FRESHNESS, QUALITY AND SAFETY IN SEAFOODS

FLAIR-FLOW EUROPE
TECHNICAL MANUAL
F-FE 380A/00

May 2000

FRESHNESS, QUALITY AND SAFETY

IN SEAFOODS

F-FE 380A/00 [May 2000]

This technical manual was prepared for FLAIR-FLOW EUROPE by:

Paw Dalgaard
Danish Institute for Fisheries Research
Søltofts Plads, DTU Building 221
DK-2800 Kgs
Lyngby, Denmark

For more information on the FLAIR-FLOW EUROPE dissemination system contact Ronan Gormley at Teagasc, The National Food Centre, Dunsinea, Castleknock, Dublin 15, Ireland. Telephone: +353-1-8059500; Fax: +353-1-8059550; E-mail: r.gormley@nfc.teagasc.ie or visit website **http://www.exp.ie/flair.html**

PREFACE

The acronym 'RETUER' was coined by the FLAIR-FLOW dissemination team to denote 'ready-to-use European research' and resulted in a series of RETUER workshops across Europe. The RETUER workshops are aimed at food SMEs, and especially at the small and very small companies. The goal is to bring results from EU-supported food research projects to food SMEs Europe-wide, in an easily understood form, thereby facilitating application and use of the results both in the short and long term. Each workshop carries a series of handouts and these have been collated into five technical manuals with the following titles:

1.   Ready-to-use fruit and vegetables
         [ISBN 1 84170 106 8]

2.   Food processing equipment design and cleanability
         [ISBN 1 84170 107 6]

3.   Managing the cold chain for quality and safety
         [ISBN 1 84170 108 4]

4.   Microbial control in the meat industry
         [ISBN 1 84170 109 2]

5.   Freshness, quality and safety in seafoods
         [ISBN 1 84170 110 6]

Ronan Gormley
Project Leader, FLAIR-FLOW EUROPE
Teagasc, The National Food Centre
Dunsinea, Castleknock
Dublin 15, Ireland
Tel: +353-1-8059500
Fax: +353-1-8059550
E-mail: r.gormley@nfc.teagasc.ie

CONTENTS

Introduction

This document summarises information on freshness, quality and safety in seafood. Information included primarily originates from EU-funded projects [Predictive modelling of shelf life of fish and meat products, AIR2 CT93 1251; Evaluation of fish freshness, AIR3 CT94 2283; Spoilage and safety of cold smoked fish, FAIR PL95 1207; Development, modelling and application of time-temperarture integrator systems to monitor chilled fish quality, FAIR CT95 1090; Development and implementation of a computerised sensory (QIM) system for evaluating fish freshness, FAIR CT97 9063]. In addition, information is included from a series of seafood FLAIR-FLOW 'RE-TU-ER' meetings organized in November 1998 in Lyngby (Denmark), in June 1999 in Dublin (Ireland), in October 1999 in Madrid (Spain) and in November 1999 in Göteborg (Sweden). Consequently, the content of the document does not exclusively reflect the opinion of the author.

Globally, the availability of fish raw material is limited and with increased transportation of seafood it has become most important to minimise seafood losses at all levels from catch and processing to storage and distribution. Improved management and food preservation technologies are also needed. The increasing interest in marketing more natural and less heavily preserved products makes this an important challenge. Like other foodstuffs and pre-packed foods, seafoods must bear a 'data of minimum durability' or, for highly perishable products a 'use by date' (EU Directive 79/112/EEC). Clearly, realistic determination and accurate prediction of shelf life of fresh and lightly preserved seafood are important to meet consumer demands and to comply with legislative requirements.

Evaluation of freshness and quality

It has been stated frequently that no single instrumental method is generally reliable for assessment of freshness and spoilage in seafood. Nevertheless, numerous microbiological, chemical, biochemical, as well as other instrumental methods, are appropriate for this purpose as long as their range of applicability in terms of fish raw material, preserving parameters, and storage conditions are realised and respected. Information on sensory and instrumental methods are given in this section.

To be useful in quality control, responses of instrumental methods should be causally related to sensory changes in seafood or they should at least correlate with sensory analyses. At the same time, the instrumental analyses must be convenient to carry out, rapid and inexpensive. However, when instrumental methods are used for set-up and verification of quality assurance programmes, requirements for ease of use, rapidity and costs are less stringent. Both classical and very rapid instrumental methods are discussed below.

Sensory methods

Sensory methods relying on trained assessors *i.e.* objective sensory methods are required for use in quality control for evaluation of freshness and for determination of remaining shelf life of seafoods.

Regulations in the European Union require freshness grading of most fish to be marketed within the Union (Council Regulation No 103/76 of January 1976; Council Regulation No. 2406/96 of November 1996). Today, freshness grading of fish relies on particular EU schemes and is usually carried out in auctions by trained personnel. Whole and gutted fish are assessed for appearance, odour of skin, outer slime, eyes,

gills and belly cavity. Fish are then placed in the grades Extra (E), A, B or Unfit (C) on the basis of schemes for different groups of species. It has been indicated by scientists that the EU sensory schemes have disadvantages because information on remaining shelf life cannot be obtained directly from the freshness grades, and because the schemes are too complicated and may not be followed in practice.

Table 1. Quality index of cod (*Gadus morhua*).

Quality parameter		Point
General appearance	Surface appearance	0 - 3
	Skin	0 – 1
	Slime	0 – 3
	Stiffness	0 – 1
Eyes	Clarity	0 – 2
	Shape of pupil	0 – 2
Gills	Colour	0 – 2
	Smell	0 – 3
	Slime	0 – 2
Flesh colour	Open surfaces	0 – 2
Blood	In throat cut	0 – 2
Sum of demerit points		

As an alternative to EU schemes, the quality index method (QIM) has been suggested. Originally, QIM was developed at CSIRO in Hobart, Australia, as a sensory method for simple and rapid evaluation of whole or gutted fish of different species. A relatively large number of fish quality attributes are evaluated in sequence by sight, smell and touch. Each attribute is scored from 0 to 3 (typically) by novice or experienced assessors (Table 1) with low scores indicating the best quality. The sum of all attribute scores is called demerit points, or QIM index points, and this value increases linearly with storage time of a given fish. The direct relationship between

QIM scores and storage time makes it easy to calculate remaining shelf life of fresh fish when stored at 0°C (Figure 1).

Recent studies have shown that QIM schemes specific to fish species are sometimes required and are now available for anchovy, brill, cod (fresh and frozen), haddock, flounder, herring, hoki, plaice, redfish, saithe, salmon, sardines, sole, spotted trevalla, turbot, whiting, and four species of warm water fish from Australia. QIM is primarily used in the evaluation of whole and gutted fish. The method is difficult to use with fish fillets and schemes for lightly preserved seafoods are not yet available.
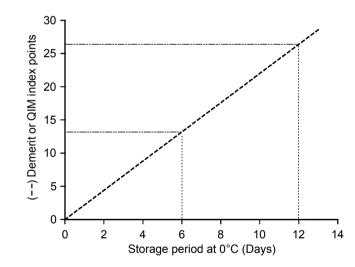

Figure 1. Typical evolution of sensory scores as determined by a QIM scheme like that in Table 1.

To facilitate and speed up the use of QIM in practice, two different application softwares have been developed. Both systems guide the users through QIM schemes, provide pictures to support the rating of various fish quality attributes, and calculate

remaining shelf life at 0°C. The 'QIM Rating System' software was developed at the Danish Institute for Fisheries Research. This internet version of QIM is accessible free of charge at http://www.dfu.min.dk/qim/. Another QIM software 'WiseFresh' has been developed in relation to the project 'Development and implementation of a computerised sensory (QIM) system for evaluating fish freshness' (FAIR CT97 9063). The WiseFresh software will be commercially available in year 2000 from TM Software (http://www.tm-soft.com/). Depending on configuration, a price of approximately 13500 Euros is expected. For further information see http://qimit.rfisk.is/.

A straight line relationship between storage time and QIM score has been determined for many species when stored at 0°C (Figure 1). For a few fish species, the straight line relationships have been observed at different storage temperatures as well. Further studies are required to determine if QIM schemes can be used in general to predict remaining shelf life of products when stored at different temperatures. Particularly, it needs to be determined (i) if QIM scores corresponding to sensory product rejection are independent of storage temperatures, and (ii) if the slopes of QIM score lines can be predicted by available mathematical models for the effect of temperature on product shelf life (See section on 'Prediction of shelf life' page 21).
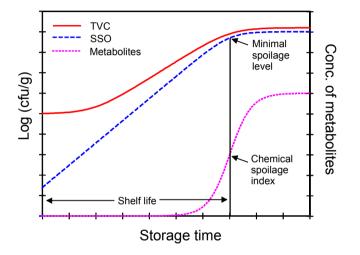
QIM is a promising sensory method and the practical usefulness of this tool would be further increased if new schemes applicable for groups of fish species or products could be developed. It also needs to be noted that different schemes provide different score-values corresponding to the end of product shelf life. This is confusing but the problem can be reduced by expressing the limit of acceptability/end of shelf life of a product as a percentage of the maximum number of demerit or QIM index points used for each scheme.

Apart from EU and QIM schemes, numerous simple sensory systems relying on the assessment of one or several quality attributes (two to ten descriptive terms and corresponding numerical marks have been suggested and have been used in research and in industry over the last decades. A very simple three-grade-system (grade I: no off-flavours, grade 2: slight off-flavours and grade III: strong off-flavours, corresponding to spoilage) has been used frequently for shelf life determination. With this simple sensory method the end of shelf life has been determined as the time when the average percentage of samples in Grade III reached a defined level, *e.g. 50%*. As compared to EU and QIM schemes, the three-grade-system has the advantage that it allows shelf life to be determined for new products where the exact changes in sensory properties with time of storage have not been previously described.

Objective sensory methods are essential for inspection/grading, quality control and shelf life determination in seafoods. At the same time, sensory methods have the disadvantages of being (*i*) expensive and time-consuming, particularly when numerous trained assessors are needed, and (*ii*) difficult to calibrate. Consequently, instrumental methods are needed to supplement sensory assessments, *e.g.* when results are to be used in specifications as part of purchase agreements.

Microbiological methods

About one-third of the world's food production is lost annually as a result of microbial spoilage (Lund *et al.*, 2000). In fact, microbial activity is responsible for spoilage of most fresh and of several lightly preserved seafoods. Possibly for this reason, the total number of microorgansims, named total viable counts (TVC) or aerobic plate counts (APC), have been used in mandatory seafood standards in some European Countries in Japan and in the USA. Furthermore, TVCs are used

extensively in microbiological specifications as part of purchase agreements. However, only a small fraction of the microorganisms present on newly processed seafood is actually of importance for product spoilage. Consequently, TVCs in seafood correlate poorly with the degree of freshness or remaining shelf life as shown below.

Figure 2. Specific spoilage organism (SSO) concept. Typical changes in total viable counts (TVCs), specific spoilage organisms (SSO) and metabolites produced by SSO during storage of fresh seafood.

During storage of seafood at particular conditions of temperature, atmospheres, % salt, a_w, preservatives, etc. specific spoilage organisms (SSO) grow faster than the remaining seafood microflora, and eventually produce the metabolites responsible for off-flavours and sensory product rejection (Figure 2). Consequently, the numbers of SSOs and the concentration of their metabolites can be used as objective quality indices for shelf life determination in seafoods. With SSOs responsible for spoilage, a

close relationship between log-numbers of SSOs and the remaining shelf life must be expected. The corresponding correlation for TVC should be reasonable when low initial TVC values predominate, but less close for higher initial TVC levels. It is also possible to predict shelf life of seafood based on knowledge about initial numbers and growth of SSOs. In relation to the EU-project 'Evaluation of fish freshness', the number of four different SSOs i.e. Shewanella putrefaciens, Photobacterium phosphoreum, Brochothrix thermosphacta and Lactic acid bacteria, were shown to correlate closely (0.90 < corr. coef. < 0.99) with remaining product shelf life, and they always correlated better than did log numbers of TVC. It was concluded that microbiological measurements can be used to evaluate remaining shelf life of fresh fish and when such measurements are needed, numbers of SSOs, whenever known, should be used to supplement or replace classical TVC measurements. Table 2 shows examples of SSOs responsible for spoilage of different seafoods and suggests methods for enumeration of these microorganisms.

Table 2. Examples of specific spoilage organisms (SSO) in different seafoods and of methods for their enumeration.

Product	Typical SSO	Enumeration method
Fresh chilled fish stored in air	Shewanella putrefaciens[1]	Iron Agar Lyngby (20-25°C, 3d)[3]
	Pseudomonas spp.[2]	Cetrimide-Fusidin-Cephaloridine (CFC) agar (25°C, 3d)[4] or by using a conductance method
Fresh chilled fish stored in vacuum or MAP	Photobacterium phosphoreum[1]	Malthus conductance method (15°C, 10-50h)
	Lactic acid bacteria[2]	Nitrite-Actidion-Polymyxin (NAP) agar with pH 6.7 (25°C, 3d)[3]
	Brochothrix thermosphacta[2]	Streptomycin sulphate Thallous Acetate Actidione (STAA) agar (25°C, 2-3d)[4]
Fresh fish stored at > 10-15°C in air	Vibrionaceae, Enterobacteriaceae	- Tryptic Soy Agar (TSA) with overlay of Violet Red Bile Glucose (VRBG) agar (30°C, 48 h)[3]
Cooked and brined MAP shrimps and possibly several other packed lightly preserved seafoods stored at 15-25°C	Enterococcus faecalis	Slanetz & Bartley agar (35/44°C, 48 h)[4]

Figure 3 shows an example of how numbers of SSOs and TVCs correlated with remaining shelf life of modified atmosphere packed cod fillets. One SSO per gram (log cfu/g = 0) corresponded to 18-19 days remaining shelf life whereas similar numbers of TVC corresponded to > 7 weeks remaining shelf life. These latter values are unrealistic due to lack of a causative relationship between TVC and spoilage.
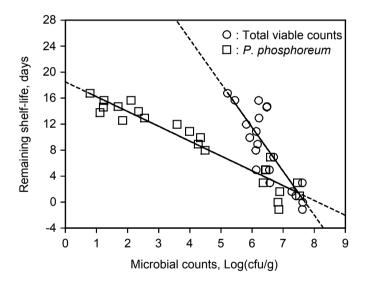

Figure 3. Relationship between remaining shelf life of modified atmosphere packed cod fillets and log-numbers of (i) TVC and of (ii) the SSO, *Photobacterium phosphoreum* (Modified from Dalgaard, 2000 with permission from the publisher).

For enumeration of TVC in fresh and lightly preserved seafood, spread plating on pre-chilled plates of Long & Hammers agar with 1% NaCl and subsequent incubation

at 15°C for 5-7 days is a useful general procedure. Many marine microorganisms in seafood are psychrotolerant, heat labile and require NaCl for growth. Consequently, pour plating with agar at about 45 °C, or the use of standard plate count agar without NaCl, or the incubation of plates at 30-37°C must be avoided when TVCs in seafood are determined. This recommendation has been supported recently by numerous experiments carried out within different EU-projects (AIR3 CT94 2283, FAIR PL95 1207, FAIR-PL95-1090).

Establishment of critical TVC and SSO limits corresponding to sensorically unacceptable seafood can be difficult. One reason for this is related to the fact that microbiological sampling from fish skin or product surfaces results in very high TVC levels as compared to samples from fish flesh. When surface sampling is used, levels of TVC and of SSO below 10^7 cfu/g will not usually be appropriate as critical limits. As shown in Figure 3 for example, MAP cod fillets with TVC of 10^6 cfu/g, can have remaining shelf life of 1-2 weeks at 0°C, and such products are of excellent sensory quality. Furthermore, TVC in lightly preserved seafoods such as cold-smoked salmon can remain at about 10^7 cfu/g for 1-2 weeks, or more, in products of acceptable sensory quality.

The microbiological methods indicated in this section are slow, with results being available after 10 h to 5 days. Modern microbiological methods, *e.g.* relying on the polymerase chain reaction (PCR), oligonucleotide probes, or antibody techniques can provide responses within one working day. However, these methods are unlikely to be used for practical seafood shelf life evaluation in the near future due to (i) lack of sensitivity when applied in food, (ii) qualitative or semi-quantitative responses, and (iii) high cost. Slow microbiological methods are inappropriate for on-line or at-line measurements in seafood processing. However, specific and sensitive, but not necessarily rapid, microbiological methods can be used to determine typical levels of

contamination and rates of growth of SSOs in products, *e.g.* as a function of storage temperature. Once, this type of information has been generated, the primary factor to be measured on- or at-line in quality control will be the product temperature; dataloggers are available at reasonable prices for this purpose.

Chemical, biochemical and other instrumental methods

Changes in seafoods during storage due to microbial activity, autolytic enzymes or chemical reactions can be useful indices of quality or spoilage.

K- and K_i-values. Adenosine triphosphate (ATP) is degraded into adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx) during processing and storage of fresh and lightly preserved seafood. IMP is formed by autolytic enzymes whereas spoilage bacteria contribute to Ino and Hx formation. Hx has a bitter taste which may be part of the off-flavour in stale fish. The K-value (Eqn. 1) was suggested by Japanese researchers in 1959 as an objective index of fish freshness. In Japan, a K-value of 20% is used as a critical limit for fish to be consumed raw. In most fish, K-values increase linearly during the first days of chilled storage and it is often an excellent index of freshness. However, the K-value cannot be used in general as an index of spoilage because maximum values, for many fish species, are reached long before sensory rejection. It may be worth mentioning that K-values in lightly preserved seafoods, e.g. cold-smoked salmon and cooked and brined shrimps, can be above 20% even for newly processed products of high sensory quality.

$$K-value \;=\; \frac{[Ino]+[Hx]}{[ATP]+[ADP]+[AMP]+[IMP]+[Ino]+[Hx]} \times 100 \qquad \text{Eqn. 1}$$

Ratios of catabolites like the K-value have been indicated to be less prone to fish-to-fish or species-to-species variability than single compound quality indices. However, extensive analyses are required. The K_i-value = ([Ino] + [Hx])x 100/([IMP] + [Ino] + [Hx]) which is quantitatively similar to the K-value reduces this problem. Use of the Hx concentration or the Hx-index = log([Hx] + 5) alone has also been suggested as a useful quality index in specific seafoods.

Concentrations of the adenine nucleotides required for calculation of The K- and K_i-value can be determined in seafood by chromatographic methods. Research into new biosensors for simple and rapid determinations of adenine nucleotides have been substantial in recent years and they may soon be available for practical use in process control and seafood inspection. K- and K_i-values have not been included in regulations of the European Union.

TVB-N, TMA and other volatile amines. In seafood, total volatile basic nitrogen (TVB-N) primarily includes trimethylamine (TMA), ammonia, and dimethylamine (DMA). Each of these compounds, as well as total levels of TVB-N, are useful indices of spoilage in different fresh and lightly preserved seafood. The European Commission (Council Regulation No. 95/149/EEC of March 1995) specified TVB-N to be used if sensory evaluation indicates doubt about freshness of different fish species. Critical limits of 25, 30 and 35 mg-TVB-N/100g were established for different groups of fish species. In processed, lightly or semi preserved, seafood levels of TVB-N at sensory product rejection are more variable. For example, 30-40 mg-TVB-N/100g has been found at sensory rejection of sliced vacuum packed cold-smoked salmon but lower levels of 10-20 mg-TVB-N/100g were determined in modified atmosphere packed, cooked and brined shrimps. However, in sugar salted herring as much as 75 mg-N TVB-N/100g was determined in products of acceptable

sensory quality. These variable levels of TVB-N at time of sensory rejection support the view that off-flavours in spoiling seafoods cannot be caused by TVB-N alone.

TMA is a microbial metabolite and it can only be used as an index of spoilage and not as an index of freshness (Figure 2). Development of TMA in seafood depends primarily on the content of the substrate trimethylamine-oxide (TMAO) in the fish raw material. Most marine animals contain TMAO with levels being generally high in elasmobranches and in deep-sea fish species. Many fresh water fish do not contain TMAO but some species like Nile Perch and Tilapia contain TMAO, and many other species have not been analysed for this compound. It is also noteworthy that the TMA concentration at sensory rejection of cod depends on the storage conditions. In modified atmosphere packed cod fillets, where spoilage is caused by *P. phosphoreum*, 30 mg-N TMA/100 g is detected at sensory rejection whereas in aerobically stored cod, spoiled by *S. putrefaciens*, lower levels of TMA are detected at sensory rejection.

In seafood with high contents of free amino acids, *e.g.* squid, crustaceans and some dark fleshed fish like herring, substantial amounts of ammonia can be formed during chill storage. The formation of DMA from TMAO is due to the indigenous enzyme TMAO dimethylase. This is a slow process but DMA can be a useful index of spoilage, *e.g.* in frozen hake.

Biogenic amines. Many seafood spoilage bacteria produce one or more of the biogenic amines agmatine, cadaverine, histamine, putrescine, spermidine, spermine, and tyramine. Biogenic amines are heat stable, and are therefore appropriate for the evaluation of freshness of raw material used in canned products. Production of biogenic amines in seafood depends on concentrations of the free amino acid substrates and is, therefore, strongly species dependent. A quality index = (mg/kg histamine + mg/kg putrescine + mg/kg cadaverine)/(1 + mg/kg spermidine + mg/kg

spermine) was suggested and validated for grading of canned tuna, rockfish fillets, lobster tails and fresh salmon steaks. More recently, a different biogenic amine index, BAI = mg/kg histamine + mg/kg cadaverine + mg/kg tyrosine + mg/kg putrescine, was found to be more valuable for the grading of tuna. End of shelf life was defined as a BAI value of 50 mg/kg. Finally, a new multiple compound quality index relying on biogenic amines and pH has been developed recently for vacuum packed cold-smoked salmon. For determination of biogenic amines, research into biosensors has also been considerable. The practical importance of quality indices relying on biogenic amines will increase substantially if such biosensors could replace the more cumbersome chromatographic techniques previously used for the detection of these amines.

Volatiles, visual spoilage indicators, and electronic noses. On the basis of numerous (~ 40) volatile compounds, determined by gas chromatography, it has been possible to grade fresh and cold-smoked salmon products in agreement with sensory analyses. Recent results further suggest that multivariate statistical methods allow identification of a limited number of the most important compounds. These volatile compounds can then be determined by visual spoilage indicators or electronic noses and in this way be used as indicators of seafood spoilage. FreshTag™ (Cox Technologies, USA, http://www.coxtechnologies.com/) is one example of an adhesive colour indicator for the detection of volatile amines in packed seafood. This type of visual spoilage indicator may be particularly useful for modified atmosphere packed seafood where consumers cannot otherwise evaluate off-flavour at the moment of purchase in the supermarket. It seems likely that gas-phase-biosensors-technology will be used in the future to develop other visual indicators for the detection of volatile compounds, in seafoods, e.g. sulphur compounds, acids, alcohols, aldehydes and ketones.

Gas sensor array systems, also known as 'electronic noses', respond to specific or groups of volatile compounds and may be used for the evaluation of food spoilage. These measurements require little sample preparation and time of analysis is relatively short, approximately 10-90 min. Different gas sensors including electrochemical, metal oxide and organic polymers are available. Responses of electrochemical and conducting polymer sensors have been shown to correlate with other objective measures of quality in fresh seafood. However, the stability of correlation between sensory data and electronic nose response still represents a problem for practical application of gas sensors in seafood shelf life evaluation.

Other instrumental methods. Measurements of dielectric properties have been tested and used during almost 40 years for quality grading and remaining shelf life determination of various fish. The Intellectron Fishtester, the Torrymeter and the RT-Freshtester represent instruments with increasing degrees of sophistication. Readings from all instruments reflect di-electrical properties of fish and they decrease with storage time, almost following a straight line. Based on these rapid and non-destructive measurements, the RT-Freshtester allows automatic grading of 60-70 fish per minute. Electrical properties of fish are not directly responsible for sensory spoilage and it is, therefore, to be expected that numerous factors influence the relationship between such measurements and seafood spoilage. In fact, these instruments need calibration depending on the season and fish handling procedures, and they are unsuitable for grading frozen/thawed fish, partially frozen i.e. superchilled fish, fish chilled in refrigerated seawater, or for fish fillets. This and the high cost of the instruments limits their practical use in the seafood sector for freshness evaluation. However, electrical measurements can also be used to test if fish was previously frozen.

Near infra-red (NIR) diffuse reflectance is another technology where measurements are rapid and have potential for on-line quality grading. This technology is useful for indirect measurement of, e.g. oil, water, and water-holding capacity in different fish. Nevertheless, it remains uncertain if the accuracy will allow it to be used for freshness grading of different seafoods.

Very rapid instrumental methods, as discussed above, may be used for freshness grading as part of on-line process control. However, fish freshness also can be determined by a traceability system with recording of product temperature from the moment of catch. This alternative, in many situations, is easier to manage, more cost efficient, and certainly more reliable.

Prediction of shelf life

By using the objective sensory and/or instrumental analyses outlined above, the degree of freshness or remaining shelf life at a constant storage temperature can be determined for many seafoods. However, the initial remaining shelf life value is of little use to determine condition of the products when they eventually reach consumers, unless changes in measured quality attributes can be predicted as a function of product characteristics and storage conditions.

The transportation of seafood and fish raw material is increasing globally and so is the need to predict effects of storage and distribution conditions on product shelf life. Empirical models for relative rates of seafood spoilage, and microbial models for

growth of specific spoilage organisms (SSOs) can be used to predict shelf life at constant and fluctuating storage temperatures as described below:

Empirical models for relative rates of spoilage

Shelf life of different types of fresh and lightly preserved seafood varies substantially (Table 3). Despite this, it is possible to develop simple and entirely empirical relative-rate-of-spoilage-models to predict shelf life of different seafoods at various storage temperatures.

The relative rate of spoilage (RRS) has been defined as the keeping time at 0°C divided by the keeping time at T(°C). Mathematical RRS models are convenient as they allow shelf life to be predicted at different storage temperatures from the shelf life at a single known temperature. Furthermore, RRS models are easy to use and require no knowledge about the types of reaction causing product spoilage. The first RRS model was developed in Scotland more than 35 years ago. This linear model included the effect of temperatures between minus 1°C and +25°C on the shelf life of cod. Today, the square root spoilage model (Eqn. 2) is the most popular for the effect of temperatures on shelf life of different fresh seafoods from cold and temperate waters. This model has been successfully validated for products stored between –3°C and +15°C.

$$Shelf\ life\ at\ T(°C) \quad = \quad \frac{Shelf\ life\ at\ 0°C}{[1 + 0.1 \times T(°C)]^2} \qquad \text{Eqn. 2}$$

Fresh tropical fish typically keep longer at 0°C than fresh fish from cold and temperate waters. The effect of temperature on shelf life is also different for the two types of products (Table 3). An exponential (Exp) tropical spoilage model has been developed for shelf life prediction of these products at different temperatures (Eqn. 3).

$$Shelf\ life\ at\ T(°C) \quad = \quad \frac{Shelf\ life\ at\ 0°C}{Exp[0.12 \times T(°C)]} \qquad \text{Eqn. 3}$$

As compared to fresh seafood, the effect of temperature on shelf life of lightly preserved seafood is more variable and less well described. Temperature influences the RRS of cooked and brined MAP shrimps much more than previously observed with fresh seafoods (Eqn. 4). However, for hot smoked cod and mackerel, RRS is much less influenced by temperature than found with fresh seafood (Table 3). The reference temperature of 0°C which is applied for the calculation of RRS in fresh fish may be inappropriate for lightly preserved products, and a different reference temperature (T_{ref}) of, for example, 5°C can be used as shown in Eqn. 4.

$$Shelf\ life\ at\ T(°C) \quad = \quad \frac{Shelf\ life\ at\ T_{ref}(°C)}{Exp[0.15 \times (T - T_{ref}(°C))]} \qquad \text{Eqn. 4}$$

Models for growth of specific spoilage organisms (SSOs)

Mathematical models for the effect of temperature, atmosphere and water activity on the growth of different seafood SSOs are available. These can be used for shelf life prediction of seafood stored at constant and fluctuating temperatures. However, this requires knowledge about the approximate initial numbers of SSOs and of their spoilage domain, *i.e.* the range of conditions within which the particular SSO caused product spoilage (see Table 2). Models for the effect of temperature on the growth of *S. putrefaciens* and for the effect of temperature and carbon dioxide concentrations on *P. phosphoreum* have been developed and, validated in seafood, and are included in the Seafood Spoilage Predictor (SSP) software (see Figure 4). Validated models

for the effect of temperature on the growth of *Pseudomonas* spp are also available. Models for the growth of *B. thermosphacta* and lactic acid bacteria have been suggested, but have not yet been validated in seafood.

TABLE 3. Shelf life of packed and unpacked seafoods at different temperatures. Modified from dalgaard (2000) with permission from the publisher.

Product	Storage temperature (°C)	Packaging	Shelf life (days)
Fresh seafood			
White-fleshed, average size, and caught in temperate and cold waters	0	air	12 - 18
Cod	-2	air	26
Cod	-2.5	MAP[a]	36
Caught in warm waters	0	air	18 - 35
Tilapia	4	MAP	> 25
Large halibut, tuna and similar fish	0	air	21- 22
Salmon	0	VP[b]	19
Dark-fleshed, small fish			
Low fat	0	air	6 - 9
High fat with large amounts of feed	0	air	4 - 6
Shellfish, temperate and cold waters	0	air	6 - 10
Pink shrimps	0	air	3 – 4
Shellfish, warm waters	0	air	8 – 12
Lightly preserved			
Cold-smoked salmon	5	VP	20 – 56
Hot smoked cod (lightly salted)	0.5	air	24 – 26
	12	air	17 – 18
Hot smoked mackerel (lightly salted)	0	MAP	8
	10	MAP	5 – 6
Brined roe (lump fish)	5	VP	75 – 90
Cooked and brined shrimps	0	MAP	230 - >308
	25	MAP	4 – 6
Sous vide cooked			
Cod	0	VP	28
Cod fillets	3	VP	15 – 21
Mussels	4	VP	> 21
Salmon	4	VP	15 – 21
Fermented			
Som fak, low-salt, from Thailand	4	air	18
Som fak, low-salt, from Thailand	29	air	7
Semi-preserved and preserved			
Cod (salted)	0	air	500
Sugar salted herring	5	brine	>360

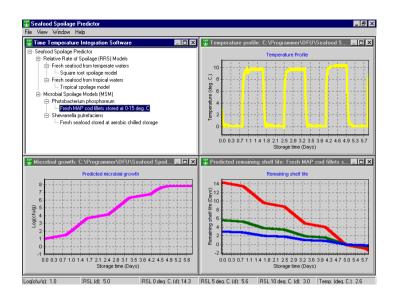[A] MODIFIED ATMOSPHERE PACKED
[B] VACUUM PACKED

Figure 4. Output from the Seafood Spoilage Predictor (SSP) software. Effect of a temperature profile (upper right window) on microbial growth (lower left window) and on remaining shelf life at 0, 5 and 10°C (lower right window).

Seafood Spoilage Predictor (SSP) software and time-temperature integration

Inclusion of mathematical shelf life models in computer software, or in automated devices, for shelf life prediction, substantially increases the practical usefulness of the models. Previously, different time-temperature integration devices have been developed based on the square root spoilage model (Eqn. 2), but these instruments are no longer commercially available.

More recently, the Seafood Spoilage Predictor (SSP) software was developed specifically to predict shelf life of fish and fishery products at constant and fluctuating storage temperatures. SSP version 1.1 contains two RRS models (Eqn. 2 and 3) and models for growth of *P. phosphoreum* and *S. putrefaciens*. This software can evaluate simple temperature profiles entered manually into the software as well as product temperature profiles recorded by several different types of temperature loggers. Remaining shelf life at 0, 5 and 10°C, and microbial growth are predicted by the software as shown in Figure 4. The SSP can be used in different ways, *e.g.* to predict the effect on shelf life of different assumed temperature profiles corresponding to likely conditions of storage and distribution. It is also possible, from actual product temperature profiles recorded by temperature loggers during distribution, to use the SSP for rapid calculation of microbial growth and/or remaining shelf life at any time points. Realistic 'date of minimum durability' can then be determined and it can be decided if improved storage and distribution conditions are needed. The SSP was developed at the Danish Institute for Fisheries Research (DIFRES) in Lyngby and the software can be downloaded free of charge from the homepage of the microbiology group at DIFRES (http://www.dfu.min.dk/micro/ssp/).

The Food Spoilage Predictor (FSP) software includes a model for the growth of pseudomonas and it allows shelf life of some seafoods to be predicted (Table 2). This software was developed at the University of Tasmania, Australia. The FSP can read and evaluate product temperature profiles recorded by Gemini Data Loggers like Tinytag® and is commercially available at a price of ~350 Euros from Hastings Data Loggers (http://www.hdl.com.au/).

Time-temperature integration (TTI) tags are another type of device for studying the effect of product storage temperatures on shelf life. TTI tags are typically simple, inexpensive devices, showing an easily measurable change that reflects their temperature history. They are available as adhesive labels to be placed in contact with individually or bulk packed food. The storage temperature influences the shelf life of different fresh and lightly preserved seafoods very differently as discussed above, and so the selection of TTI tags with appropriate characteristics is highly important for successful application of this technology. TTI tags from VITSAB® (Visual Indicator Tag Systems AB) and MonitorMark^{TM}, and the Smart Label version from 3M^{TM} can be used with many fresh and lightly preserved seafoods, except with products having a very long shelf life (see Table 3).

Application of mathematical shelf life models and TTI tags is increasing in the fishery sector although these techniques are still not frequently used. However, the focus on traceability in food manufacturing is increasing and loggers for collection of temperature data are becoming both cheaper and more powerful. The combined use of traceability systems and mathematical shelf life models will reduce the need for costly product evaluation by sensory and instrumental methods and so mathematical shelf life models will be used substantially in the future within the seafood sector.

Processing for increased safety and extended shelf life

Prediction of safety

Assurance of seafood safety includes numerous factors and considerations, and only a few are mentioned here. Food safety cannot be assured by inspection alone and knowledge about factors that influence growth, survival and inactivation of pathogenic microorganisms is an essential element in the design of processing, storage and distribution systems that provide safe seafoods. Over the last decade numerous mathematical models for the effect of environmental factors on

growth/probality of growth, and the survival or inactivation of various pathogenic microorganisms have been developed and included in software packages. The pathogen modeling program, developed by the usda in philadelphia, can be downloaded free of charge (http://www.arserrc.gov/mfs/_pathogen.htm/), and the food micromodel, developed by maff in the uk, is commercially available at approximately 800 euros/year (http://www.lfra.co.uk/lfra/micromod.html). These software packages include models for a substantial number of pathogenic micro-organisms. The combined effect of environmental factors such as temperature, water activity, pH, organic acids and atmospheres can be predicted. Successfully validated models (see below) are useful in product development, haccp-plan development, and for the assessment of risks. However, the programmes do not yet allow the effect of fluctuating storage conditions to be evaluated.

Successful product validation, *i.e.* comparison of predicted and observed responses in seafood, is most important for the practical application of predictive models. Differences between observed and predicted growth of a pathogen can be quantified by the so-called bias- and accuracy factors. These indices of performance of predictive models are convenient and enable the users of models to verify that (i) the model has been validated in a product with similar microbial ecology, and (ii) that the bias factor in the validation studies was within the range 0.75 –1.25.

Sous vide cooking

This technology can produce minimally processed seafoods with a fresh appearance together with some extension of shelf life. Sous vide cooking is defined as 'Raw materials that are cooked under controlled conditions of temperature and time inside heat-stable vacuumized pouches'. To obtain a substantial reduction in numbers of the pathogenic microorganism *Clostridium botulinum,* heat treatments equivalent to 90°C for 10 min in the centre of products have been recommended (Harmonization of safety criteria for minimally processed foods, FAIR CT96-1020, http://www.alma.kuleuven.ac.be/onderzoek/harmony). Such heat treatments are used with blue mussels where a considerable extension of shelf life has been obtained (Table 3). However, sensory properties of fish like cod and salmon are substantially reduced by this amount of heat treatment. To obtain good sensory properties in fish, sous vide cooking at 65-75°C is required and this heat treatment does not inactivate all pathogenic and spoilage bacteria. Consequently, sous vide cooked seafoods require the same strict control of chill temperatures during distribution as fresh seafood, and only a modest extension of shelf life should be expected (Table 3).

Targeted inhibition of specific spoilage organisms

Another new approach for the development of mildly preserved seafood with extended shelf life originates from the SSO concept (Figure 2). Mild preservation procedures that reduce the growth of SSOs will extend shelf life even if the treatment does not influence the other microflora. This approach, *i.e.* targeted inhibition of SSOs, has been used to extend the shelf life of MAP cod fillets. The SSO, *P. phosphoreum*, was inhibited by low levels of antimicrobials in one study and by freezing in another. Compared to fresh MAP cod fillets, shelf life of thawed MAP cod fillets was extended from 11-12 days to more than 20 days at 2°C. In addition, products could be stored and distributed in the frozen state which provides flexibility for the choice of species and origin of fish to be sold as MAP products in supermarkets. With increased interest in mild and natural preservation methods, it seems logical to use various other procedures for targeted inhibition of SSOs in seafoods.

Concluding remarks

This manual summarises information on methods for the **determination** and **prediction** of shelf life in seafoods and provides examples of methods and techniques for shelf life **extension** in seafoods. Numerous methods for the determination, prediction and extension of shelf life in seafoods, and models for prediction of safety, are available but the range of applicability of many of the techniques is poorly described with respect to fish raw material, product characteristics and storage conditions. To use these methods in a more efficient way in the future, new information on the growth of microorganisms in seafood, on seafood spoilage reactions, and particularly on spoilage domains of specific spoilage organisms must be collected; underutilised species should be included in these studies in view of their likely future importance.

Recent literature for further details

Year 2000:

Dalgaard, P. Fresh and lightly preserved seafood. In: *Shelf life Evaluation of Foods*. 2nd edition. Man, C.M.D. and Jones, A.A. (Eds.) Aspen Publishing Inc. Gaithersburg, Maryland, USA. pp. 110-139.

Gram, L. and Huss. H.H. Fresh and processed fish and shellfish. In: *The Microbiological Safety and Quality of Foods*. Lund, B.M., A.C. Baird-Parker and G.W. Gould (eds). Aspen Publishers, Inc. Gaithersburg, Maryland, USA, pp. 472-506.

Lund, B.M., Baird-Parker, A.C. and Gould G.W. *The Microbiological Safety and Quality of Foods*. Aspen Publishers, Inc. Gaithersburg, Maryland, USA, 1885 p.

Year 1998:

Olafdottir, G, Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdottir, E. and Heia, K. *Methods to Determine the Freshness of Fish in Research and Industry.* Int. Inst. Refrig. Paris, France, 396 p.

Year 1997 and before:

Luten, J.B., Børresen, T. and Oehlenschläger, J. (1997) *Seafood from Producer to Consumer, Integrated Approach to Quality*. Developments in Food Science 38. Amsterdam: Elsevier, 712 p.

Ashie, I.N.A., Smith, J.P. and Simpson, B.K. (1996) Spoilage and shelf life extension of fresh fish and shellfish. *Cri. Rev. Food Sci. Nut.* 36, 87-121.

Huss, H.H. (1995) *Quality and quality changes in fresh fish*, Rome: FAO Fisheries Technical Paper No. 348. 195 p.

Huss, H.H. (1994) *Assurance of Seafood Quality*, Rome: FAO Fisheries Technical Paper, No. 334. 169 p.

Colby, J.-W., Enriquez-Ibarra, L.G. and Flick, G.J. (1993) Shelf life of fish and shellfish. In: *Shelf life studies of foods and beverages*, Charalambous, G., (Ed.) Elsevier, Amsterdam. pp. 85-143.

Acknowledgements