Microbial Fish Spoilage and Its Biochemical Changes

Ni Ketut Suriasih
Laboratorium llmu Produksi Ternak Perah
Fakultas Pcternakan UNUD

ABSTRACT

Microbial spoilage of fish and its biochemical changes has been reviewed. Naturally fish of tropical region was dominantly contaminated by gram positive mesophilic bacteria such as bacillus, micrococci and coryneform, while fish from temperate region was contaminated dominantly by gram negative psychrotropic bacteria such as pseudomonas, moraxella, achromobacter, cytophaga and flavobacterium. Growth of these microorganisms in fish caused spolilage of the fish. Microorganisms predominate spoilage of cold storage fish were pseudomonas and alteromonas. Spoilage of dried fish was usually due to *Polypaecium piscae, Basidiospora halophila* and the pink bacteria. Fish preserved by hot smoke usually spoiled by micrococci, coryneform, while cold smoked fish spoiled by pseudomonas. Soon after a fish die, enzymatic reaction proceed which used glycogen as energy sources, and stop as the glycogen depleted from the tissue. This process was named " rigor mortis". Fish spoilage start at the end of rigor mortis. So, fish spoilage coul be delayed by retaining flesh glycogen through reduction in energy consumption. It can be done by preventing vigorous moving of fish during hauling/catching. Delay in spoilage was also due to lactic acid production resulted from glycolisis, which reduced fish flesh pH, so that inhibit microbial growth. Immediately after rigor mortis the flesh pH increase to normal pH, caused contaminating microorganisms grow, degrade NPN protein such as trimethylamineoxida (TMAO) to trimethylamine TMA. This TMA was ammoniacal. Later stage of spoilage contaminating microoeganisms degrade protein compound result in compoun that exert putrid smell such as H_2S, methyl mercaptan and dimetyhyl sulfide.

Key words: fish, spoilage,biochemical changes

I. **INTRODUCTION**

Fish is widely recognised as a very good source of protein in the -human diet. Itprovides-14% of the world's animal protein intake (Pedrosa-Menabrito and Regenstein, 1988). This protein is high in essential amino acids, and has a good amino acd structure. The fish is also a good source of mineral and vitamin. About 80% of weight of frersh fish consist of water, 18% of protein, carbohydrate content of 1% and lipid content of 1% (Hobbs, 1983).

The muscle tissue of flesh live healthy fish is truly sterile. The microorganisms of normal marine fish are present in the gut cavity and on all the

outer surfaces including the gills. It is generally accepted that little or no bacterial activity occurs until the period of "rigor mortis" is passed. However, fresh fish is extremely perishable due to its high pH, normally the pH is 6.4-6.6 due to low reserve of glycogen in the flesh (Buckle, *et. al*. 1978) and generally spoils within 12-24 hours if not stored under refrigeration or at temperature less than 5^{o}C (Adams 1986), since it provides a favorable medium (high water activity, neutral pH and high level of soluble nutrients) for the growth of spoilage microorganisms (ICMSF, 1980).

The spoilage fish is the result of a series of change brought about in the fish tissue immediately post mortem by the action of endogenous enzymes (autolysis), microorganisms, primarily bacteria and chemical reactions such as oxidation of lipids (Reay and Shewan, 1949; Hobbs, 1983). These actions result in undesirable alteration of flavor, odor and appearance of the fish, which lead to objectionable quality of fish product. This means post harvest losses of fish while people especially in developing countries including Indonesia need it as a major source of protein in their diet (Wheeler, *et al*. 1986). The spoilage pattern of fish depends on initial bacterial flora, the flora acquired during handling and processing, the condition to which the fish has been subjected during storage and processing, and on the chemical composition of the fish (Shewan and Hobbs, l967; ICMSF, 1980).

The purpose of this paper is to evaluate various scientific data concerning the various factors affecting the spoilage of fish (wet fish), effect of chilling, curing, smoking and drying on the microflora of the product, spoilage patterns and the biochemical changes of the spoilage process. These are important in order to indicate where insufficient knowledge is present and research is required and to establish quality control on processing lines.

II. INITAL BACTERIAL FLORA

Although the flesh and internal organs of freshly caught, healthy fish are generally sterile, it is known that, the skin, gills and in the intestine of freshly fish harbour high bacterial loads (ICMSF, 1980; Burgess, *et al*. 1965). The majority of bacteria associated with fresh and spoilage fish come from the environment (Kraft, I992) and belong to the water soil types (Reay and Shewan, l949) which has a lower

optimum temperature of generation time than that of normal pathogen. The equipment used such as catch boxes, bins, holds, dressing surfaces, decks and cultery handles and seawater used for washing gutted fish and cleaning the equipment and decks at sea also become the source of contaminating microflora
(Ayres *et al*. l980; Nickerson and Sinskey, 1972).

Generally, marine fish is dominantly contaminated by halophilic bacteria, but in most cases the fish will come into contact directly to the ice used for chilling. And when the ice start to melt the salt concentration decrease, the predominating bacteria will be psychrotrophic and psychrophilic of fresh water origin and euryhaline type bacteria (i.e. able to grow over a wide range of salt concentration) (Shewan and Hobbs, 1967; ICMSF, l980; Kraft, l992).

In case, there is no ice available for chilling, the types of bacteria contaminate the fish is greatly affected by temperature (ICMSF, I980). In the north region such as Sweden where the ocean water temperature is about 10^{o}C and the wheather temperatureis about 15°C the psychrophilic gram negative rods bacteria will predominate while in Indonesia, where the temperature of the seawater and air of about 20^{o}C and 25^{o}-35°C respectively, the mesophilic gram positive bacteria become predominating contaminants (Len,1986; Shewan, 1977).

Moreover, Shewan and Hobbs (1967) showed that bacterial population oi fish from northern hemisphere ocean dominated by the genera of Moraxella, Acinerobacter, Alteromonas, Pseudomonas, Flavobaclerium, Cytophaga and Vibrio. Among the population Pseudomonas and Alteromonas constitute about 30 to 60 %; Moraxella and Achromobacrer about 20 to 30 % (Kraft, 1992). While from tropical, subtropical and southern hemisphere it is found that gram-positive bacteria of Bacillus, Micrococcus and Coryneform group (Shewan, 1962; ICMSF, 1980; Ayres, *et al*.,l980). Similarly, Cann (1977) cited by Gorczyca, *et al*.(l985) shown that the predominant genera present in the freshly landed prawns from the gulf of Thailand (Tropical climates) were Micrococcus, Coryneform and Achromobacter.

Shewan (l977) stated that both quantitatively and qualitatively the bacteria initially contaminate marine fish reflects the effects of environmental factors (Table. l). The bacterial load on marine fish from tropical or subtropical and polluted areas appears to have greater numbers than that of the clean and cold region (ICMSF, l980). Variation in the numbers of bacteria on the slime and gills occur due to some factors

such as season an methods of fishing. Acording to Reay and Shewan (l 949) in North sea, during summer (the period of June to August) bacterial loads is of $10^{4.5}$ to 10^{5} sq cm^{-l} while during the rest of the year the counts range from $10^{3.5}$ to $l0^{4}$ $cm2^{-1}$. The difference of bacterial population of gut contents may be due to source of feed consumned by the fish. Fish used to feed on the sea bottom, muddy condition has higher bacterial loads on the gut compare to that of feeding on plankton in clean ocean water. In term ot fishing techniques, fish caught by line has lower bacterial counts than that of caught by trawl net (Reay and Shewan, l949). Further, Shewan cited by Shewan and Hobbs (1967) reported that trawled fish usually harboured bacteria l0-l00 times heavier than that caught fish. The reason is the fish caught by trawl is dragged along the bottom of the sea where there are huge numbers of bacteria and while hoisting the trawl net onto the ship the gut content of the fish is express by forces among them.

Table 1. Bacterial loads on newly caught marine fish

Area	Species	Temp (^{o}C)	Skin (/cm^{2})	Gills ($^{-g}$)	Gut content ($^{-g}$ or $^{-cm2}$)	Author
Temperate Zone	North sea fish	20	10^{2}-10^{5}	10^{3}-10^{7}	10^{3}-10^{8}	Shewan (1962)
Tropical	Indian sardine	30 37	10^{5}-10^{7} 10^{4}-10^{7}	10^{6}-10^{8} 10^{8}-10^{9}	10^{7}-10^{9} 10^{6}-10^{8}	Kartiayani Iyer (1967, 1971)
Subtropical	Japanese flatfish Australian mullet	20	10^{4} 10^{4}	10^{3}-10^{5}	10^{3}-10^{7}	Simidu et al.(1969) Gillespie and Macrae (1975)

Regardless of the flora t initially present, if the fish is removed from water, the manner in which it is handled will introduce other flora which come from the worker, the ice used for chilling, the deck as well as the equipment. Therefore in addition to Pseudomonas, Achromobacter, Flavobacterium and Bacillus, in the fresh fish or chilled fish, other bacteria such as Stphylococcus aureus, Micrococci, coliform and Salmonella is found both in fish from tropical and temperate region.

III. PRESERVATION AND SPOILAGE OF CHILLED FISH

Generally in Indonesian fishing boats, after the fish are caught they are placed on the deck, sorted and placed directly into the containers and added broken ice. However, there is usually a delay between the fish being brought into the deck and being placed in to the containers. This often take about 3 hours and it is done on the deck where the temperature can be as high as 28-30°C (Kamari *et al*. 1976; Barile *et al*., l985). The high ambient temperature as well as inadequate chilling due to in sufficient availability of ice of Indonesian fleet (Barile *et al*., 1985) or inadequate cold sea water in Sweden fishery boats as both fishing boats in Indonesia as well as in Sweden, experience moonstruct. There is also a delay in returning to the land for both of them. These phenomena affect the growth kinetics of the contaminating organisms occur in the fish

Generally there is a lage phase then followed by log phase, stationary phase and death phase in the growth curve of microorganisms. If the fish is properly chilled at temperature about 0^oC, the microorganisms will not grow during the first two days. It is also means that there is no increase in total viable counts of contaminating organisms in the fish. Also low temperature will slow the growth of contaminating organisms which is psychrotrophic (grow
at 12-20^oC). This means that, the storage life of the chilled fish can be extended to more than two days. This is in agreement with Shewan and Hobbs (1967) who reported that, if the ice chilling is enough, the chilled fish will be stale after 14-15 days.

However, after one day chilling in sea there is no new ice supply for chilling. Therefore the fish is then exposed to the higher temperature of (melted ice) say of about 15-20^o C. This means a rapid growth of contaminating organisms occur. According to Lone Gram *et al*.(1987) the storage life of the fish at 20°C only l day. Therefore, it can be said that in the first day in the sea there is no bacterial growth occur, but on the second day when the fish is exposed to higher temperature the contaminating bacteria will grow fast and leads the fish to the spoilage stage in 24 hours.

Spoilage of fish post-mortem is caused by endogenous enzyme action known as autolysis, by microorganisms which invade the fish to start degradation of protein,

and lastly by chemical reaction in which the fat of the fish is attacked by oxygen result in rancidity (Hobbs and Hodgkiss, 1982; Clucas, 1981). Spoilage lipid oxidation oftenly occurs in fatty fish such as cod, which result in offensive odors and flavors (Shewan and Hobbs, l967).

Microbial spoilage of fish will occur if the bacterial count definitely reach $l0^7$-10^8/g but not at 10^4-10^5/g. At this later stage the fish is free of spoilage (Kraft, 1992). Moreover, Shewan and Hobbs (1967) stated that, the maximum values of 10^3-10^7 cells per gram of flesh fish will be reached after 9 to 10 days if the fish is stored at 0°C and there is a lag phase during the first two days of this storage. For cod and haddock it is usually considered stale after 14-15 days.

Shewan at id Hobbs (1967) reported that exposure of the fish for 9 hour at 14°C after chilling result in the bacterial load of about $l0^7$cfu/g. Nickerson and Sinskey (1972) stated that, if the bacterial load of the fish as high as 10^7-10^8/g, there may an obvious invasion of bacteria into the flesh through the skin or even through the arterial system of the gills. Therefore it can be said that, the fish begin to undergo microbial spoilage which means degradation of amine compounds as well as protein occur resulting in odour changes as well as appearance of the fish. Then, as the spoilage proceeds there may be a production of ammoniacal, sulphide volatile odour and odoriferous amine of TMA (trimethylamine).

Organoleptic, chemical and bacteriological shows that this duration of exposure equivalent to 3 days storage in ice. Therefore, chilling is a good preservation technique for raw product as well as fresh product.

IV. SPOILAGE OF DRIED FISH

Sun Drying is the most convenient and cheap form of fish preservation in Indonesia. Dried fish, a term used to denote both salt and unsalted dried fish. In Indonesia a large quantities of small fish are dried in the sun. These include some oily fish as shown in Table 2. Salting and drying the fish in the sun is done to reduce deterioriation and provide as microbially stable products. The principle effect of drying and salting on microorganisms is due to the lowering of aW (water activity), through NaCl itself in

higher concientration may be lethal for some bacteria and yeast (Lion, 1980; Hocking, 1988). As shown in Table 2. the range of water activity of the Indonesian salted dried

Table 2. Dried Fish Species from Indonesia.

Latin name	Engglish name	Indonesian name	Number of samples	aW range of samples
Sardinella fimbriata	Fringe scale sardine	Tembang	16	0.71-0.79
Katsuwonus sp.	Skipjack	Cakalang	1	0.71
Loligo sp.	Squid	Cumicumi	9	0.69-0.76
Rastrelliger kanagurta	Chub mackerel	Kembung lelaki	3	0.72-0.73
Opheocephalus striatus	Snakehead	gabus	7	0.73-0.75
Scomberomorus sp	Spanish mackerel	Tenggiri	4	0.72-0.76
Leiognathus sp	Slipmouth ponifish	Peperek, selar	2	0.72-0.73
Trygon sp	Ray	Pari	1	0.72
Lutjanus sp	Red snapper	Ikan jangki merah	1	0.73
Paraplotosus sp	Catfish eel	Sembilang	1	0.76
Anadara sp	Blood cockles	Kerang darah	1	0.76
Chanos chanos	Milk fish	bandeng	1	0.74
Pseudosciaena sp	Croaker	Gulamah	3	0.71-0.74
Tachysurus sp	Sea catfish	Jambal	2	0.75
Saurida sp	Lizard fish	Beloso	1	0.76
Decapterus sp	Scad	Layang	2	0.74
Puntius Japanicus	Carp	Tawes	3	0.73-0.75
Stolephorus sp	Anchovy	Teri	5	0.645-0.72
Trichiurus sp	Hairtail	Layur	1	0.73
Hremirrhampus sp	Halfbeakgarfish	Julung-julung	1	0.74
Trichogaster pectoralis	Snakeskin gourami	Sepat siam	1	0.72
Sepia sp	Cuttle fish	Sotong	1	0.72
Tachyurus sp	Dried fermented catfish	Jambal roti	1	0.78
Cynoglosus lingua	Tongue fish	Ikan lidah	1	0.72

Source : Wheeler *et al.* (1986)

In general the aW range from intermediate moisture foods products make it unlikely that both gram negative and Gram positive bacteria will grow but cocci, some spore formers and lactobacilli (Jay, 1986). Eventhough the *Staphylococcus aurcus* which fish range from 0.73-0.75, may contaminate the products initially will still grow at aW of 0.83 but it can not produce enterotoxin below aW 0.86 (ICMSF, 1980).

Liston (1980) stated that microbiological changes in drying fish occur mainly during the salting and drying process with growth sometimes taking places in the early stages while the aW level more than 0.90. However in Indonesia, the fish is dried after salting, and the final products generally have water activity about 0.73-0.75. This means that there is no growth of bacteria but moulds and halobacterium (ICMSF, l980). The main microflora of Indonesian dried fish and seafood are fungi which describe as *Polypaecilium piscae* (Pitt and Hocking, l985 cited by Hocking

1988) and halophilic fungus *Basipetospora halophila*. Wheeler er al. (1986) reported that, there are about 22 genera of moulds found in dried salted fish in Indonesia. Among them Aspergillus, Eurotium and Penicllium species accounted for more than 70%, 85% of *Polypaecium piscae*. The other 20% from '18 different genera (Table.3).

Table 3. Genera of fungi isolated from Indonesian dried fish

Genus	Percentage of isolate	Number of species
Aspergillus	34.1	17
Eurotium	20.9	6
Penicillium	16.5	20
Cladosporium	4.4	2
Polypaecium	8.5	1
Chaetomium	3.0	NI
Talaromyces	2.3	1
Manascus	1.6	NI
Basipetospora	1.4	1
Paicilomyces	0.8	1
Syncepalostrum	0.8	1
Trichoderma	0.8	2
Ulocladium	0.6	NI
Absidia	0.6	1
Alternaria	0.6	NI
Eupenicillium	0.5	2
Wallemia	0.3	1
Botryotrichum	0.3	NI
Corynascus	0.3	1
Dactylosporium	0.3	NI
Dreschclera	0.3	1
Fusarium	0.3	NI

NI : not identified to species level. Source : Wheeler (1986)

Aspergillus niger has been found to be one of the most commonly reported fungi from foods as it more prevalent in warmer climates and is highly resistant to sunlight (Pitt and Hocking, 1985 cited by Wheeler *et al*., 1986). Fortunately it is found that, there is no aflatoxin production in the products as this fungi occur in low numbers. Therefore it is concidered as contaminants rather than growing on the fish.

Moreover Buckle *et al.* (1988) showed that, in dried salted fish from Indonesia, moulds and pink bacteria have been found to be the microflora of the products. These pink bacteria were actually a hallophilic bacteria which can produce a distinctive pink to rose-red colour. If these bacteria were let to grow, then another

pink group of bacteria began to grow and vigorously attack the fish flesh and produce a characteristics off putrid odours. At this stage of spoilage of the produt is describe as stale, sweety or like over ripe cheese (Buegess er al,. 1965).

In addition to microbial spoilage, there is also chemical change in this dried fish. Lipids compound in fatty fish can undergo oxidation which leads to rancidity. Also physical damage and discoloration such as non-enzymic browning are important as they reduce the quality of the products.

In addtion the increase in aW of the product usually due to poor storage, will leads to the growth of fungi as well as bacteria. In these case packaging of the product: as well as good storage condition is of prime important.

V. SPOILAGE SMOKED FISH

In lndustrialized countries such a Sweden, smoking is primarily designed to produce a convenience products, but in much of the world it is still used as a preservative process. Preservation is achieved essentially by drying. There are two types of smoking i.e. hot smoking and cold smoking. In hot smoking the internal temperature of the products is normally exceed 60^{o} C while in cold smoking it rarely exceec 35^{o}C (Liston, 1980).

The fish to be smoked is brined. This process will change the initail microflora of the products since the salt used for brining bring about bacterial flora consisting of bacillus spp mainly B. megaterium and B. subtilis, Micrococcocci and Sarcina (Liston, 1980; Shewan and Hobbs, 1967). This is especially important if the salt use for brining is solar salt which contain those bacteria and also if the brining result in high salt content. With light cured fish, since the overall effect of smoking will reduce the proportion of gram negative bacteria and increase the gram positive types particularly micrococci and coryneform in hot smoked fish(Liston, 1980), while in cold smoked a typical Pseudomonas spoilage group develops during subsequent storage. The reason is that the high temperature will kill the initial microflora of the fish (Pseudomonas and Aeromonas spp) but it does not happen in cold smoked fish. Temperature of about 3-5^{o}C is good for Pseudomonas group to grow. However, the aW of the products will be too low for the growth of this bacteria. Therefore smoked fish is

generally spoiled by moulds such as Aspergillus, Penicillium spp (Graikkoski, 1973 cited by Liston, 1980).

During smoking processes there is a reduction of bacterial load due to phenolic compounds released in the smoked. These compounds include guaiacol, creosol, pyrogallol as well as its methyl and propyl isomer, have a high phenol coeficient against *Salmonella typhii* and *Staphylococcus aureus* (Shewan and Hobbs, 1967). However, this product sometimes reported to cause *Clostridium botulimun* type E out-breaks. This may be due to the aW of the product still above 0.93, which promote the growth of these microorganisms.

VI. BIOCHEMISTRY OF FISH SPOILAGE

As fish contains high percentage of water, high in essential amino acids, and has relatively high ultimate pH (6.2-6.5), it is easily undergo spoilage. Processes of fish spoilage involve indigenous enzymatic reactions, biochemical changes microbially induced activity and chemical reactions which result in change of flavour/odour, texture and appearance of the fish.

A.Enzymatic and Biochemical changes

A.1 Rigor Mortis

Immediately a fish die, the supply of food ceases and the energy resources soon become depleted. But the enzymes do not die. Some of the enzymes continue to operate their function, including those that maintain the muscle to contract. This contraction needs energy, which obtained from reduction of adenosin triphosphate (ATP) to adenosin diphosphate (ADP), which come from the glycogen stored in the fish flesh, resulting in a phosphorous group (P) by Mg-activated actomyosin ATPase (Figure l). The reaction occur continuously and cease when the ATP is absence resulting in the stiffening of the fish flesh. This stiffening is known as rigor mortis . The onset and duration of rigor mortis is important as it determines the onset of microbial spoilage of the fish. Spoilage by microorganisms will not start unless the rigor mortis resolved (Clucas, 1981). Rigor mortis in fish is affected by some factors such as condition of the fish at hauling, temperature and species (Reay and Shewan, I949).

Fish in good condition (not fatique) in hauling will experience rigor mortis late and it last longer than that experienced by fatique fish. Fish caught by trawl net move vigorously during the capture. This movement used a lot of energy in the form of ATP which is obtained from aerobically metabolisms of glycogen of the fish flesh. This also means less glycogen and less ATP available in the fatique fish. Since gor mortis will start as the ATP production from glycolisis is ceased (Hobbs, 1982), the more fatique the fish the rapid the rigor mortis last. Reay and Shewan (1949) also mentioned that the fish caught by hand-line killed and pitched immediately, remained stiff for 30 hours at 8^{o}C while fish caught in two hour haul by trawl net passed through rigor mortis within hours.

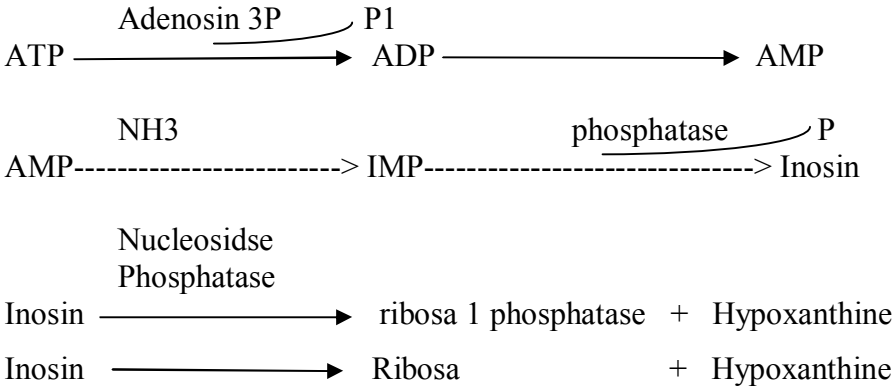

Figure 1. Degradation of adenosin triphosphate during postmortem. .

Rigor mortis is an enzymatic processes. Since the enzyme activity is affected by temperature, the rigor mortis process should also temperature dependant. The higher the temperature of the flesh the fast the enzymatic reaction is, which mean hasten the disappearance of ATP as well as creatine phosphate (CP) from the fish flesh. This rigor mortis last fast.Therefore chilling the fish prolong the final resolution of rigor mortis and this remains until enzymatic activity such asproteolysis release the tension, which means the fish flesh become pliable again. At the same time of occurrence of rigor mortis, glycolisis also happen (Hobbs, l983, Jcober and Rand, l982).

A.2. Glycolisis

The lack of oxygen after the fish die, causes metabolisms of glycogen anaerobically resulting in lactic acid production (Figure 2). Accumulation of lactic acid in the tissue leads to a reduction in the tissues pH, suppressing the development of microorganisms. Unfortunately glycogen content of the fish fles is low.

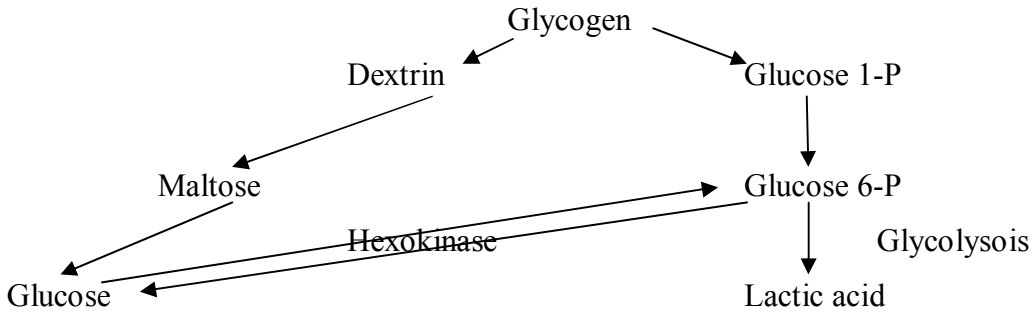

Figure 2. Glycogen degradation post mortem

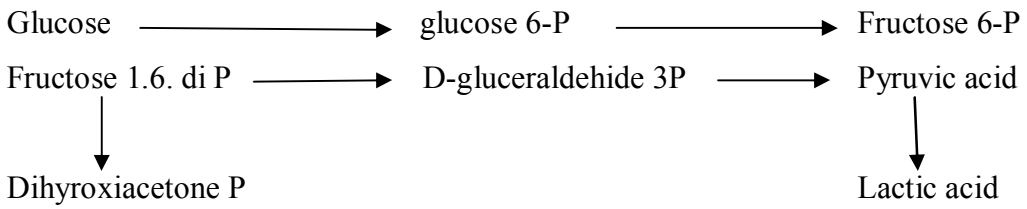

Figure 3. Glycolisis pthway post mortem

Therefore the pH of the fish flesh is ultimately high. However, should the pH of the fish goes down below normal pH of the fish (6.2-6.5) then the fish will lose water easily and this leads to chalky fish. Glycogen content of the fish after death is affected by the condition of the fish. Fish undergo many struggle movement during capture will have less glycogen reserve in its flesh then that of having more rest; and glycolisis continue with an apparent increase in lactic acid formation in the fish flesh, which means drop in pH tissue of the fish also result in the liberation and activation of inherent acid cell proteases, cathepsins, peptidases (Eskin *et al.,* 1971).

A.3. Autolytit Enzyme Action

As the glycolisis reaction stop due to exhausting in glycogen reserve, autolytic enzymes (proteases, peptidases and cathepsins) activity begin. These enzyme activities play an important role in degradation of peptides and proteins

resulting in metabolite in the form of amino acid providing an optimal medium growth and reproduction of the spoilage microorganisms. In addition digestive tract of the fish also has as a strong protease enzyme, which can invade the fish tissue through the abdominal cavity, degrade protein compound to amino acid compound. This occurrence could lead to rapid availability of the microbial growth medium, which means increase succeptibility of the fish to microbial spoilage. Therefore it is highly recommended to gut the fish as soo as the fish caught, in order to avoid this enzyme invade the fish flesh. It is also reported that, species that have high amounts of stomach and gut content is more succeptible to autolytic tissue degradation due to having higher activities of digestive enzyme. This digestive enzyme can leak out post mortem through the belly walls and degrade the surrounding tissue, but this can be prevented by cooling and processing the fish quickly. It can be seen that all the autolytic enzyme activity produces metabolite i.e amino acid compound which is very important in growth of the spoilage microorganisms.

B. Chemical Changes Due To Microorganisms

Fish flesh is composed of protein, fats, carbohydrate, water and amino acids compounds such as triniethylamine oxide (TMAO), urea, taurine, creatine, anserine, free amino acids and trace glucose etc. The amount of this substrate vary with fish species. Skate and other elasmosbranchs which has higher content of urea (l,500-2,000 mg percent), triniethylamineoxide (500-1,000 mg percent), creatine 300-500 mg percent, compare to cod which has 230-390 mg% trimethylamine (Shewan, 1962). The high percentage of urea in elasmosbranchs and readily attacked by manmy species of marine bacteria result in strong ammoniacal odour characteristics in its spoilage products.

In chilled/iced fish the Pseudomonas, Achromobacter predominating groups rapidly metabolized most amino acids, dipeptides found in the non protein nitrogen (NPN) fraction of the flesh. This action result in ammonia and volatile fatty acids (Liston, I982). This process occurs in the early stage of spoilage. As spoilage of fish proceeds, there is a gradual change in the p-hisical, microbiological and organoleptic. In raw fish undergoing spoilage. There is a chractcristics squence of odour changes as the following (Clucas, l98l). Initially it is fresh, it then becomes sweetish, sometimes fruity, later ammoniacal and fishy odors predominates untill finally putrefactive

become evident. As these later changes occur, than the fish is inedible. The ammoniacal odour consist of ammonia, TMA and other amine, while putrid elements composed by H_2 S, indole etc (Hebbert and Shewan, 1976 cited by Pedrosa-menaberto and Regenstein 1988; ICMSF, l980).

B.1.Trtiniethylamine oxide (TMAO) Reduction

Trimethylamine oxide (TMAO) is found in a large number of marine fish and shellfish, generally in large amounts in elasmosbranch fishes. Reduction of TMAC to TMA possibly done by endogenous enzymes in fish, but mainly by the enzyme activity of certain bacteria. Reay and Shewan (1949) showed that only fraction of the total bacterial population reduces TMAO is facultative anaerobic Achromobacter, which are capable of growth in interior or on he surface of the fish. Tarr (1939) cited by Reay and Shewan (1949) showed that bacterial enzyme will activate the oxide of the TMAO rendering it succeptible to reduction by many of dehydrogenases of the cell. This enzyme called triamine-oxidase. This triamine (TMAO) so that the bacterial dehydrogenase can reduce it to TMA. Figure 4 illustrated the degradation of TMAO

TMA associated with fishy odour spoilage and is part of spoilage pattern of many fish species. When TMA reacts with fat in the muscle of the fish, a characteristic ishy odor of low quality fish is produced. The odor will appear when the levels of TMA is about 4-6 mgN/ 100 ml of muscle extract and it will be definitely smell at the level l0 mgN/100 ml of the muscle extract (Pedrosa-menaberto and Regenstein, 1988).

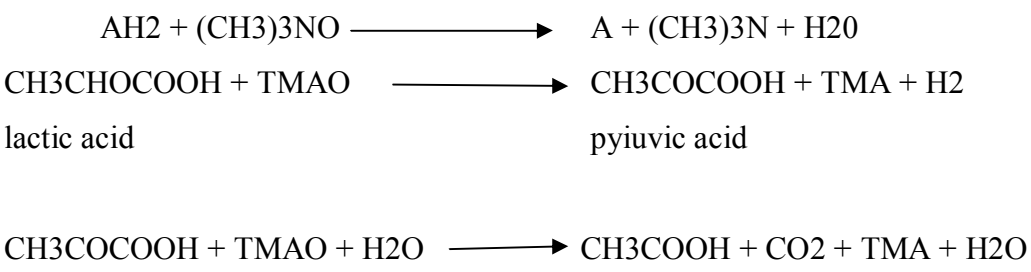

Figure 4. TMAO degradation reactions.
Source :Pedrosa-Menaberto and Regenstein (1988)

In evicerated or fillet fish which is stored at refrigerated and undergo bacterial spoilage, TMA content will increase as the number of bacteria increase (Nickerson and Sinskey, 1972) and the fillets eventually take on trimethylamine-like odor prior to the development of ammoniacal and putridiodors. Further they stated that Pseudomonas putrfaciens has responsible for TMA production.

B.2. Proteolysis

Following the phase of TMAO reduction, during which there was no appreciable change in amino-nitrogen, deamination process which result in the formation of ammonia. In anaerobic storage condition on boards (under cold sea water) fish may develop an odor which has been describe as bilgy. This odor is especially offensive odor usually reminiscent of some hydrogensulfide mixed with other odors. In anaerobic condition (when oxygen is depleted) TMAO can be used by spoilage bacteria as an electron acceptor or oxidising agent, thus stimulating bacterial growth. The bacterial growth will cause degradation of cysteine and methionine, resulting in H2S, methyl mercaptan and dimethyl sulfide which are responsible to the sulfide off odors assocoated with stale chilled-stored cod.

B.3.Spoilage of Fat

Fish contain fats which is proportionately high in unsaturated fatty acids which are subject to attack by atmospheric oxygen leading to deteriorative changes especially in fatty fish. The fat oxidation causes changes in flavor, colour and possibly texture of the fish flesh associated with rancidity (Hobbs, 1982). ln addition to lipid oxydation, fish fat can also undergo hydroysis due to lipolytic activity of fish tissue, but it ilso can be promoted by bacterial lipases during fish spoilage. These types of spoilage generally happen in the later stage of spoilage and it is usually occur at chilling and freezing temperatures (ICMSF, l980).

VII. CONCLUSION

Fish is a very good source of protein in human diet because tontains about 18% protein, which is mostly composed of essential amino acids. But fish contain

very low glucose, which make them to have high ultimate pH (6.2-6.5). These condition caused the fish suceeptible to microbial spoilage easily.

Microbial spoilage of fish is affected by some factors such 2 initial microflora, handling, processing and environment such as temperature, season, handling such as gutting; processing such as chilling, drying and smoking.

Environment give variation in the type of microorganisms contaminate the fish (initial microflora). In temperate region, psychrophilic and psychrotropic bacteria such as Pseudomonas, Achromobacter, Alteromonas, Flavobacterium predominate, while in tropical area the mesophilic gram positive i.e. bacillus, aeromonas. Micrococci, coliform will predominate. During handling staphylococcus will introduce to the fish.

Drying and smoking will changes the microflora of the fish. In drying fish, mould will be the most contaminant if the aW of the product less then 0.75 as no bacteria can grow in this aW. This also happen in smoking, but in addition to moulds smoked fish also bring about Closrridium botulinum.

As the fish die, it will undergo rigor niortis. Microbial growth will not occur unless the rigor mortis ceases. As rigor mortis finish, microorganisms on the surface of the fish grow because the fish support the growth by nutriennt availability. The microorgnaisms grow on the surface of the fish as well as invade the fish flesh through the gills or kidney and also their enzyme invade the fish flesh causing degradation of amino acid result in chemical change, odor. Initially the odor is fresh then become sweetish, sometimes fruities, later ammoniacal or fishy odor dominate untill finally putrefactive odors dominate which appear as a result of microbial spoilage.

The fish usually become inedible when the mixture of ammoniacal (Ammonia, TMA) and putrid elements (H2S) appear. In freezed and chilled stored fish there is also oxidation of fat result in rancidity of the fish.

REFFEREN CES

Adams, M.R. 1986. Fermented flesh foods. Adams M.R. ed. Progress in industrial microbiology. Vol. 23.Microorganisms in the production of food. Amsterdam. Elsevier: l59-198.

Ayres, J.C., J.O. Mundt, and W.E. Sandine. 1980. Microbiology of Foods. W.H. Freeman & Co. San Fransisco

Buckle, K.A., R.A.Souness, S. Putro and P. Wuttijumnong. l988. Studies on the stability of dried salted fish. Sew.CC. eds. Food preservation by moisture control. Elsevier appl. Sci.

Barile, K.E.; A.D. Milla; A. Reilly and A. Villadsen. l985a. Spoilage patterns of Mackerel (rastrelliger faugni Matsui). l Delays in icing. Asean Food J. Vol.l No.2. ,

Barile, K.E.; A.D. Milla; A. Reilly and A. Villadsen. 1985b. Spoilage patterns of Mackerel (rastrelliger faugni Matsui). 2. Mesophilic and psychrophilic T spoilage. Asean Food J. Vol.1. No.3. l2l-127.

Burgess, G.H.O.; C.L. Cutting; A. Lovern and J.J. Waterman. l965. Fish handling and processing. Ministry of technology.'Torry research station Edinburgh

Clucas, J.J. 1981 . Fish handling preservation and processing in the tropics: part l. Tropical products institute. London.

Eskin. N.A.M. 1990. Biochemical changes in raw foods: meat and fish. Eskin, N.A.M. eds Biochemistry of foods. 2nd ed. Academic Press. New York. Pp.337.

Goeczyca, J.L. Sumner; D. Cohen and P. Brady. l985. Mesophilic fish spoilage. Food Tech. In Aust. Vol. 37(1): 24-26.

Hobbs, G.and W. Hodgkiss. 1982. The bacteriology of fish handling and processing. Davies. R. ed. Development in food microbiology I. London Appl. Sci. Publ.

Hobbs. G. 1983. Microbial spoilage of fish. Roberts. T.A. and Skinner, F.A. eds. Food microbiology: advanced and prospects. Academic press London.

Hocking, A. 1988. Mould and Yeast associated with food of reduced water activity: ecological interaction. Seow,C.C. eds. Food preservation by moisture control.

ICMSF. 1980. Microbial ecology of foods. Food comodities. Vol.2. New York. Academic Press.

Jacober, L.F and A.G. Rand. 1982. Biochemical evaluation of seafood. Martin, R.E. et.al.eds. Chemical and biochemistry of marine food products. Avi Pub. Co.

Jay. M. 1981. Modern food microbiology. New York Van Nostrand.

Kraft, A.A. 1992. Psychrotrophic bacteria in foods. Disease and spoilage; CRC Press.

Len Pek Poh. 1986. Mesophilic spoilage of marine fish: by trout, bream and mullet. Food tech. 1n Aust. Vol 39(6):277-282.

Liston, J. 980. Microbiology of fishery Science. Connel, J.J. eds. Advances in fish science and Technology.

Lone Gam, G. Trile and H.H. Huss. 1987. Detection of specific spoilage bacteria from fish stored at low (10^o C) and high (20^o C) temperature. Int. J. of food microbiology, 4:65-72.

Nickerson, LT. and A.J. Sinskey. 1972. Microbiology of food and food processing. New York. American Elsevier Press.

Pedrosa Menaberto.A. and J.M. Reggenstein. 1988. Spoilage of fish. J. food. Quality. 11:117-127.

Rcay. G.A. and J.M. Shewan. 1949. Advances in food research. Vol.I1. Stewart. Eds.

Shewan, J .M. 1962. The bacteriology of fresh spoiling fish and some related chemical . changes. Recent advances in food science. London: Butterworth.

Shewan, J.M. 1971. The microbiology fish and fishery products. A progress report. J. Appl. Bacteriology. 34: 299-315.

Shewan,J.M. 1977. The bacteriology of fresh and spoiling fish and the biochemical changes induce by bacterial action. Proceeding of conference on handling , processing and marketing of tropical fish : 5-9 july 1976. London tropical products institute.

Shewan, J.M. and G. Hobbs. 1967. The bacteriology of fish spoilage and preservation. Prog. Ind. Micro. 6: 169-208.

Wheeler, K.A.; A.D. Hocking,; J.J. Pitt and A.M. Anggawati. 1986. Fungi associated with Indonesian dried fish. Food microbiology

KONSORSIUM BIOTEKNOLOGI INDONESIA

THE 3rd INDONESIAN BIOTECHNOLOGY CONFERENCE 2004

This Certificate is presented to

Ir. Ketut Suriasih, M.App.Sc

In recognition of participation as

PRESENTER

In the 3rd Indonesian Biotechnology Conference
An International Conference and Exhibition,
held at Inna Grand Bali Beach Hotel – Sanur, Bali
December 1st – 3rd, 2004

INDONESIAN BIOTECHNOLOGY CONFERENCE 2004

A. Syaifuddin Noer, Ph.D
Chairman of the Indonesian Biotechnology Consortium

Prof. Ir. I G.P Wirawan, Ph.D
Chairman of the Organizing Committee