NON-SENSORY ASSESSMENT OF FISH QUALITY

The eating quality of fish is one of the important attributes that influence the acceptability of fish as food to the consumer. Price, appearance, availability and perceived nutritional value are important also, but if the fish is not good to eat the customer will not buy it again. The quality of fish begins to deteriorate quite soon after capture, and so it is essential that everything possible be done at all stages in the handling of fish to slow down such deterioration. It is important, then, to be able to measure the eating quality of fish at all stages.

Methods of measuring quality can be described as sensory or non-sensory. Sensory methods use the senses directly, principally those of taste, smell and sight, in much the same way as the ultimate consumer will, and they are described in more detail in another Note in this series. Non-sensory methods may be chemical, physical or microbiological. This Note deals with chemical and physical methods; the values derived by such tests are often called freshness indices. Microbiological methods can give information on the state of spoilage, but are applied much more in assessing hygiene, safety and conformance with standards; microbiological methods are described in more detail in another Note.

What do the tests measure?
The eating quality of fish becomes less pleasant as a result of various spoilage and deteriorative changes. Unfrozen fish become unacceptable to the consumer principally because of the activities of bacteria; some of the chemical tests used to estimate quality actually measure the products of bacterial growth. In the early stages of spoilage, when the numbers of bacteria are still low, certain enzymes, or biological catalysts, that have essential functions in the living fish remain active for some time after death, and their effects can be measured and used as indicators of freshness.

Oily fish like herring and mackerel can become rancid; this is due to the reaction of the oil with oxygen in the air to create unpleasant odours and flavours. Chemical tests can measure the extent of such oxidation. Oxidation occurs quite slowly in iced fish, and the products of bacterial growth can render fish inedible before oxidation.
contributes much to the off odours and
flavours.
In frozen fish, bacterial action is
reduced to negligible levels but
oxidation of the oil, especially in oily
fish, will continue during storage and
will lead to a loss of eating quality.
Some of the proteins in fish undergo
changes, not fully understood, during
long periods of frozen storage which
lead to undesirable toughening of the
flesh.

Do non-sensory tests have
advantages?
Since sensory methods of assessment
apply the same senses as the consumer
uses when deciding whether a piece of
fish is pleasant to eat, they are likely to
predict the consumer’s reaction better
than non-sensory methods; the latter
do, however, have certain advantages.
Since sensory assessment may require
the use of a taste panel, non-sensory
methods can be cheaper and often
quicker. Taste panels need to be
trained and kept in training, which can
be time-consuming. Non-sensory
assessments should give the same
result no matter where they are carried
out, whereas sensory evaluations may
depend on subjective responses of the
panellists to the fish being examined.
Non-sensory methods can appear more
objective and reliable than sensory
methods, although this need not be the
case. When specifications are being
prepared it is easier to insert numerical
limits based on non-sensory tests than
on sensory tests, especially in
international trade, and this is widely
done. Courts of law may find it easier to
accept the results of chemical or
physical tests, being based on impartial
instrumental readings, than the results
of sensory tests.
Non-sensory tests have disadvantages,
et. They measure usually only one
aspect of spoilage or may even assess
some change in the fish not directly
related to spoilage: sensory methods
can take many aspects of quality into
account in arriving at a single value.
Again, although non-sensory test
results should be independent of the
method of measurement used, this is
not always the case and can lead to
disputes. Chemical methods and some
physical methods need laboratory
facilities and trained staff and are
necessarily destructive, i.e. the fish
once examined cannot then be sold.

What methods are there?
Most of the remainder of this Note
describes the principles of a number of
non-sensory quality tests that are in
common use. Many other tests have
been suggested over the years but few
have found practical application: some
that have are briefly listed later.
It would unduly lengthen this Note to
give full practical details for carrying
out the tests described; in some cases a
choice of methods is available and to
describe only one method might give
an unbalanced view. Further details of
the methods employed at Torry
Research Station are available.
The tests are described separately for
chilled, unfrozen fish and for frozen
fish, but these are not strict divisions. It
is possible, for example, to apply some
tests for chilled fish to frozen products;
the results will then indicate broadly
the state of spoilage of the fish before
freezing, while additional tests may be
applied to estimate the degree of
deterioration during frozen storage.

Methods for chilled fish

Hypoxanthine
A substance adenosine triphosphate,
ATP, is important in the utilisation of
energy in most living things. When fish
der the ATP is broken down over a
period of days by enzymes present in
the flesh, through a succession of
different substances. The final stage of
this process is the formation of a
compound called hypoxanthine, which
gradually increases in amount as time
goes on and can be used as a measure
of the duration of icing. The rate of
accumulation of hypoxanthine is not the
same in all species and this must be
remembered when interpreting the
results. The amount of hypoxanthine
present is measured either by an
enzymic method that converts
hypoxanthine into uric acid, or by
separating the hypoxanthine from any
remaining ATP and the intermediate
compounds by a technique called high
pressure liquid chromatography
(HPLC). In both cases the last stage is
to measure how much of a particular
wavelength of uv light is absorbed by
the solution of uric acid or
hypoxanthine itself; the instrument used
is a spectrophotometer.
**K value**
Like hypoxanthine, the K value measures the extent of the breakdown of ATP; it is the percentage of the initial ATP present at death that has been converted by enzyme action into hypoxanthine and its immediate precursor, called inosine, in the chain of decomposition of ATP. The HPLC procedure used to measure hypoxanthine can allow the K value, also, to be calculated.

**Trimethylamine (TMA)**
Most marine fish contain a substance called trimethylamine oxide (TMAO). Certain bacteria that occur naturally on the skin and in the guts of fish and in sea water can break down TMAO to trimethylamine. The amount of TMA produced is a measure of the activity of spoilage bacteria in the flesh and so is an indicator of the degree of spoilage. TMA can be measured by a chemical method that produces a coloured solution; the amount of the coloured product is measured using a spectrophotometer. Alternatively, TMA can be separated from similar compounds, and its amount measured, by gas chromatography (GC).

**Ammonia**
Bacteria can generate small amounts of ammonia in spoiling fish, mainly from free amino acids; the amount of ammonia can give an indication, though not a particularly accurate one, of the extent of spoilage. Much larger amounts of ammonia are produced during spoilage of the elasmobranch fishes, skate and dogfish for example, because they have large amounts of urea in their flesh. Shellfish, also, may develop more ammonia than most marine fish and at an earlier stage. There are several chemical and enzymic methods for measuring ammonia.

**Total volatile bases (TVB)**
Ammonia and trimethylamine are examples of bases; another base, dimethylamine (DMA), can also be formed during spoilage of fish, together with traces of others. These bases, other than ammonia, are known chemically also as amines. The combined total amount of ammonia, dimethylamine and trimethylamine is called the total volatile base content of the fish and is a commonly used estimate of spoilage. The increase in the amount of TVB parallels the increase in TMA but the analysis is

A range of methods are used to measure TVB. In all of them the fish, or an extract of the fish, is made alkaline, the bases are distilled off and collected, then measured by titration. Some of the substances used to make the fish, or the extract, alkaline can convert other substances present in the fish to ammonia during the distillation, so that the apparent amount of TVB increases as distillation proceeds. Several other factors can affect the result so that the measured TVB depends quite significantly on the
by two different methods, to get results on the same sample that differ by a factor of 2. For this reason TVB, though widely used, is not a particularly good index of spoilage: when a limiting value of TVB is included in a specification or standard for any particular species, it is important that the method of measurement to be used is described in detail.

**Histamine**

Certain families of fish, notably the mackerel family, contain histidine, an amino acid, in larger amounts than other families. During spoilage of these fish, especially if the temperature rises to above 10°C, histidine may be converted to histamine. Histamine is a substance that is produced by the body as part of the allergic response to foreign substances, as in hay-fever. When spoiled mackerel is eaten, any histamine present is usually inactivated in the stomach and rendered harmless (except in rare cases where certain medicines are being taken). There is evidence, however, that some other, unidentified substance is produced in the spoiling fish along with histamine and this substance causes marked gastrointestinal disturbance.

Measurement of the amount of histamine in fish is used as a guide to the potential of the sample for causing this form of food poisoning, the so-called scombroid poisoning.

To measure histamine a protein-free extract is first prepared; the histamine is separated from interfering substances by extraction first into an organic solvent followed by back extraction into an aqueous solution. The histamine is treated with a substance that gives a fluorescent product and the amount of this product is measured using an instrument called a fluorimeter. Histamine can also be measured by HPLC, along with certain other amines including putrescine and cadaverine; the term “biogenic amines” is often used to describe these substances.

**Physical methods**

The electrical properties of fish skin and muscle change systematically after death and can be used as the basis of an instrument; two models are commercially available. The change in electrical properties is not caused directly by bacterial action or other spoilage mechanism, but the instrumental readings on iced fish can be correlated with the stage of
spoilage, as measured by sensory methods or by one of the non-sensory methods already described. The instruments can be used only on whole fish or fillets with skin. Frozen fish, when thawed, give no response to the meter and this can be used as a basis for checking whether fish have been previously frozen.

What do the numbers mean?
It is not possible to lay down rules for deciding what values of any of the freshness indices should be regarded as indicating any particular stage of spoilage or acceptability. There are differences between species, the kinds of bacteria causing spoilage may vary, and the methods of analysis, as noted for TVB, can affect the values and the mode of handling may influence the results. Ideally, the relationship between the freshness as measured by sensory assessment and the various freshness indices described should be derived for the species of interest, using well-defined methods, and for the particular handling procedure concerned. This is not always done: it is common for a particular level of, say, TMA or TVB to be taken as indicative of an unacceptable degree of spoilage in a range of species and without reference to the handling procedure or the measurement technique.

Purely as a guide to the relative magnitude of certain indices in iced cod, values of hypoxanthine, TMA and TVB, measured by methods used at Torry Research Station, and readings on the Torrymeter (one of the instruments mentioned that measure the electrical properties) are compared in the Table below with the time in ice and a sensory score.

<table>
<thead>
<tr>
<th>Days in ice</th>
<th>Sensory score</th>
<th>Hypoxanthine</th>
<th>TMA</th>
<th>TVB</th>
<th>Torrymeter</th>
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</table>

Days in ice means days in boxes since caught, well iced
Sensory score means the raw odour freshness score on the Torry wet fish scale
Hypoxanthine is measured in mg/100 g flesh
TMA is measured in mg N/100 g flesh
TVB is measured in mg N/100 g flesh (by a method that gives relatively

Other methods
A wide range of compounds arising from bacterial spoilage have been proposed as freshness indices but only those in regular use are described above. Other indices that may be met with include:
- free fatty acids
- indole
- lactate
- putrescine and cadaverine
- tetrazolium test
- volatile fatty acids
- volatile reducing substances.

Details of these analyses are available.

Methods for frozen fish

Dimethylamine (DMA)
Trimethylamine oxide (TMAO), as mentioned above, is decomposed to TMA by bacteria in iced fish. In frozen fish of many species, this reaction is replaced by a slow conversion by an enzyme to dimethylamine and formaldehyde. The amount of DMA produced depends on the storage temperature and time, and can be used as an indirect measure of cold storage deterioration. Some DMA is also produced in the wet fish, so, if the fish is badly spoilt before freezing, the measured DMA will not all have arisen during frozen storage. The value of TMA could be used in this case as a guide to the state of spoilage before freezing: if TMA is high then use of DMA as an index of frozen storage deterioration is less satisfactory.

The best method of measuring DMA is by gas chromatography after a preliminary separation. The method measures both DMA and TMA.

Formaldehyde
Since formaldehyde is produced along with DMA by the enzymic breakdown of TMAO, measurement of formaldehyde is a possible alternative means of assessing the extent of this reaction. As in the case of DMA, the possibility of some formaldehyde having been formed before freezing should be borne in mind.

Formaldehyde can be measured either on an extract or on a distillate of the fish. Formaldehyde combines readily with proteins and other substances in fish; the apparent formaldehyde content as measured by most methods is less than the total amount of formaldehyde formed during frozen
Extractable protein
The main structural protein of muscle, actomyosin, can be dissolved in a salt solution. The changes in the protein that cause toughening of the flesh during poor cold storage also reduce the solubility of the protein, and this reduction can be applied as an indicator of deterioration. The amount of protein that can be extracted from fish depends not only on the solubility of the protein but also on the apparatus and procedure used to extract the protein, the concentration of the salt solution, the ratio of the volume of solution to the weight of fish, and the temperature of extraction. Provided all these remain constant, the amount of protein extracted can be correlated with sensory assessments of toughness.

Peroxide value (PV)
Oxidation of the oil, in oily fish like herring, gives rise to rancid odours and flavours; these can limit the storage life of such species more quickly than the protein changes that govern the extractable protein value. An important stage in the oxidation is the addition of oxygen to the fatty acid molecules to form hydroperoxides; the amount of these can be used as a measure of the extent of oxidation in the early stages. The correct term hydroperoxide value is frequently shortened to peroxide value.

To measure peroxide value the oil must first be extracted from the fish by a method that does not itself encourage further oxidation. The oil containing peroxides is treated with potassium iodide; iodine is liberated and measurement of the amount of iodine enables the peroxide value to be calculated.

Increase in the peroxide value is most useful as an index of the earlier stages of oxidation; as oxidation proceeds the peroxide value can start to fall.

Thiobarbituric acid value (TBA value)
The hydroperoxides, mentioned above, can react further to give a wide range of compounds, some of which are responsible for the rancid odours and flavours in oily fish and for cold storage odours and flavours in white fish. One such compound, called malonaldehyde, and a number of related compounds can be separated from the fish either by distillation or by preparing a protein-free extract. Reaction of these compounds with 2-thiobarbituric acid gives rise to coloured products, the intensity of which, the TBA value, is measured using a spectrophotometer. The increase in the TBA value is a measure of the extent of oxidative deterioration in oily fish, but, as in the case of peroxide value, the TBA value can fall again at a later stage of spoilage.

What do the numbers mean?
It is not possible at present to attempt to relate the indices of deterioration to each other or to sensory test values as was done in the Table earlier for iced fish. There are several reasons. The number of useful tests is small, and different tests are applied to oily and to non-oily fish. The two tests described for oily fish, peroxide value and TBA value, are useful at different stages of oxidation and cannot readily be correlated. Again only two tests, DMA (or the equivalent, formaldehyde) and extractable protein are useful for non-oily fish and insufficient experience has yet accumulated to allow comparison.

Other methods
As in the case of chilled fish, a wide range of methods other than those described have been proposed for estimating deterioration in frozen stored fish. The following may be met with:
  - cell fragility
  - colour test
  - expressible fluid
  - extract release volume
  - fluorescence
  - texture measurements using a variety of instruments
  - thaw drip
  - water holding capacity.

Further information
Further information can be obtained by contacting:

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This Note is one of a new series, prepared by staff at Torry Research Station. New Notes are:

91 Sensory assessment of fish quality
92 Non-sensory assessment of fish quality
93 Handling and processing scad
94 Temperature measurement in the fish industry
95 Who does what - advice for the fish industry

Most Notes in the original series (numbered 1 to 90) are still in print, but are being replaced; a list is available, free of charge.

Copies of all Notes are obtainable, for a small handling charge, from the above address.

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