

# FISH PROCESSING

Since 1978, New Zealand has had exclusive fishing rights to all its surrounding waters up to 320 km away from the land. This has resulted in fish becoming an important resource, and much research has been done to understand the processes involved in fish ageing, and ways to prevent these processes.

After a fish dies, the flesh quickly becomes rigid, in a process known as rigor mortis. This rigidity then dissolves, and the fish flesh decomposes.

## **Rigor mortis**

When muscles contract, they absorb calcium ions. After a fish has died, calcium ions leak into the muscles, causing them to contract. However, ATP, the biochemical energy carrier that provides energy to relax the muscle again, is no longer present, so the muscles remain rigid. This locking of the muscles, called rigor mortis, can be slowed down by chilling the fish immediately after death.

## **Dissolution of rigor**

Over time this rigidity disappears, but by then the fish has significantly deteriorated.

## **Autolysis**

The decomposition of the fish occurs as its constituent compounds break down (called autolysis). The proteins, nucleotides and sugars break down, bases are released, the pH falls and the fats are oxidised. These make the fish smelly, rancid and tough.

## **Tests for freshness**

By measuring the pH of the fish and the levels of various compounds present the amount of autolysis that has occurred, and hence the freshness of the fish, can be measured.

## INTRODUCTION

Since the 320 km Exclusive Economic Zone was established in 1978, giving New Zealand one of the largest fishing zones in the world, the significance of fish as a resource has risen greatly. Many areas of research, such as the best processing methods, the quality and composition of the fish landed, and the effects of handling, storage and packaging on fish quality, have become of great importance.

The Seafood Research Unit, Crop & Food Research was established in 1979 in response to the industry's need for research. Laboratories are located in Auckland and Nelson. Major areas of interest include:

*Catching and handling.* The understanding and control of the physiological changes occurring during the handling of fish and their effect on quality.

*Chemistry and biochemistry.* Detection of spoilage indicators and toxic components and quality evaluation of seafood to establish management guidelines for quality systems.

*Microbiology.* Improvement of product safety and quality, and assistance with hygiene and sanitation.

*Seafood toxins.* Depuration of algae from shellfish bioassays.

*Fish proteins.* Changes in the structure and biochemistry of proteins and hence the texture of fish muscle during frozen storage.

*Sensory evaluation.* Determination of shelf-life changes and consumer evaluation of seafood.

In this article we shall discuss the major biochemical and microbiological changes occurring *post mortem*, their effects on the quality of the fish, plus the handling, storage and processing procedures by which these can be reduced and the quality of the fish maintained.

### **CHANGES IN FISH FLESH BIOCHEMISTRY *POST MORTEM***

The demise of a fish begins a series of irreversible changes which lead to spoilage and loss of quality.

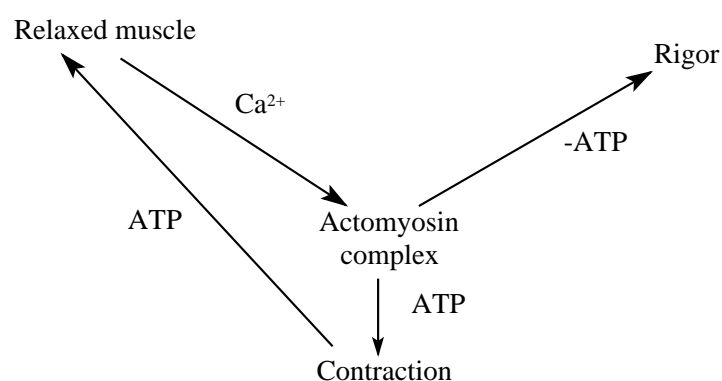
The natural process:

*Rigor mortis* → *Desolution of rigor* → *Autolysis*

can be slowed down if correct handling and storage procedures are followed.

#### **Step 1 - Rigor mortis**

Muscle consists of several proteins actively involved in contraction (**Figure 1**). The two major proteins, actin and myosin, combine in the presence of calcium ions to form actomyosin. ATP then supplies the energy for contraction, and later also the energy for the removal of the calcium ions via a calcium pump. This breaks the actomyosin complex, leaving the muscle ready for a further contraction.



**Figure 1 - Muscle contraction reactions**

On death, the circulatory system stops and the ATP levels drop. Calcium ions leak, forming actomyosin. However, there is insufficient ATP for the calcium pump to operate, and so the actomyosin complex remains unbroken. The muscle is now in a continual state of rigidity, known as rigor mortis.

## **Step 2 - Autolysis**

Enzymes in the flesh and gut previously involved in metabolism now catalyse autolytic reactions, in which various compounds decompose. Enzymes in the flesh break down desirable compounds into tasteless or bitter ones, whilst gut enzymes attack the internal organs, turning them into a soupy mess and allowing bacteria to enter the flesh.

### *Bacterial attack*

In a living fish, bacteria are present in the gut and skin, but the flesh, which they are prevented from entering, remains sterile. Once autolysis begins, however, the bacteria are able to enter the flesh, whereupon they multiply rapidly and decompose the muscle. Anaerobic bacteria (those which operate in the absence of oxygen) produce a particularly foul type of spoilage which results in an inedible fish.

A number of chemical changes take place during autolysis, and these are outlined below.

### *Protein Denaturation*

Denaturation of protein involves the destruction of its secondary, tertiary and quaternary structure, reducing the protein to a simple polypeptide chain. A number of factors, including slow freezing and variability of storage conditions, cause this denaturation. A denatured protein has not only lost its ability to function as an enzyme, but also its "water-holding" ability. This results in denatured fish flesh dripping excessively when thawed (a situation known as "drip-thaw"), and appearing white, dull and spongy, and upon chewing becoming fibrous and tasteless.

### *Decreasing flesh pH*

A living fish has a flesh pH of 7.0. However, after death residual glycogen is broken down *via* glycolysis to pyruvic acid and then lactic acid. As this happens, the flesh becomes more acidic. If the pH remains above 6.6, the texture is reasonably soft, but below this level the flesh becomes firm and eventually unacceptably tough.

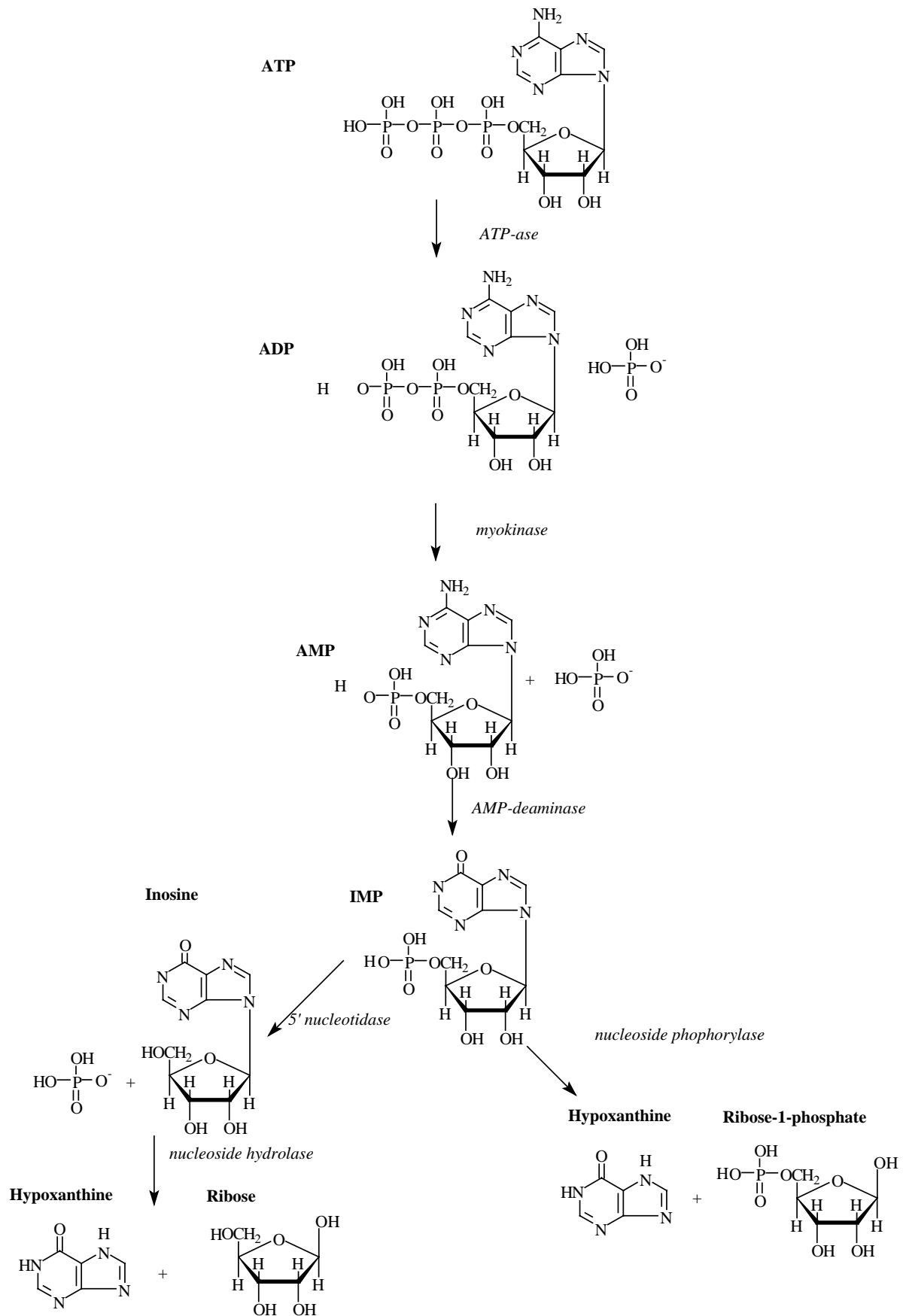
### *TVB-Total Volatile Base*

TVB is a measure of the total amount of a variety of nitrogen-containing substances which are produced during storage. An example of a volatile base present in the flesh is a trimethylamine (TMA), which is formed from the reduction of trimethylamine oxide. Marine fish contain a small amount of trimethylamine oxide, the function of which is unknown. This odourless and tasteless compound is reduced by invading bacteria to TMA, which is characterised by its "fishy" smell. TMA, though, only becomes useful as a quality index during the middle and late stages of spoilage after the bacteria have invaded the fish.

Trimethylamine oxide is converted in the muscle tissue into dimethylamine (DMA) and formaldehyde by enzyme action during frozen storage. This formaldehyde is able to cross-link with protein, denaturing the muscle structure. This fish loses water when it is thawed, and when cooked has a tough and fibrous texture.

### *Nucleotide Breakdown*

This involves the enzymatic breakdown of the energy carrier ATP, as outlined below:

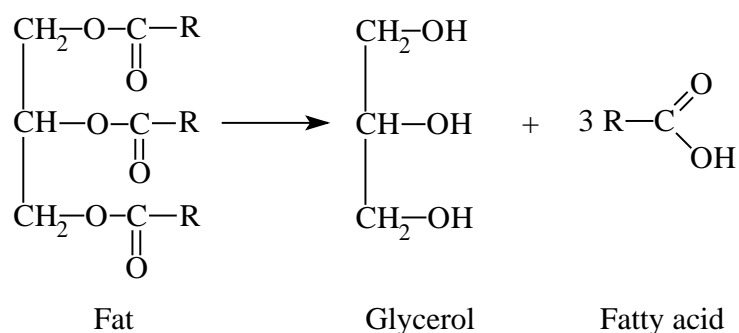


Thus as spoilage proceeds the amount of ATP present decreases, causing rigor mortis.

### *Liquid Oxidation and Hydrolysis*

The two major deteriorative changes which occur in fish are:

- (i) the enzymatic hydrolysis of lipids (fats) to produce free fatty acids and glycerol:



- (ii) the oxidation of fish oils yielding the rancid odours and tastes which are the major problem encountered in fish storage.

## **TESTS FOR FRESHNESS**

Several of the changes described above can be chemically monitored to determine the freshness of the fish, and these are used in conjunction with taste testing.

### *Protein denaturation*

During cold storage the proteins undergo molecular cross-linking with formaldehyde (see above), causing the muscle protein to become less soluble. Deterioration can be assessed by adding 5% NaCl (common salt) and then separating and measuring the soluble and insoluble proteins present. Increasing insoluble protein indicates longer storage and greater deterioration, mainly of texture.

### *Nucleotide breakdown*

The concentrations of ATP, ADP, AMP, IMP, inosine and hypoxanthine (i.e. the different compounds formed over time as ATP breaks down - see above) can be measured by high performance liquid chromatography. The ratio of inosine and hypoxanthine to the total amount of the above substances is represented as a percentage value, *K*. A *K* value of less than 20% represents fresh fish, whilst anything greater is indicative of spoilage.

### *Decreasing pH*

pH is a possible test of textural strength, with anything below 6.6 resulting in noticeably firmer flesh than that of fresh fish.

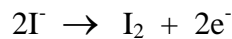
### *TVB - Total Volatile Base*

The amount of TVB is measured by distilling a fish extract, and determining the base concentration by titration against acid. A fresh sample of Jack Mackerel would have a value of 19-21mg TVB N/100g, whilst an ageing sample would be nearer 30 mg TVB N/100g.

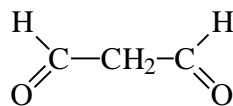
The amount of DMA can be measured by spectrophotometry, giving an indication of the storage time.

*Lipid oxidation and hydrolysis*

Oxidation leads to rancidity, the degree of which is commonly evaluated by measuring the free fatty acid and peroxide concentrations. Hydroperoxides can be measured by mixing the fish oil with potassium iodide, and measuring the amount of iodine liberated by titration against thiosulphate. The hydroperoxides oxidise the iodide to iodine, which is liberated according to the following equation:



A further measure of oxidation is the TBA test. This involves extracting some fish muscle into trichloroacetic acid and treating it with thiobarbituric acid (TBA). The TBA reacts with malonaldehyde, a substance formed during oxidation, to form a red compound. The intensity of the red colour, which is proportional to the concentration of the malonaldehyde, can be measured using a spectrophotometer.



malonaldehyde

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