# Fish Quality Declining Based on Changes in Biochemical Properties (a Review)

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**ABSTRACT**

*The protein and fat components of fisheries products will quickly undergo a number of metabolic changes during storage, in addition to the creation of amine compounds (volatile and biogenic) and the buildup of hypoxanthine. As a result, the sensory quality will decrease, the nutritional value will decrease, and detrimental changes in the physical characteristics of fish muscles will take place. Fish with a high protein content are at risk as they decompose. Protein cleavage generates peptides and amino acids that can be broken down further to create biogenic amine compounds, which are frequently present in protein-rich foods. Endogenous enzyme activity causes post-mortem ATP breakdown in fish flesh muscle. The development of hypoxanthine in fish tissue points to an early stage of autolysis quality degradation. Early postmortem alterations in fish tissue are also connected to lipid oxidation. The generation of free radicals and the removal of protons from the carbon atoms of unsaturated fatty acids start the process. Additionally, by measuring a number of substances, including nucleotide content, TVB (Total Volatile Base), TMA (Trimethyl amine), TBA (Thiobarbituric Acid), and other substances, whose amounts will change during the stages of deterioration, one can observe changes related to quality deterioration chemically.*

**Keyword:** *biogenic amines, deterioration, fish, nucleotide, volatile base*

## 1. INTRODUCTION

The quality of fish is easy to decline after the fish itself die. During the storage of fishery products, a series of biochemical changes will immediately occur in the protein and fat components, besides the formation of amine compounds (volatile and biogenic) and accumulation of hypoxanthine. As a result, there will be a decline in sensory quality, loss of nutritional value and negative changes in the physical properties of fish muscles will occur. This degradation process is initially carried out by enzymes contained in fish muscles and then followed by enzymes derived from bacteria [1], [2], [3].

The main content of fish meat is water (66-81%), protein (16-21%), fat (0.2-25%) and ash (1.2-1.5%). Fish is an important source of protein. Water-soluble proteins (20-35%) in fish are mostly enzymes and the proportion does not change during frozen storage [4]. On the other hand, high protein content poses a risk during the decomposition process. Protein breakdown produces peptides and amino acids that are susceptible to further breakdown and produce biogenic amine compounds that are widely found in protein-rich foods [3],[5].

In addition to protein, fish is also rich in fatty acids that are beneficial for the intelligence of the human brain. The benefits of its unsaturated fatty acid content have also received great attention because it can prevent heart disease in humans. Fish is a major contributor of 3 fatty acids for human consumption. The frequency of fish consumption should be at least twice a week to have a heart disease prevention effect. Therefore, the consumption of freshwater fish and seawater fish is highly recommended [3].

Given the significance of understanding the loss in the quality of fisheries products, this paper will provide a quick overview of the theories underlying the general drop in fish quality, along with findings from a number of international journals. The results of the journals presented include biochemical changes and quality deterioration

during cold storage of turbot fish (*Psetta maxima*) [1]; Lipids changes in sardine meat (*Sardinella gibbosa*) during cold storage [6]. Biogenic amine in carp (*Cyprinus carpio*) meat which is packaged and stored at different temperatures [5]; Production of biogenic amines and nucleotide ratios in sea bass (*Dicentrarchus labrax*) stored in ice and packaged [7]. In the expectation that different kinds of damage and quality deterioration might be prevented, this review intends to provide information on quality deterioration experienced by fishing commodities in terms of changes in chemical and biological processes.

## 2. BIOCHEMICAL CHANGES IN POST MORTEM OF FISH

### 2.1 Autolysis

Autolysis is a fish decomposition process as a result of enzyme activity contained in the fish body and is not related to quality changes caused by microorganisms. This process plays a role in the overall loss of quality and also as a process that can support the process of further damage by microorganisms [8].

In addition to the occurrence of nucleotide catabolism by enzymes in muscle, in this autolysis process, protease enzymes that can soften tissue also play a role. Nucleotide degradation by enzymes generally does not play a role in changing fish texture, unlike protease enzymes which can cause softening, belly burst and others. The protease enzymes involved in the autolysis process include catepsins (D and L), calpains and collagenases, each of which has a specific function [8].

### 2.2 pH value

The decrease in pH occurred in the early post-mortem period caused by the accumulation of H+ ions. Then the pH of the meat will increase again in the post-mortem period as a result of the activity of decomposing microorganisms to protein into volatile amines and other basic components that become dominant in fish meat. Changes in pH however will depend on the type of fish, fishing methods, food and other physiological conditions [9].

Post mortem glycolysis causes accumulation of lactic acid which will lower the pH of fish meat with different values depending on the type of fish but rarely lower than 5.1. The amount of lactic acid formed will depend on the content of carbohydrate reserves (glycogen) stored in live fish tissues. Glycogen reserves will be affected by fish nutrition and the amount of stress and movement carried out by fish before death. The decrease in pH during post mortem will affect the physical properties of the muscles. Along with the decrease in pH, the protein will be partially denatured so that it loses its water binding capacity [8].

The pH value in the research of [10] regarding the storage of three types of fish at a temperature of -26$^{o}$C showed a decrease depending on the type of fish. The decrease in pH is caused by the applied temperature so that there has not been any decomposition that produces amino acid derivative compounds. While in research [3], the pH value increased until the 5$^{th}$ day, on the 8$^{th}$ and 12$^{th}$ days it showed a decrease and then increased again, the length of storage of turbot fish in ice increased up to 19 days. This indicates that there has been a breakdown of protein into basic derivative compounds after the 12$^{th}$ day of storage.

### 2.3 Lipid Oxidation

Lipid oxidation is associated with early post mortem changes that occur in fish tissue. The process is initiated by the removal of protons from the carbon atoms of unsaturated fatty acids and the formation of free radicals. Lipid oxidation is more likely to occur in frozen products than in refrigerated products and can occur enzymatically (lipoxygenase, peroxidase) and non-enzymatically (iron ions) [11],[12].

Fish meat is very susceptible to damage caused by microorganisms and chemical reactions. The decline in the quality of fish that has a high fat content is the main cause of attack by microorganisms and fat oxidation [13]. Fat conversion is easy and limits the shelf life of high-fat fish. Hydrolytic and oxidative rancidity in fish muscles are closely related to quality deterioration. Hydrolysis influenced by lipases and phospholipases produces free fatty acids which will undergo further oxidation and then produce low molecular weight components that give rancid aroma and aberrant taste to fish and fishery products [6],[14].

Fish fat components in post mortem muscle conditions are very susceptible to oxidation because fish fatty acids are more unsaturated than mammals and birds. The basic mechanism of fat oxidation can be divided into three distinct steps: initiation, propagation and termination reactions. This phenomenon can be influenced by intrinsic and extrinsic factors such as fatty acid composition, concentration of pro-oxidants, endogenous iron, myoglobin, enzymes, pH, temperature, ionic strength and oxygen consumption. The formation of metmyoglobin has a relationship with fat oxidation [6].

Myoglobin in sardines becomes very difficult to extract during cold storage ($4^oC$), this is due to the presence of fat oxidation, the formation of oxidation products such as aldehydes and the occurrence of fat bonds with proteins. Cold-stored sardines undergo fat oxidation rapidly, possibly due to the high content of unsaturated fatty acids and prooxidants in the red meat. Fat oxidation is a complex process in which unsaturated fatty acids react with molecular oxygen usually through free radical mechanisms to form hydroperoxides, the main product of oxidation [6].

During frozen storage, the triglyceride and phospholipid content of sardines decreased while the content of fatty acids, diglycerides and monoglycerides increased with increasing storage time. This shows that triglycerides and phospholipids are hydrolyzed into free fatty acids, diglycerides and monoglycerides. In the study of storing sardines in ice, it was found that there was an increase in the free fatty acid content at the end of the storage period ($15^{th}$ day) [6].

Fat hydrolysis occurred during cold storage of turbot fish and increased until the end of the storage period (day 26) with a concentration of 19.4 g $kg^{-1}$ fat while the initial concentration was below 5 g $kg^{-1}$ fat [1]. The presence of free fatty acids caused by oxidation and hydrolysis of fats is undesirable because fatty acids can turn into aromatic volatile compounds [13]. Based on research on horse mackerel stored in ice powder and ice in the form of flakes for 22 days, it can be concluded that fat hydrolysis can be prevented properly by storage using ice in powder form as evidenced by the low amount of free fatty acids formed [2].

Post mortem changes in fish such as decreased ATP content, increased ATP breakdown products, changes in xanthine to xanthine oxidase, loss of reducing components such as ascorbate, NAD(P)H, changes in ferrous ions to ferric, loss of structural integrity of the membrane, loss of antioxidants. of the membrane and muscle cells lose the ability to maintain a calcium gradient will affect the occurrence of fat oxidation, possibly because these changes can make the tissue more susceptible to oxidation [11].

**2.4 Thiobarbituric Acid (TBA)**

The detection of the presence of reactive TBA compounds was caused by the second stage of the autoxidation process during which the peroxide was oxidized to aldehydes and ketones. High TBA content is undesirable because it can cause a rancid aroma [13]. The TBA value may not give an idea of the actual level of fat oxidation because malonaldehyde can interact with other fish components such as nucleotides, nucleic acids, proteins, phospholipid amino acids and other aldehydes which are end products of fat oxidation. These interactions can differ in different fish species [3].

In a study on storage of sardines (*Sardinella gibbosa*) in ice, the TBA value at the beginning of storage was 17.2 mg malonaldehyde/kg sample and increased by 97% at the end of storage period ($15^{th}$ day). The increase in TBA content indicates the formation of secondary products of fat oxidation. TBA has been used to measure the concentration of relatively polar secondary products, especially aldehydes. TBA increased sharply on the day of storage after the $6^{th}$ day of storage, while the value of the peroxide value showed a decrease caused by the breakdown of hydroperoxides into secondary oxidation products, especially aldehydes at an advanced stage of fat oxidation [6].

The TBA values of the three types of fish (whiting, mullet, anchovies) which were kept frozen for 9 months showed an increase depending on the storage time. The maximum TBA value that indicates the quality of frozen and refrigerated fish is 5 mg malonaldehyde/kg, while fish can be consumed up to a limit of 8 mg malonaldehyde/kg [10].

**2.5 Nucleotide Degradation**

The most important nucleotide in animal muscle is adenosine 5'-triphosphate (ATP). After the fish die, ATP is degraded by enzymes sequentially into adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP) and inosine (HxR) by autolytic enzymes, followed by a slower oxidation breakdown of HxR to hypoxanthine (HxR).) resulting from the activity of microbial enzymes. Such nucleotide degradation has a strong correlation with the loss of freshness of fish [9],[12],[15].

Nucleotides are nitrogen-containing extracts, with the amount of content being the second largest after amino acids in fish. ATP-related components in fish have been analyzed as an indicator of freshness for a long time since Saito et.al. published his findings in 1959 namely "A New Method for Estimating Fish Freshness" [4]. The ATPase enzyme plays a role in muscle contraction, is activated by $Ca^{2+}$ cations and splits ATP between actin and myosin filaments causing the release of energy. The enzymes involved in the degradation of ATP and their associated components in fish are shown in Figure 1.

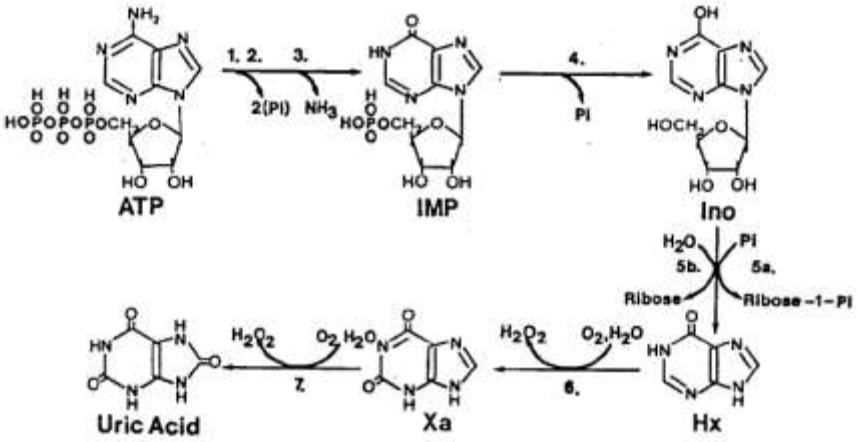


Figure 2. Post mortem ATP degradation in fish. Enzymes include: 1. ATPase, 2. Myokinase, 3. AMP deaminohydrolase (or AMP deaminase), 4. IMP phospohydrolase (5'-nucleotidase), 5a. nucleoside phosphorylase, 5b. inosine nucleosidase, 6,7. Xanthine oxidase. Source: [16]

Nucleotide degradation is generally measured using a K-value. The K value describes the decrease in the freshness of the fish. Determination of the nucleotide content proved to be an excellent means of measuring the loss of freshness during the fish's edible period. The value of K increases with increasing storage time, depending on the type of fish and tissue used in the measurement [1],[2],[3],[9].

Post mortem degradation of ATP in fish meat muscle occurs due to endogenous enzyme activity. The accumulation of hypoxanthine in fish tissue indicates an early stage of deterioration in the quality of autolysis and also damage caused by bacteria. A bitter, aberrant aroma is characteristic of spoiled fish and is associated with the production of hypoxanthine [17].

The application of a single nucleotide degradation measurement (measuring hypoxanthine levels only) has limitations in its use due to variations between fish species which may be caused by various intrinsic enzyme activities and nucleotide concentrations. However, when these nucleotide breakdown measurements are combined, they can be a useful way of determining fish freshness. Measurement of other values besides the K value is carried out because adenosine phosphate changes very quickly. The Ki value was calculated without including ATP, ADP and AMP, in addition there were H and G values that have been used as an index of freshness quality as a result of the many patterns of nucleotide catabolism [7].

In a study on storing sea bass in ice, there was an increase in the value of K to 82%, which was caused by a sharp decrease in the IMP content. IMP will be degraded into inosine and hypoxanthine so that it will cause the loss of the desired fresh fish components. Along with the decrease in the organoleptic quality of fish, the K value will actually increase. K value is a reliable indicator to measure freshness in frozen fish, smoked fish and fish stored in a modified atmosphere [7],[17].

The inhibition of bacterial growth will result in a decrease in the concentration of inosine and hypoxanthine, which will directly reduce the values of K, Ki, H and G. Loss of freshness in fish can be objectively measured by changes in K values and other related values. In the study of storing sea bass in ice for 21 days, the K, Ki, H and G values showed an increase with increasing storage time. On the 20th day the values respectively were 77%, 83%, 16% and 97% where the samples had been rejected on the 16th day of storage [7]. A gradual increase in the K value (>70%) also occurred in the storage of turbot fish at cold temperatures ($4^{o}C$) for 19 days. After the $19^{th}$ day there was no change in the K value, possibly because the IMP content had been exhausted after the $19^{th}$ day [1].

**2.6 Total Volatile Base-Nitrogen (TVB-N)**

The main components of volatile bases are ammonia, trimethyl amine (TMA), and dimethylamine (DMA). Volatile bases result from the degradation of protein and non-protein nitrogen components, mainly as a result of microbial activity. TVB is widely used as an indicator for fish quality deterioration. The increase in TVB levels was caused by a combination of deamination reactions caused by microorganisms and the amino acid autolysis process and the reduction of trimethylamine oxide (TMAO) to TMA by microbes. The highest TVB producer in smoked salmon was *Enterobacteriaceae*, *Photobacterium* spp. and *Lactobacillus* spp. [9],[13,[17].

The TVB content in freshly caught fish is generally between 5 and 20 mg N/100 g muscle. However, a content of 30-35 mg N/100 g is considered the limit for acceptance of refrigerated fish [18]. The critical limit of TVB ranges from 25-35 mg per-100 g applied to four different groups of fish, if the fish is processed the limits will be more diverse [9],[12].

TVB content in three types of fish (mullet, anchovies and whiting) which were filet, weeded and frozen, showed an increase during the storage period. Differences in fish species showed an effect while filet and weeding treatments did not show a significant effect [10]. Meanwhile, the TVB measurement results for turbot fish stored in ice had an initial value of 12.1 mg/100 g sample, then decreased until the 8$^{th}$ day and again increased to 31.1 mg/100 g sample at the end of storage period (day 19). Fluctuations in TVB values were also seen in other types of fish such as cultured sea bass and turbot and gilthead sea bream, indicating that TVB is not a good indicator for these fish.

**2.7 Trimethyl Amine (TMA) and Trimethyl Amin Oxide (TMAO)**

Reduction of trimethylamine oxide (TMAO), an osmoregulatory component in marine fish, is generally the result of bacterial activity but in some species an enzyme is present in muscle tissue and is capable of breaking down TMAO into dimethylamine (DMA) and formaldehyde. Formaldehyde can form cross-links of muscle proteins which will cause muscles to become hard and lose their ability to bind water [8],[12].

TMA is formed in damaged fish as a result of bacterial activity against TMAO. The final concentration and developmental rate of TMA will depend on the storage temperature; Increasing the storage temperature will cause a higher TMA concentration. It is very difficult to determine the safe limit of TMA in aquatic products because of the differences in the content of TMAO in each organism. There is no maximum TMA limit set by the European Union. Changes in TMA have a correlation with sensory values, storage temperature, storage time and the number of live anaerobic and aerobic bacteria [9],[17].

TMA accumulation is the result of TMAO breakdown and this occurs to a significant degree only in the logarithmic phase of bacterial growth. In the study of [9] there was an increase in TMA values in frozen storage of three types of fish for 9 months. Changes in this value will depend on the ambient temperature. Frozen storage will prevent bacterial activity so that it is expected to inhibit the accumulation of TMA. The lower the temperature, the TMA accumulation becomes more inhibited. Usually, a TMA of 10-15 mg TMA of nitrogen is the limit at which refrigerated fish is considered too polluted [12].

Unlike cultured turbot, the TMA content of wild turbot increased sharply from its original value of 9.36 mg/kg on day 0 of ice storage, to 38.9 mg/kg on day 19 [3]. Fish (especially seawater fish) contain trimethylamine oxide and the quantity depends on the type of fish and the environment. TMA is associated with the smell of spoiled fish and part of the spoilage pattern. Marine fish contain 1-100 mg of TMAO for every 100 g of muscle tissue, whereas freshwater fish generally contain 5-20 mg/100 g (Stansby and Olcott, 1963).

**2.8 Biogenic Amin**

Biogenic amines are generally produced from the decarboxylation reaction of microorganisms to certain free amino acids in fish. The importance of determining the concentration of biogenic amines in fish and fish products will relate to their effects on human health and food quality. The most common biogenic amine compounds found in food are histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, spermidine and agmatine. Histamine is produced in fish during bacterial breakdown due to bacterial decarboxylation of the amino acid histidine via the enzyme histidine decarboxylase. Arginine is easily converted to agmatine as a result of bacterial activity. Lysine can be converted to cadaverine. Tyramine, tryptophan and 2-phenylethylamine can be formed from tyrosine, tryptophan and phenylalanine. Putrescine is a precursor of ornithine [7],[9],[17],[20].

Among the biogenic amine compounds, histamine is a potential hazard and is an intermediate for histamine poisoning associated with seafood consumption. Cadaverine and putrescine have been shown to increase histamine toxicity. Histamine is the only biogenic amine that has a content limit set by the European Union for tuna and fish from the families Scombridae and Scomberesocidae which is around 10 mg/100 g, but due to the high probability of poisoning, the FDA has reduced the histamine content contained in it. allowed to be 5 mg/100 g [7],[17].

Fish muscle conditions are able to support the formation of various types of amine compounds derived from amino acid decarboxylation. Biogenic amines are produced in very low amounts in fresh fish and their formation is associated with bacterial damage to fish [21]. Together with the amine compounds produced by bacterial attack until the end of the fish's shelf life, the content of these compounds is considered as an index of damage rather than as an indicator of freshness. Biogenic amines are quality indicators that are useful in observing fish decomposition [7].

Cadaverine and putrescine show a steady increase in content after bacterial damage begins, therefore these amines are considered as potential indicators of fish quality [21]. The most important factor affecting the production

of biogenic amine compounds is storage temperature. The easiest method to prevent the accumulation of amines is by cooling quickly after the fish is caught and keeping the temperature low until the time the fish is consumed. Hygienic handling of fish from capture to consumption is important to reduce the formation of biogenic amine compounds [7].

The number and type of amine compounds formed are influenced by the composition of the food, the type of bacteria and several parameters that will increase the growth of bacteria during storage such as temperature, ripening and packaging. The reasons for determining the content of amines in foods are due to their potential toxicity and the possibility of using amines as markers in food. Biogenic amine compounds in low concentrations are needed to carry out various physiological functions but if ingested in large amounts it will cause health problems [5].

During digestion in the human intestine, biogenic amines are detoxified by certain enzymes such as diamine oxidase (DAO), but this enzyme cannot perform its function efficiently if the amount of biogenic amine consumed is too high. Amino biogenic compounds such as histamine and tyramine are considered as antinutritional components. Biogenic amines in fish can be used as indicators of spoilage [5].

The microorganism content of fish meat will depend on the method of cultivation and the sanitary conditions of its handling. When fish are weeded, bacteria from the gills and intestines will contaminate the edible parts of the meat, which in turn is related to the biogenic content of amines in fish. In research on goldfish storage at several different temperatures, the biogenic amine content of carp is known to not affect human health because the content of harmful amine compounds such as histamine and tyramine is quite low. The formation of biogenic amines is known to have a relationship with the activity of mesophilic and psychrotropic bacteria [5].

During the storage of turbot fish (19 days), eight types of biogenic amine were observed, namely histamine, putrescine, cadaverine, spremidine, spermin, tryptamine, tyramine and 2-phenylethylamine. Three types of amines (histamine, tyramine and tryptamine) were not detected in the samples during the storage period and with increasing storage time putrescine and cadaverine became the dominant amine compounds in the amount of 22.7, and 16.9 mg/kg on the $19^{th}$ day of storage in ice [3]. During the storage of sea bass, it was found that aluminum packaging had no effect on preventing the accumulation of biogenic amines and the content of these compounds increased with increasing storage time [7].

## 3. CONCLUSIONS

The quality of fish is easy to decline after the fish die. During the storage of fishery products, a series of biochemical changes will immediately occur in the protein and fat components, besides the formation of amine compounds (volatile and biogenic) and accumulation of hypoxanthine. As a result, there will be a decline in sensory quality, loss of nutritional value and negative changes in the physical properties of fish muscles will occur. This degradation process is initially carried out by enzymes contained in fish muscles and then followed by enzymes derived from bacteria. At the stage after sensory changes, the process of autolysis as a result of enzyme activity in fish muscles will also affect the sensory assessment of the fish. In addition, changes related to quality deterioration can also be observed chemically by measuring a series of substances whose amounts will change during the stages of deterioration, such as nucleotide content, TVB (Total Volatile Base), TMA (Trimethyl amine), TBA (Thiobarbituric Acid) and other substances. After and during the autolysis process, nutrients in the form of components resulting from the breakdown of macromolecules become available to microorganisms and after this stage takes place in general the fish have been damaged and are no longer fit for consumption.

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