and proteases.

Most cathepsins and other proteolytic enzymes are highly active at acidic pH. They are also active at a wide range of temperatures from –10 to 60°C. Thus, much of the lysosomal enzymes released into fish muscle, have the most favourable conditions for vigorous activity in post-mortem fish muscle and bring about partial breakdown.

2.3.6 Role of Gut Enzymes

Another group of enzymes actively engaged in autolysis of whole fish is the digestive enzymes of the gut. Fish gut contains a wide spectrum of enzymes capable of hydrolyzing protein, fat and carbohydrate. The major enzymes of fish gut are proteases, including pepsin, trypsin and chymotrypsin, followed by lipases and carbohydrases. Once the fish is dead, the enzymes hydrolyze and perforate the stomach and intestinal walls and leak into the surrounding tissue, causing general hydrolysis. This is the mechanism of belly bursting seen in fatty fishes like capelin and oil-sardine. This is a clear index of spoilage.

However, the potent autolytic enzymes of the gut are effectively contained by the stomach and intestinal walls for about 4 to 7 days in fish stored at 0°C- 4°C. Moreover, for processing and storage, gut enzymes can be effectively removed by evisceration.

The autolytic changes described above relate to the spoilage of fish muscle. There are also some autolytic changes which are considered beneficial for fish as food material. An important group of enzymes responsible for such beneficial changes in post-mortem fish are the nucleodepolymerases. They are enzymes responsible for hydrolyzing nucleic acids to mononucleotides. Two important enzymes of this category are acid ribonuclease and acid deoxyribonuclease. As the name suggests, these enzymes are also active in acidic pH and are of lysosomal origin.

The nucleotides produced as a result of the activity of these enzymes greatly enhance the flavour of fish and meat in general.

Thus, as a result of lowering of pH, glycolysis and lipolysis, cathepsins and other proteolytic enzymes are activated and released into the tissue. These enzymes start degrading the tissue proteins tenderizing the muscle (6-10 hours after death). Up to this stage, the fish will be in ideal condition for cooking and consumption. As time passes, the autolytic process proceeds further and results in gradual loss of texture, taste and flavour due to the formation of polypeptides, peptides, free amino acids, free fatty acids, hypoxanthine, etc. All these are spoilage indices which are chemical in nature. Due to these changes, the fish skin will become easily permeable to bacteria. Bacteria also enter through the gills. The products of autolysis provide a nutrient-rich medium for bacteria to grow and multiply. Once bacterial access to tissue is established, spoilage becomes rapid, resulting in total breakdown of fish tissue producing a multitude of metabolites, including the foul-smelling amines, characteristic of spoiled fish.

Check Your Progress 2

Note: a) Use the space given below for your answers.

b) Check your answers with those given at the end of the unit.

1) How does bacterial spoilage occur?

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2) Define autolysis?

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3) What is the end result of Glycolysis?

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4) What causes belly bursting?

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2.4 MICROBIAL SPOILAGE OF FISH AND SPOILAGE INDICES

A live fish will have very few bacteria on its surface. However, once the fish dies and it is not preserved by ice, bacterial colonies multiply and increase in numbers. Let us now learn about bacteria and their role in the spoilage of fish.

2.4.1 Microflora in Fishes

Every fish has a native flora of micro-organisms. The nature of this native microflora largely depends on the habitat of fish. Based on salinity, the habitat of fish can be classified into three categories. They are the Marine, brackish and fresh water habitats. The nature of micro-organisms in these habitats are found to vary. About 80% of the bacteria in marine water are gram negative (-ve). They are highly salt tolerant and in fact they require 2-3% salt in the medium for normal growth. Even though marine microbes are potent spoilers, they are not pathogens.

The microbes of fresh water are mostly a mixture of gram positive (+ve) and gram –ve organisms. They are less tolerant to salt, rather, they are killed or inhibited by salt concentrations above 0.5%. The fresh water microbes are also found to be a mixture of spoilage organisms and human pathogens. The presence of human pathogens can be traced back to the close association of human life and fresh water availability.

The brackish water is actually a mixing area of fresh water and marine water. Consequently, it will have salinity and microbial characteristics in between that of fresh water and marine water. Thus, the brackish water will have a salt content of 0.5 to 2% with a microbial flora with and without salt tolerance, characterized by the presence of gram +ve and –ve species. However, due to the salinity the existence of human pathogens are rare in brackish water.

The bacteria in these waters are free swimming and they occur in association with plankton. As fish move in such waters, they exchange the water through gills and consume plankton. As a result of this, the skin, the gills and gut of the fish get a compliment of the micro-organisms present in the water. Even though the brackish water and fresh water microflora vary widely due to several types of contamination, the microflora of marine water show a stable pattern, with reference to various species.

As long as fish is live, whatever be the type of bacteria they cannot attack the fish muscle, due to the immune systems and the intact membranes. Consequently, the muscle of live fish is sterile. On death, the immune system fails and the membranes breakdown due to autolysis exposing the sterile fish muscle to bacterial invasion. Post-mortem changes, particularly autolysis brings about a partial breakdown of the macro-molecules, converting fish muscle into a fertile medium for bacteria to thrive. Marine fish with a high content of non-protein nitrogen (NPN) will be a better nutrient medium for bacteria and hence is more susceptible to bacterial spoilage than fresh water fish with a lesser NPN content.

The growth and multiplication of microbes will bring about an all-round hydrolysis of various biological molecules in fish muscle. Of the different molecules affected, the changes in proteins are of paramount importance as they form the basis of fish spoilage. The products of bacterial spoilage on carbohydrates and lipids, though give some spoilage indices, their contribution to changes in organoleptic properties is insignificant.

On bacterial spoilage, the proteins will be broken down to polypeptide, peptide and to amino acids by bacterial proteases and peptidases. The amino acids are further metabolized by bacteria in different routes depending on certain conditions. The final effect of these metabolic reactions is either a decarboxylation to give a corresponding amino compound or deamination to give an acid. The major metabolic reactions thus occurring as a result of microbial activity are:

1) ***Reductive deamination of amino acids:*** In this, a molecule of amino acid is reduced to produce ammonia and an acid. In other words, during reductive deamination, amino group of an amino acid combines with hydrogen to form free ammonia and an organic acid.

$$R-\underset{\substack{|\\ NH_2}}{CH}-COOH \xrightarrow[\substack{\text{reductive}\\ \text{deamination}}]{H_2} R-CH_2-COOH + NH_3$$

Some bacteria bring about hydrolytic deamination of amino acids to produce free ammonia and a hydroxy acid as shown below:

$$R-\underset{\substack{|\\ NH_2}}{CH}-COOH \xrightarrow[\substack{\text{Reductive}\\ \text{deamination}}]{H_2O} R-\underset{\substack{|\\ OH}}{CH}-COOH + NH_3$$

The above reactions mostly occur in the absence of free oxygen.

2) ***Oxidative deamination of amino acids:*** In presence of oxygen, some bacteria can bring about removal of amino group of an amino acid to produce ammonia and a keto acid. Such reactions are called 'oxidative deamination'.

eg: $$R-\underset{\substack{|\\ NH_2}}{CH}-COOH \xrightarrow[\substack{\text{oxidative}\\ \text{deamination}}]{O_2} \underbrace{R-\overset{\substack{O\\ \|}}{C}-COOH}_{\text{keto acid}} + NH_3$$

The acids, hydroxy acids and keto acids formed by deamination are further metabolized by bacteria to give carbon dioxide and water. Ultimately, the deamination reactions will lead to production of ammonia, carbondioxide and water.

3) ***Oxidative decarboxylation of amino acids:*** In this, the bacteria bring about oxidation of amino acids to produce carbon dioxide and an amino compound.

eg: $$R-\underset{\substack{|\\ NH_2}}{CH}-COOH \xrightarrow[\substack{\text{decarboxy-}\\ \text{lation}}]{\text{Oxidative}} \underbrace{R-CH_2-NH_2}_{\text{Amine}} + CO_2$$

When the basic amino acids (amino acids with two amino groups) arginine and lysine are subjected to oxidative decarboxylation, the amine formed is the diamine putrecine and cadaverine with typical putrid smell and giving a clear indicator of spoilage.

On the other hand, if tryptophan undergoes this type of decarboxylation, the products can be indole and skatole which produce faecal smell and their presence indicates advanced spoilage.

The amino acid histidine undergoes this type of decarboxylation on spoilage and produces the typical allergen called histamine also known as scombrotoxin, which is responsible for the Scormbroid poisoning from eating spoiled tuna, mackerel etc.

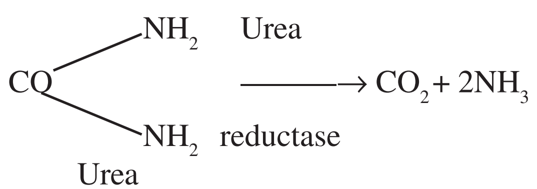
Similarly, decomposition also takes place in sulphur amino acids like cysteine, cystine and methionine leading to production of foul smelling hydrogen sulphide and mercarptans.

Eg: Cysteine and cystine	$\rightarrow H_2S$	Hydrogen sulphide
Methionine	$\rightarrow CH_3\text{-}SH$	Methylmercaptan

The effect of bacterial growth and multiplication on nucleoproteins and sub-units also cause damage to the quality of fish. Bacterial decomposition of nucleoproteins, produce xanthine and hypoxanthine along with a host of other compounds. These two compounds add an unpleasant bitter taste to fish. In extreme condition, these compounds also breakdown further to give ammonia. All these are clear chemical indices of spoilage.

Bacteria are also known to decompose the non-protein nitrogen fraction of fish. The most important compounds of this category are trimethyl amine oxide (TMAO) in marine fish and urea in elasmobranches. Marine bacteria elaborate the enzyme called TMAO reductase, which decompose TMAO to trimethyl amine. Trimethyl amine is responsible for the typical spoilage smell of marine fish, while TMAO gives the typical sea weedy smell of fresh marine fish. These are also clear indices of spoilage of fish. Similarly, bacteria decompose the urea in elasmobranches to produce carbon dioxide and two molecules of ammonia.

$$2\,\text{TMAO} \xrightarrow[\text{reductase}]{\text{TMAO}} 2\,\text{TMA} + O_2$$



The growth and activity of microbes in fish also bring about changes in lipid and carbohydrate components of fish. But, in most cases, these changes are marginal and insignificant compared to the changes in protein. The contribution of these products to spoilage and spoilage indices is insignificant compared to the products of protein decomposition by bacteria.

Thus, as a result of bacterial spoilage a variety of bases like ammonia, mono-amines, di-amines (putrecene, cadaverine, histamine), tertiary-amine (TMA), etc. are produced which the bacteria is unable to decompose further. With extent of spoilage, these compounds accumulate in fish tissue. All these compounds are basic and together they will increase the pH of fish muscle to neutral or above neutral (pH=7). The moment pH goes above 7 these amines will be slowly released from muscle as volatile bases. Most of these amines as well as the sulphur containing compounds are toxic and foul smelling and so impart stale or putrid odour to the spoiled fish. Most of the bases produced being volatile in nature, a measure of total volatile bases is found to increase with spoilage. This is the basis of using total volatile basic nitrogen (TVBN) as a spoilage index.

A careful analysis of the concentration of certain products of autolysis and bacterial spoilage with extent of spoilage is shown to provide a direct relationship between spoilage and their concentration. A measure of such products of spoilage will give a clear objective idea about the extent of spoilage. Since these products are chemical compounds they are called chemical indices of spoilage. The most important chemical indices of spoilage are:

1) pH: As a result of increasing spoilage, the amounts of basic substances produced in the fish tissue *increase* steadily producing an increase in pH from acidic side to neutral and to alkaline pH. Thus, an alkaline pH (>7) for fish muscle is a sure indication of spoilage.

2) The *content* of total volatile bases or content of ammonia (TVBN) in fresh water fish/content of TMA in marine fish also increases with spoilage.

   For fresh water fish, the total volatile base nitrogen (TVBN) content will be <20-25mg per 100g fish. On the other hand, total volatile base nitrogen content of fresh marine fish will be <30 to 35mg per 100g muscle. TVBN values above these tolerance limits are an indication of spoilage.

3) The nucleotide degradation product hypoxanthine is also found to increase with spoilage. Consequently, a measure of hypoxanthine will give an index of spoilage and in fresh fish hypoxanthine content is found to be less than 25 mg per 100 g fish muscle.

4) Free ammonia and hydrogen sulphide are also found to emanate from spoiled fish. The presence of ammonia can be detected by white fumes when exposed to hydrogen chloride gas. Similarly, the emanation of hydrogen sulphide gas can be detected by blackening of a piece of lead acetate paper exposed to the vicinity of fish.

? **Check Your Progress 3**

Note: a) Use the space given below for your answers.

b) Check your answers with those given at the end of the unit.

1) What happens to proteins due to bacterial spoilage?

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2) What is oxidative deamination?

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3) How can you use ammonia as a spoilage index?

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Activity 1

Visit a nearby market and buy some fishes which are not very fresh. Using the methods and suggestions used in this unit, try to assess the freshness/ spoilage percentage of the fishes. Make small tables and give marks on freshness grades.

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2.5 LET US SUM UP

Spoilage of fish begins as soon as the fish dies. The major changes occurring during spoilage are biochemical, microbial and autolytical changes. Another factor responsible for the deterioration in quality of fish results from oxidation of lipids. The net result is the spoilage making the fish inedible for man. Off-odours and off-flavours, slime, production of gas, discoloration and softening of muscle are the signs of spoilage. Thus, spoilage of fish is caused by the cumulative action of autolytic and microbial parameters. As a result of their action, complex chemical substances are produced and their presence helps us to find out the extent of spoilage in fish. This unit helps you in understanding these complex changes happening in fish after death and how some of these act as indices in estimating spoilage of the fish.

2.6 GLOSSARY

Anabolism : The phase of metabolism in which complex molecules, such as the proteins and fats that make up body tissue, are formed from simpler ones.

Arginine : An amino acid.

Catabolism : The metabolic breakdown of complex molecules into simpler ones, often resulting in a release of energy.

Catabolic products : Conversion of complex molecules into simple ones.

Deterioration : To diminish or impair in quality, character, or value.

Elasmobranches : A fish with a cartilaginous skeleton.

Endogenous Enzymes : Produced inside organism.

Flabby : Sagging.

Gills : Breathing organ of fish.

Glycolytic : Production of energy from carbohydrates.

Habitat : The area or environment where an organism or ecological community normally lives or occurs.

Impermeable : Water resistant.

Inedible : Not suitable for food; not fit to be eaten; uneatable.

Intracellular : Within a cell or cells.

Metabolism : The chemical processes occurring within a living cell or organism that are necessary for the maintenance of life. Here, some substances are broken down to yield energy for vital processes while other substances, necessary for life, are synthesized.

Microflora : Bacterial colony.

Moulting : Periodical removal of outer cover.

Opaque : Unclear.

Perceptible : Detectable.

Salinity : Concentration of dissolved sodium chloride.

Scavenging : Feeding on dead material.

Scombroid : A group of fishes such as mackerels.

Slime : Slippery liquid.

Spoilage : The process of becoming spoiled.

Vulnerable : Defenseless.

2.7 SUGGESTED FURTHER READING

Mukundan, M.K. and Balasubramaniam, S. 2007. *Sea Food Quality Assurance*. CIFT Training Manual 1.

2.8 REFERENCES

Seafood Safety. 2002. *Proceedings of the Symposium on Seafood Safety: Status and Strategies*, 28-30 May, 2002, Cochin, India

2.9 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

1) Colour, odour, appearance, texture and taste.

2) It is widely used for deciding the quality of fish and shell fish.

3) The gills of fresh fish will be bright red and free from mucus deposit. With spoilage, the bright red colour turns brown and then gets bleached. The gills also get covered with thick mucus. This mucus covering also changes its thin transparent nature to thick and yellow in colour on spoilage.

Check Your Progress 2

1) Bacterial spoilage results from growth and multiplication of micro-organisms at the expense of muscle constituents.

2) In simple terms, autolysis is defined as the degradation of muscle and skin constituents by endogenous enzymes.

3) As a result of the glycolysis, a considerable amount of lactic acid accumulates in fish muscle. This lactic acid which is neither neutralized nor removed from the site of formation and therefore reduces the pH of the tissue. pH of tissue increases as the spoilage increases. Hence, pH is one of the chemical indices for spoilage.

4) The major enzymes of fish gut are proteases, including pepsin, trypsin and chymotrypsin, followed by lipases and carbohydrases. Once the fish is dead, the enzymes hydrolyze and perforate the stomach and intestinal walls and leak into the surrounding tissue, causing general hydrolysis. This is the mechanism of belly bursting seen in fatty fishes like capelin and oil-sardine.

Check Your Progress 3

1) On bacterial spoilage, the proteins will be broken down to polypeptide, peptide and to amino acid level by bacterial proteases and peptidases.

2) In presence of oxygen, some bacteria can bring about removal of amino group of an amino acid to produce ammonia and a keto acid. Such reactions are called ‘oxidative deamination’.

3) Free ammonia and hydrogen sulphide are also found to emanate from spoiled fish. The presence of ammonia can be detected by white fumes when exposed to hydrogen chloride gas.