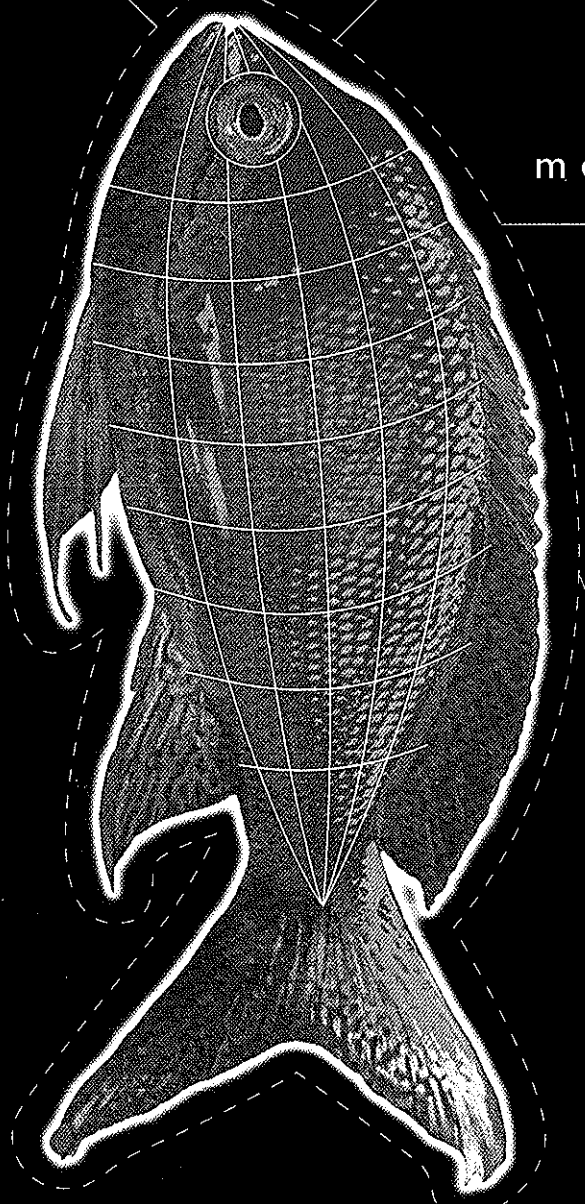
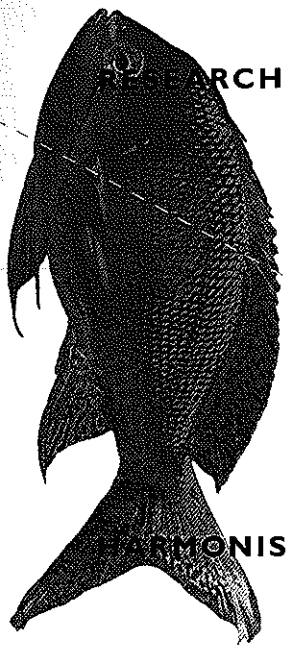
AIMING AT FAST AND OBJECTIVE ANALYTICAL METHODS



methods to determine the freshness of fish in research and industry

evaluation of fish freshness



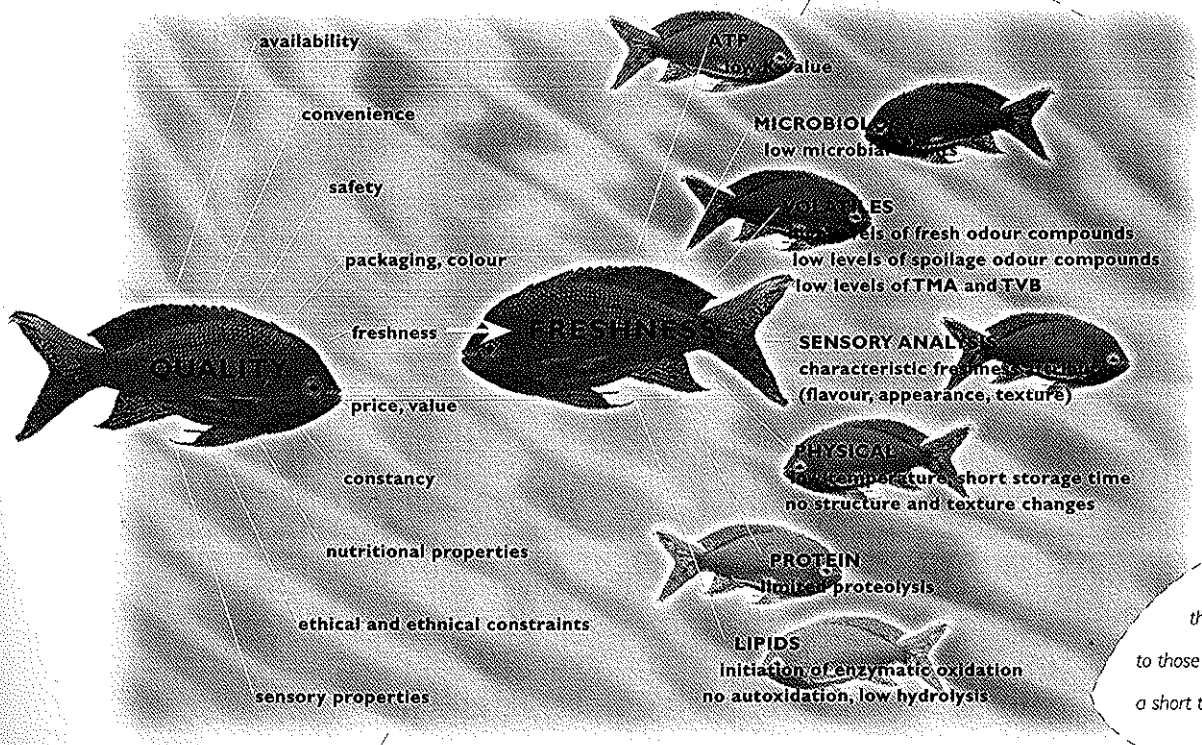
RESEARCH FOR THE FISHING INDUSTRY

Freshness is essential for the quality of fish. Leading fish laboratories in Europe are cooperating in order to find fast and objective analytical methods to evaluate fish freshness. The overall aim of their concerted action is to validate methods and define criteria for fish freshness assessment. The researchers are working in subgroups covering microbiology, physical and chemical measurements and sensory analysis. Leading fish laboratories in Europe expect that their concerted action will support the fishing industry in their own activities in the field of quality control.
This brochure summarises the present state of research and the aims for the future.

HARMONISING RESEARCH ACTIVITIES

For centuries the freshness of fish was measured exclusively by subjective criteria. But, freshness can also be determined by objective characteristics. Controlled storage experiments can show the changes of properties of fish from the time of harvest until spoilage. Freshness, loss of freshness and spoilage can thus be monitored resulting in a model that predicts the freshness and the remaining shelf-life of fish.

The correlation of a number of measures obtained by different methodologies with sensory assessments, such as smell, colour or taste, results in more accurate models. Current work in this European concerted action project focuses on harmonising research activities in seven different areas to evaluate fish freshness. The overall aim is to develop general methods that will provide the fishing industry with practical instruments to evaluate the freshness and freshness changes of fish and fishery products and give directives for production.



Above all freshness means that the entire properties of a fish are close to those of the living state. In other words: only a short time has passed since the fish was caught or harvested.

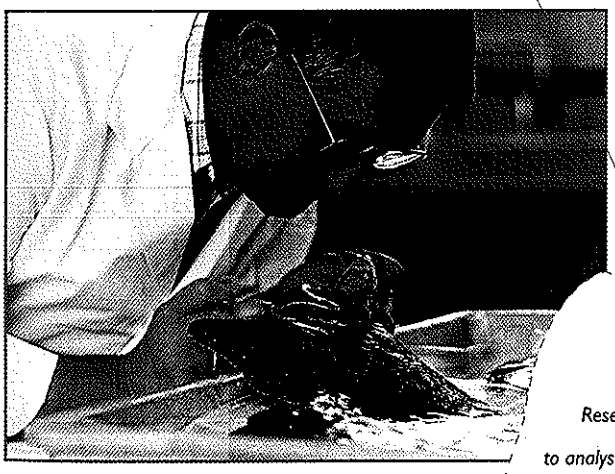
SENSORY EVALUATION

World-wide the sensory test is still the most commonly used scale to grade the freshness of fish. Sight, smell, taste and touch of the fish represent the main components of this analysis. The sensory test can be divided into three groups:

- discriminative tests give the difference between samples;
- descriptive tests, describing every observation in an objective manner, for example by grading on a scale from 1 to 10;
- tests measuring the acceptance by consumers.

There are various ways to describe the sensory changes that occur in fish. In Europe the most commonly used method for quality assessment of raw fish in the inspection service and fishing industry is the EU-scheme. The alternative objective Quality Index Method (QIM) for raw fish is increasingly being adopted. The QIM methods are described in a model that is used to predict the keeping qualities of fish. In the fishing industry and fish laboratories it is also common practice to cook fillets for sensory evaluation. In these cases the Torry scheme is the most used scale.

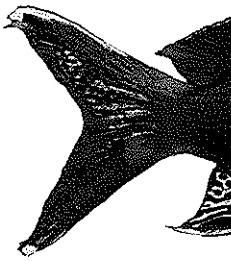
European research laboratories are discussing guidelines for the sensory evaluation of the freshness of fish. Standardisation of this kind of research will contribute to the growing importance of specially trained panels in the fishing industry to control the quality of fish production.



SENSORY EVALUATION OF FISH

Research used to analyse and interpret characteristics of fish as perceived through the senses of sight, smell, taste and touch.

2 MICROBIAL METHODS AND PREDICTIVE MODELLING



Newly caught fish contain a diverse micro-flora but the activity of specific groups of micro-organisms determines the shelf-life of fresh fish.

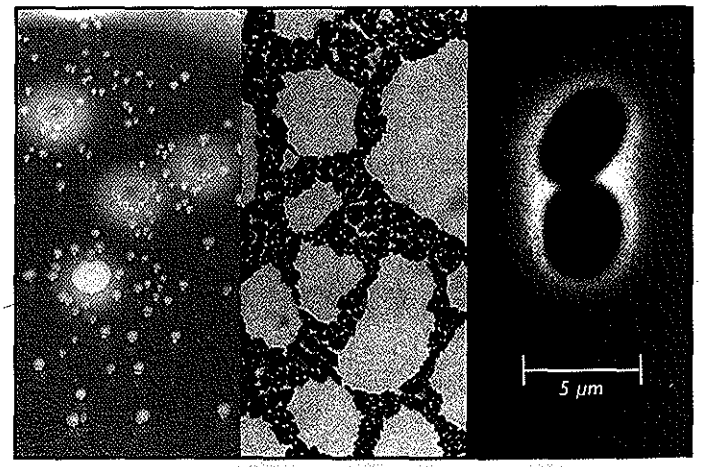
The European researchers study the different applications of microbial methods. They use fish from various countries, stored under different conditions of temperature and atmosphere.

During chill storage psychrotolerant micro-organisms are selected. A good correlation was found between these micro-organisms, the storage conditions and the remaining shelf-life of fish. Differential counting of the selected groups of micro-organisms is used as a measure of fish freshness. The organisms measured include *Pseudomonas spp.* and *Shewanella putrefaciens* in chilled fresh fish as well as *Brochothrix thermosphacta* and *Photobacterium phosphoreum* in some modified atmosphere packed fish.

The best results are still obtained from the relatively slow detection methods such as plate count and other techniques involving incubation. Due to their long response time these techniques are not optimal for practical use in industry. Researchers are therefore focusing on the development of new rapid methods for concentration, separation and measurement of micro-organisms.

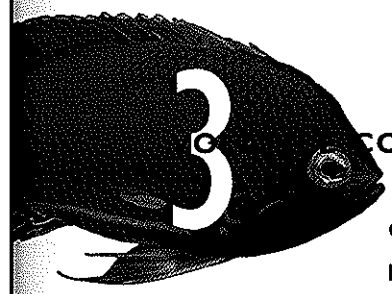
Mathematical models used in combination with microbial methods allow the remaining shelf-life of fresh fish to be predicted under the fluctuating temperature conditions often observed in practical seafood processing.

PREDICTIVE MODELS



MICROBIAL METHODS

The remaining shelf-life of fresh fish storage at constant temperature and atmosphere conditions can be determined by microbial methods.

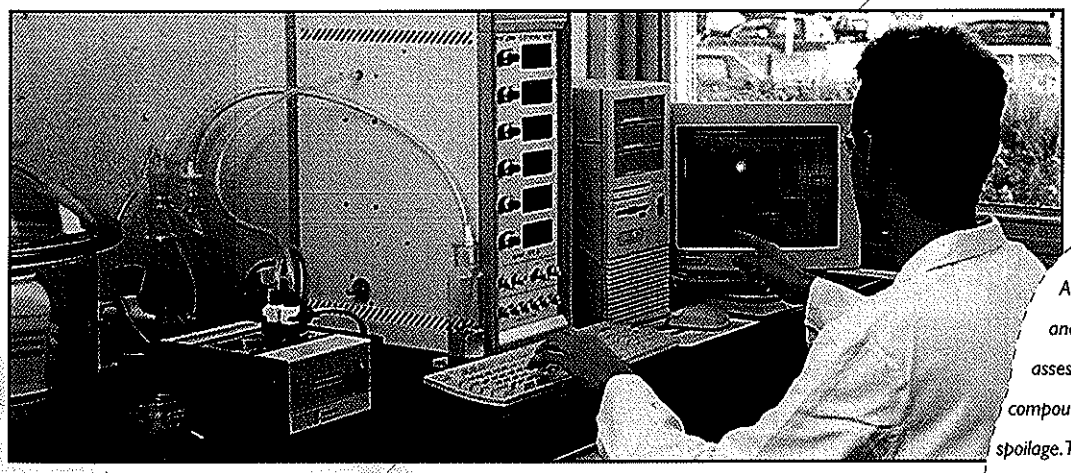


3 COMPOUNDS

Odour is one of the most important parameters used to evaluate fish freshness. Analytical headspace methods to measure volatile compounds can be applied to identify the various groups of odour compounds. Depending on the concentrations of volatiles present in the headspace, different methodologies are used to sample and measure them. Though highly automated instruments are available for trapping and chromatography steps, the complexity, costs and time required in these methods, mean they are feasible only for specialised research laboratories.

Classical chemical methods for the analysis of TVB (total volatile bases) and TMA (trimethylamine) in the fish muscle have traditionally been used by the fishing industry.

The food industry needs a rapid assessment method for volatile compounds to evaluate freshness or spoilage that can be related to odour. The use of arrays of gas sensors, so called electronic noses, is of increasing interest. Assessment of the quality of salmon and whiting with an electronic nose has shown that samples can be effectively classified in the categories good, acceptable and not acceptable.



ELECTRONIC NOSE

A rapid and automated assessment of volatile compounds in food to detect spoilage. The use of rapid arrays of gas sensors offers promising possibilities. However, for future development of rapid gas sensor techniques for the fishing industry, it will be necessary to define standard and validated methods.

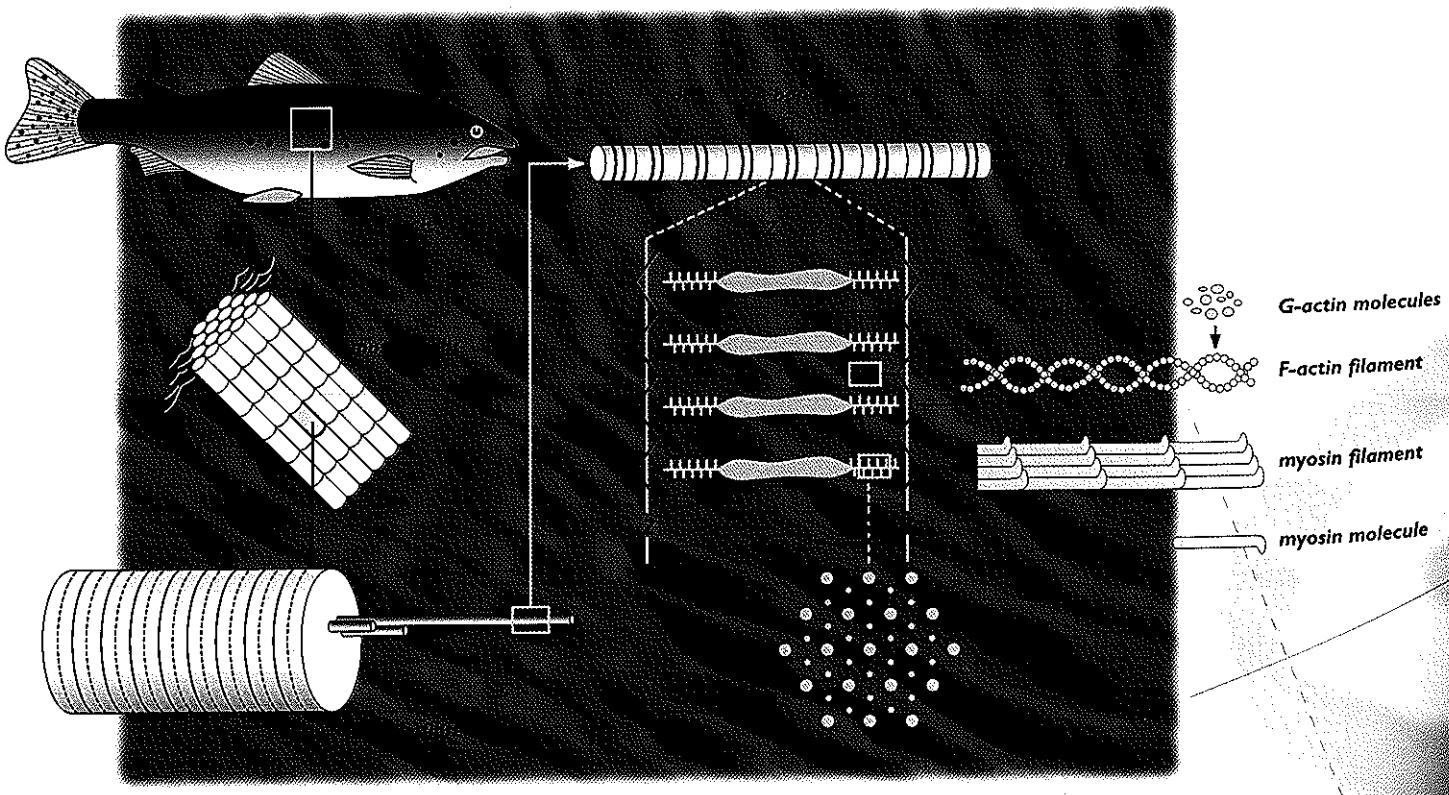
4 FISH MUSCLE PROTEINS

Currently there are no rapid methods for determining changes in muscle proteins during post mortem storage. There is little prospect of such methods suitable for industry emerging. Instrumental methods for measuring texture, however, are proving more promising.

Proteins are the major organic constituents of fish flesh. Researchers have investigated the effect of post mortem storage of fish on proteins.
The proteins found in fish muscle can be categorised as

- water-soluble proteins (of cell plasm);
- contractile proteins, that execute movement of the muscle and are extractable in solutions of relatively high ionic strength;
- collagens, that are insoluble and form the main part of connective tissue.

Investigations have shown that on post mortem storage there is no change in the water soluble proteins in either composition or enzymatic activity. There is no change either in the major contractile proteins. The muscle cells in fibres however are broken down by the action of enzymes leading to the softening of the texture. The collagen proteins are relatively minor components of muscle but are important for structural arrangements of muscle cells. Though the evidence is conflicting, it is generally agreed that some degree of breakdown by enzymes also takes place contributing to the overall softening of the flesh.
In the main the proteins of the muscle are not affected during storage. Researchers explain that the softening of muscle is caused by the breakdown of minor cell components which link the major structural units together. These changes can be observed by microscope. It is difficult to measure them due to the inherent nature of muscle proteins. Changes in the characters of proteins can only be determined after isolation involving lengthy and specialised techniques, only suitable for research laboratories. Hence these methods are not suitable for industrial use.

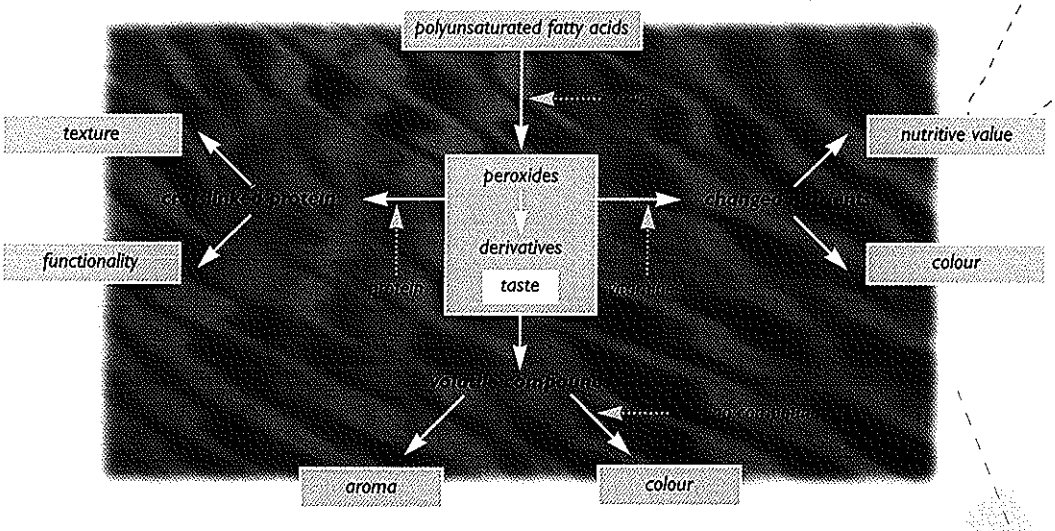


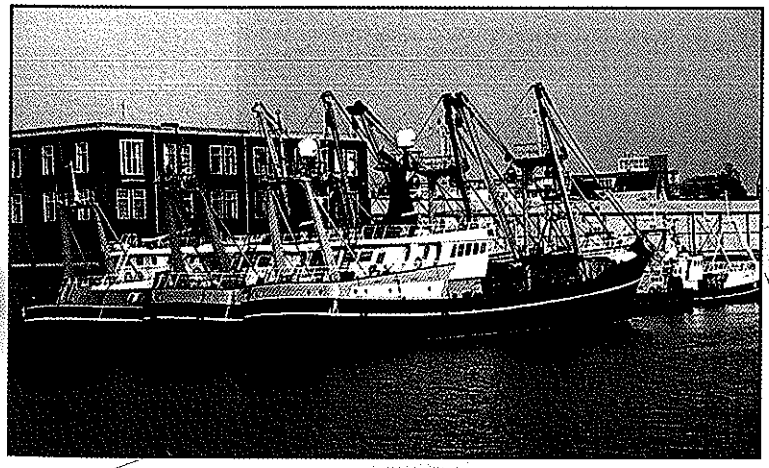
5 LIPID OXIDATION IN FISH

The highly unsaturated lipids of fish are easily oxidised, resulting in alterations in smell, taste, texture, colour and nutritional value. At temperatures below 0°C lipid oxidation is the determinant factor for shelf-life.

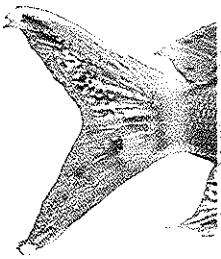
Various techniques to monitor the progression of lipid oxidation are used in research laboratories. It is recommended to use more than one technique, since the different oxidation products are very unstable. If only one technique is used, the results might be difficult to interpret and are often misleading. In the fish industry, only a few methods are routinely applied to follow lipid oxidation. Among these, the measurements of peroxide value and of aldehydes constitutes are important examples.

More fundamental research is necessary to establish new techniques in this kind of investigation.





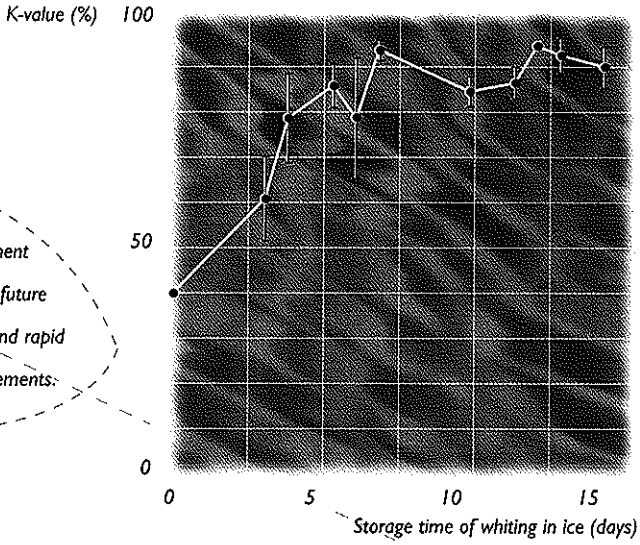
6 DEGRADATION OF ATP AS FRESHNESS INDICATOR

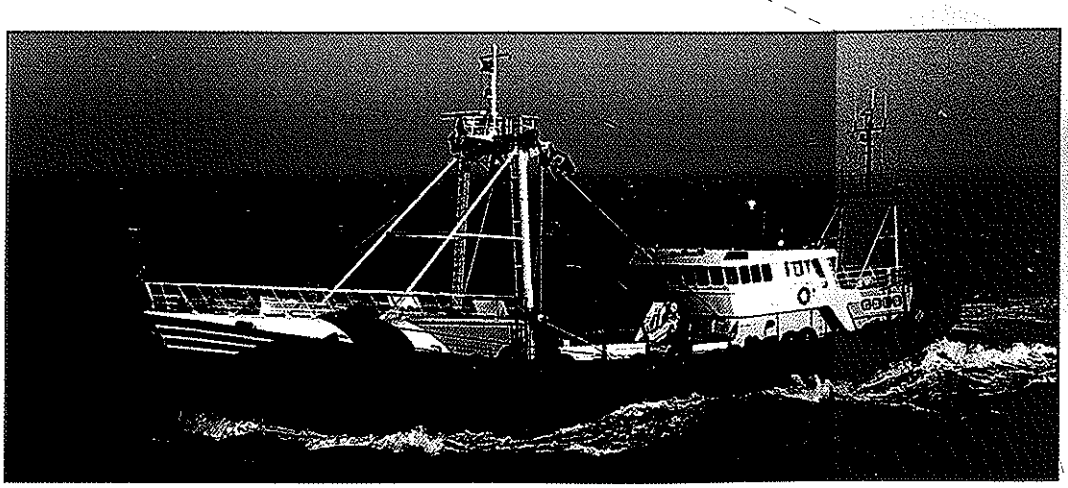


After death several chemical processes take place in fish. Adenosine triphosphate (ATP) plays an important role in this process. After death ATP is rapidly degraded to inosine and hypoxanthine.

The extent of ATP-degradation is expressed as the K-value. In fresh fish the K-value is low. The K-value is a reliable freshness indicator for frozen and smoked fish and of fish stored under modified atmospheres. The K-value depends on a variety of variables. It varies between kinds of fish, the method of killing and the time/temperature conditions during storage and handling. This implies that for each species and for its specific handling and storage, a profile of the K-value must be established, before K-value measurements can be used to establish freshness. Due to the time and expense involved with this technique, the K-value is not widely used in industry.

It is generally accepted that the degradation of ATP will be an effective instrument to measure fish freshness in the near future. For future development there is need for cheap, reliable and rapid methods for ATP breakdown measurements.





7 AN EYE FOR STRUCTURE AND COLOUR MEASUREMENTS

Physical changes in fish resulting in decline of fish freshness are mainly related to - structure and colour. There are several methods to determine physical changes.

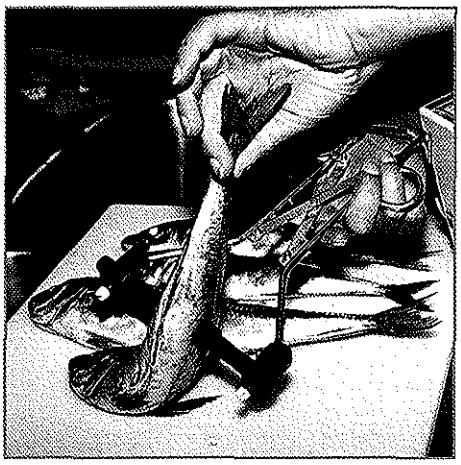
Texture measurements

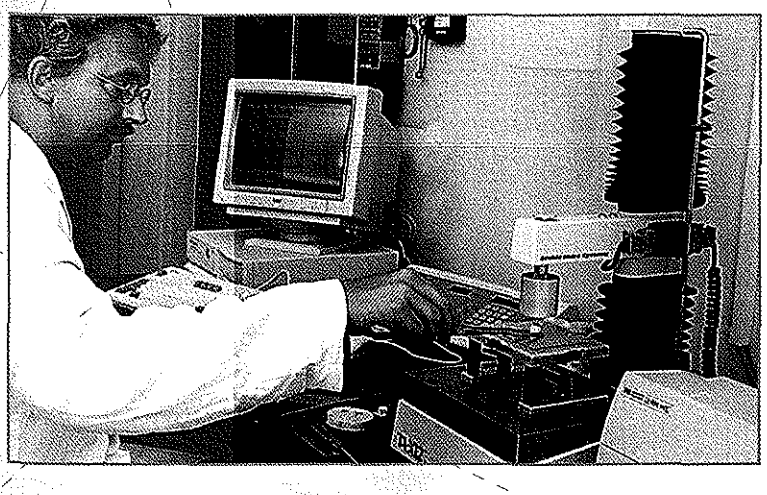
Texturometers are instruments to determine textural changes in the flesh of fish. The texture of fish is difficult to measure because of the lack of a uniform structure. It is not easy to prepare standard samples. This has led to a variety of sample preparation procedures and some variation in results. Texture measurements of fish have been compared to the result of sensory analysis and some results have shown good correlation.

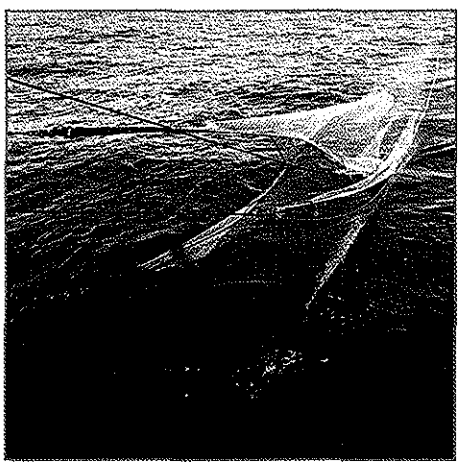
Microstructural characterisation

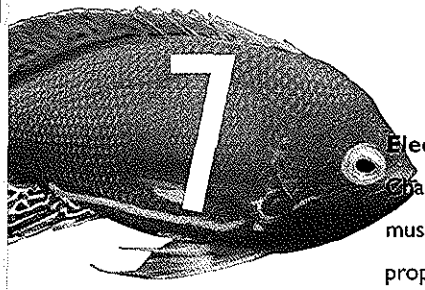
Another way to assess the structure of fresh fish is by microstructural characterisation of the fish muscle. There are different techniques available: light and laser scanning and electron microscopy. A weakening of the connective tissue is shown to be one of the reasons for post mortem tenderisation of fish muscle. Post mortem changes of the microstructure of cod and salmon have also an impact on the liquid holding capacity.

There are several physical measurements that give information on parameters related to fish freshness. None of them gives the fish industry a unique and unambiguous answer to the question whether the fish is fresh or not.









Electrical properties

Changes in fish freshness can also be measured by the electrical properties of the fish muscle. Three different instruments are available to measure the change in electrical properties: the Torrymeter, the Intelectron Fish-tester and the RT-Freshness Grader. These methods all show good correlation with the sensory score of fish freshness. But, these meters cannot be used in thawed fish or fish stored in chilled seawater. The use on fillets is limited to a few days. High salt content in water-ice and mechanically damaged fish cause erroneous results. The advantage of these instruments is their practical and simple field use and their immediate response.

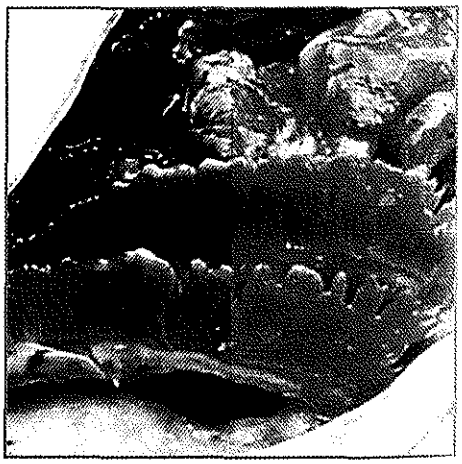
Colour measurement and spectroscopic methods

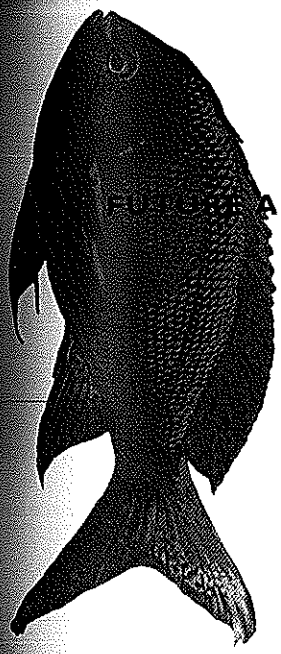
Instrumental colour measurements are becoming more important in quality control in the food industry. Advanced technologies allow simple performance of these methods. Spectroscopic methods have gained importance in evaluation of food quality parameters. The advantages of spectroscopic methods are rapid analysis, simultaneous evaluation of several parameters and the potentials for on-line use.

So far spectroscopic methods have proven to be insufficient to fully characterise the properties of fresh fish. Developments in instrumentation and evaluation techniques of spectral data however are promising. As for example the application of near infrared spectroscopy (NIR) that reveals information on the storage time of fresh fish.

Time-temperature indicators (TTI)

TTIs are devices or materials that can be attached to or incorporated into food to give an indication of its time-temperature history. The mechanism of recording the TTI is through biological, physical or chemical processes that depend on time and temperature. The record can be used in appropriate models for shelf-life as affected by bacterial or enzymatic spoilage. It is likely that TTIs will gradually be introduced into the wholesale and retail food chain starting with temperature-sensitive high value foods such as meat or fish.





FUTURE AIM FOR FISH FRESHNESS EVALUATION

By combining the results of the various European investigations of the freshness of fish it has become clear which methods are the most promising for the near future. Experts from different disciplines are co-operating to facilitate and improve freshness determination for the processor.

Sensory tests are the most important methods today for freshness evaluation in the fish sector. Standardisation of this evaluation will result in the use of specialised and trained panels for quality control in the fish industry. The Quality Index Method is promising.

The development of ***microbial methods*** results in mathematical models expressing the effect of storage conditions in order to determine the relationship between the growth of relevant spoilage micro-organisms and the shelf-life.

Rapid assessment of ***volatile compounds*** in fish using gas sensors to determine freshness is of increasing interest. The development of the electronic nose offers a suitable perspective to determine the shelf-life of fish by its odour.

More fundamental research is necessary to determine fish freshness by ***degradation in muscle proteins or lipid oxidation.***

The European investigations show promising results in the development of rapid, cheap and reliable techniques for ***ATP metabolites***.

In conclusion various physical measurements (electrical properties, spectroscopic methods, and time / temperature indicators) give reasonable reliable information to determine the freshness of fish.

The wholesale and retail food industry is showing a growing interest in effective and objective methods to evaluate the freshness of fish. The aim of the European research laboratories, co-operating in the concerted action programme is to combine various standard methods using rapid measurement techniques and a mathematical model to evaluate fish freshness in an objective way. These techniques will be used to complement sensory analysis in the near future

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