THE EFFECTS OF HIGH SUB-ZERO STORAGE TEMPERATURES ON THE QUALITY OF FROZEN HAKE (MERLUCCIUS MERLUCCIUS)

by

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Abstract

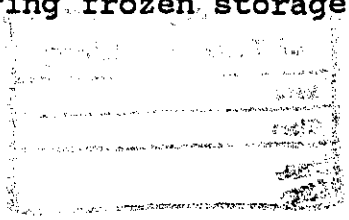
Frozen hake (Merluccius merluccius) is highly susceptible to cold storage damage, and its quality may fall to unacceptably low levels, particularly as a consequence of the development of tough texture. This is a major problem for the fish supply industry in Portugal, the author's home country. The present work aimed to elucidate the mechanism of such deterioration, and was based on the hypothesis that it is promoted by autolytic processes occurring during frozen storage at temperatures above those normally recommended. During distribution and retail display, the correct storage temperatures are often disregarded and frozen fish can be subjected to temperatures in the range of -5°C to -15°C, often with fluctuating temperatures within that range.

In the present work, frozen hake was stored at constant temperatures of -6°C and -11°C, and the effect of temperature fluctuation was assessed using a daily fluctuation between -4°C and -16°C. Fish were also stored at -60°C as controls. In order to evaluate the reliability of methods for assessing quality under such conditions, the fish was assessed for some of the methods most used in similar research. The parameters selected for use in the storage experiments were: protein extractability, free fatty acid, trimethylamine and dimethylamine content and objective measurement of texture, coupled with sensory evaluation.

Free fatty acid content proved to be the best chemical way of assessing the quality of frozen hake, and the use of the Instron for objective textural measurements also proved to be useful.

Storage temperature played an important role in determining the storage life of frozen hake, but fluctuating temperature had little effect.

The amounts of DMA produced were negligible, even in the poorest quality samples, and hence it can be concluded that the formation of formaldehyde, concomitant with the production of DMA, is not a major cause of quality loss. The correlation between free fatty acid and sensory assessment suggests that the build-up of free fatty acids may be an important factor in textural deterioration during frozen storage of this species of hake.



To Bertrand, with love.

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1. Introduction

Freezing is now a well established food preservation technique throughout the world. Its effects on the characteristics of the frozen products have been intensively studied, however such studies have been mainly carried out in those countries which have high technology food industries. In the case of frozen fish, most research on the effects of freezing and cold storage conditions has been undertaken either in the United States, the United Kingdom or Japan.

As a consequence, the most studied fish species are those from the North Atlantic and North Pacific, and the environmental conditions that have received greatest attention are those occurring in cold climates (in spite of the great variety of weather conditions in the U.S.A.). It is not surprising therefore that cod (*Gadus morhua*) is by far the most studied fish among the lean fish, and herring (*Clupea harengus*) and mackerel (*Scomber scombrus* and *S. japonicus*) the most studied among the fatty fish.

Species characteristics are, however, very important in determining the behaviour of fish during freezing and frozen storage and, compared with the relatively small number of species landed from Northern waters, there is now a very wide range of fish species landed from warmer waters. This is partially as a consequence of stock exhaustion of many cold water species. With the recent development of many new fishery industries in developing tropical countries, some warmer water fish species have now been studied, and also the implications of much warmer environmental conditions on the normal storage life and quality of frozen fish has received attention.

However, many problems concerning frozen fish still remain to be solved in countries which fall between the two climatic extremes. Several countries with mild climatic conditions (e.g. Mediterranean climates) catch fish simultaneously in cold, temperate and warm waters, and transport and retail their frozen fish through environmental conditions which approach those of the coldest climates during the winter, and are not very far from those of the tropical countries in the summer. The effect of ambient temperatures on the "cold chain" temperatures tends to be underestimated in these countries, since it is not as drastic as in tropical climates, and frozen fish can often be seen in retail stores exposed in open freezers with the top layers completely unprotected from exterior heat damage.

Frozen fish submitted to such conditions might suffer from high frozen temperatures and even thawing and refreezing. Such high sub-zero storage temperatures, particularly those in the range between -5°C and -10°C, are known to be critical in terms of enzymatic activity and protein denaturation. Storage at these temperatures has not been widely studied, because it is generally assumed that they occur only very rarely during frozen fish storage. This is not the case however in the intermediate climate countries referred to here.

Ideally, such storage conditions should not be permitted and the recommended temperature of -30°C for long term commercial frozen storage should be enforced. However it will take a great many years to institute such changes and, in the meantime, it is important to know how such storage conditions can influence frozen fish quality, in order to be able to assess the scale of the problem, and to try to alleviate the effects.

2. Literature survey

2.1 Fish preservation by freezing

2.1.1 General aspects

Although freezing of foodstuffs has been utilised by the inhabitants of Earth's coldest regions probably since the Stone Age, frozen foods as we perceive them nowadays, i.e., products which keep most of their "fresh" characteristics over several months of storage, are quite a recent idea.

The commercial freezing of fish was first attempted in the middle of the 19th century, first in the United States of America, and then in the United Kingdom, but the products obtained were so different from the fresh products that it took almost a century for the process to be widely accepted by the consumer. Nowadays, good quality frozen fish is nearly indistinguishable from recently caught "fresh" fish. Freezing appears to be the most acceptable fish preservation method, capable of offering to the consumer a good quality product, no matter the season nor how far from the fishing grounds.

However, freezing can never improve the quality of the raw material and only truly fresh, good quality fish should be frozen. Often this is achieved by freezing at sea, or inshore but as near the catching point as possible, using ice whenever necessary to keep the fish cool until it can be frozen (Greenwood-Barton and Cole, 1966).

Following the end of World War II, the world fishing industry made rapid progress, as a consequence of several factors, such as: the good biological condition of international fishing grounds, the freedom of access to those grounds, and the utilisation of new fishing technologies, including the use of

diesel motors, fish detection equipment and new net designs.

Following closely on this boom in the fishing industry, expansion took place in the fish freezing industry, in two different ways. On one hand, freezing plants were built in big fishing ports, allowing the fish to be frozen as soon as it was landed, and then to be distributed in the frozen state; on the other hand, freezing factory boats were built, which permitted much longer fishing trips during which the fish was prepared and frozen and then landed in the frozen state. In both cases the existence of so called "cold chains" was necessary to make possible the inland distribution of the frozen fish in good conditions.

This "golden age" of the fishing and fish freezing industries did not last long and by the late sixties and early seventies, the world fish production rates suffered a dramatic decrease, due mainly to fishing ground exhaustion by overcatching, inevitably followed by a decrease in the growth of the freezing industry.

In spite of this, the consumption of frozen fish, according to FAO statistics, has consistently continued to increase at the expense of other preservation techniques (FAO, 1984).

2.1.2 Fish freezing in Portugal

Portugal has an extensive and storm-free coast, with rich fishing grounds nearby and, by virtue of this, has been able to develop a large fishing industry. In the second half of the 19th century, the industry consisted mainly of tuna fishing near the Algarve coast, cod fishing in the North Atlantic and fishing by trawl on the North African coast for mixed fish species. In addition, a large fleet of artisanal fishing boats fished near the whole coast mainly for small pelagic fish.

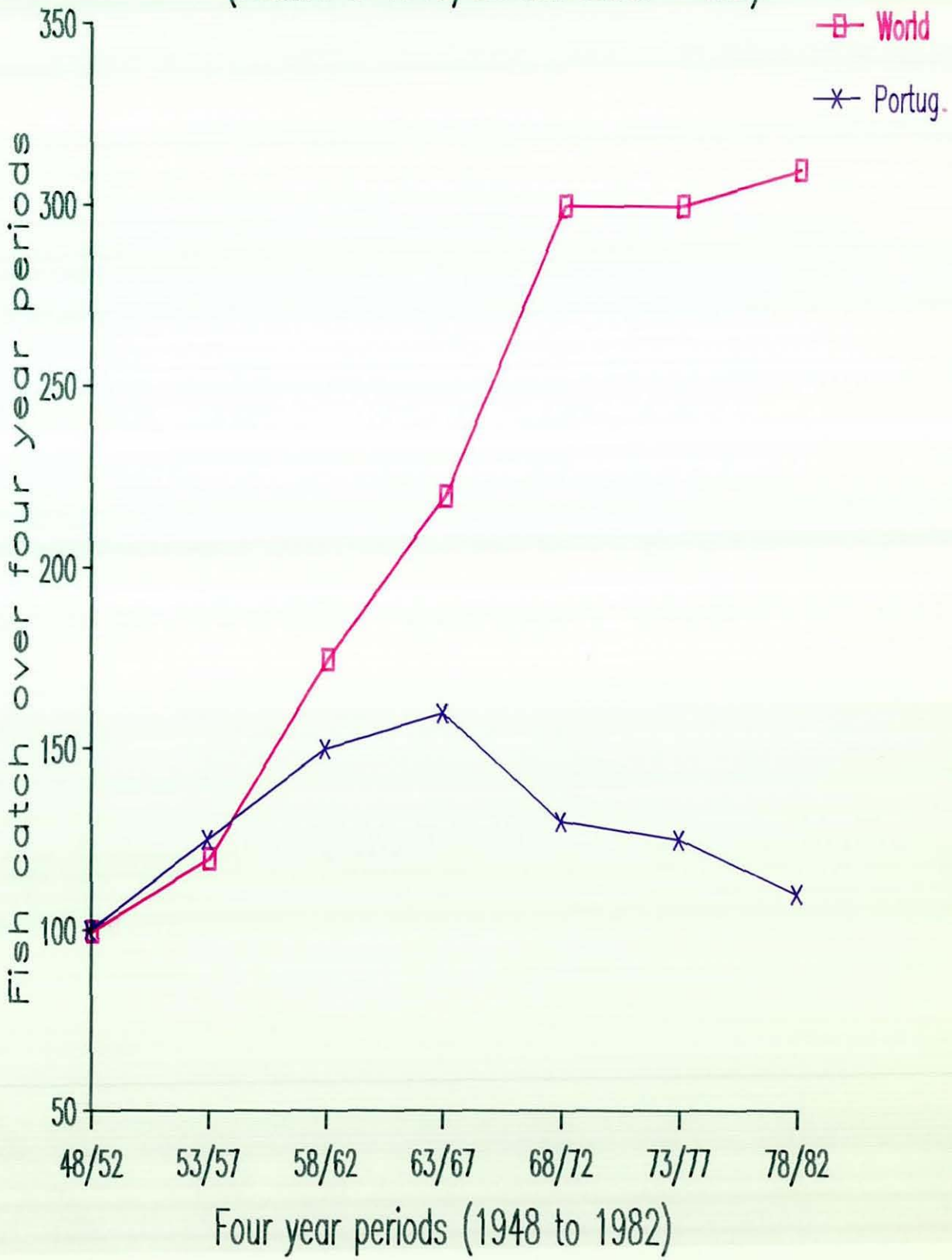
As in the rest of the world, in Portugal there was an expansion after the end of World War II, due mainly to an increased industrialisation of the fisheries, especially distant waters fisheries, but this was followed by a decline in the early sixties (see Fig. 1).

In 1967, a new fleet began fishing in distant waters which had not previously been fished, such as the coasts of South Africa and Angola. This fleet consisted of modern freezer ships, capable of a production of 20 to 25 tons of frozen hake. This, however was not enough to stem the overall decline in fish production, in Portugal. It would appear that the fish populations could not withstand the greater catching capability of the new technologies. In Portugal, where consistent economic control of the fishery industry was absent, the effects of such ground depletion rapidly became apparent.

For the coastal fisheries, the evolution was slightly different, since sardine (Sardinella sp.), mackerel and horse mackerel (Trachurus sp.) stocks remained in good condition until

Fig.1: World and Portuguese fishery production

(relative to 1948/52 fish catch = 100)



1965, and in the case of sardine, after a decline over 5 years, production rose again in 1970.

In spite of the overall decline in fish production, Portugal has a high per capita fish consumption, occupying the 8th place in the world consumption tables, above all the EEC members, with the single exception of Denmark (Leal, 1984). Statistics up to 1977 indicate that fish contributes about 13 to 14% of protein consumption and 30% of animal protein consumption in Portugal, with the declining production by the Portuguese industry being compensated for by imports.

Freezing is the only fish preservation technique that is increasing in capacity in Portugal. More than 60 000 metric tonnes of raw material are processed annually and the proportion of frozen fish has now reached 25% of the total fish consumption. Frozen fish imports are about equal to the national production.

Frozen fish has been widely accepted, particularly in places far from the coast, mainly on the basis of availability and low cost. However, in the coastal regions, fresh fish is still preferred.

Most of the Portuguese fish catch is either frozen at sea in well equipped vessels, as happens with hake (Merluccius merluccius), or caught very near the coast and frozen inshore after a few days in ice, as happens with sardines. Products might therefore be expected to reach the consumer in good condition, but this is not always the case. Although the Portuguese "cold chain" is not far from the necessary minimum, problems can arise because of the unequal distribution of cold stores throughout the country and the lack of trained staff (Leal, 1984; Quintela, 1980). Also, Portuguese retailers do not respect strictly the

recommended frozen storage conditions and frozen fish is sometimes kept in freezers where the temperature is allowed to fluctuate and can rise to very high values (Teixeira, 1985, personal communication).

2.1.3 Freezing techniques, cold storage and thawing

The main objective of any food preservation process is to retard the spoilage induced by microbial, autolytic, spontaneous chemical or physical processes.

During freezing, most of the water in the fish flesh is converted into ice. In the early stages of this process, the concentration of salts and enzymes in the remaining water phase increases, and so increases their activity (Greenwood-Barton and Cole, 1966). Another factor of importance in inducing spoilage is the action of ice crystallization. If freezing is slow, ice develops first in the intercellular spaces and the resulting hypertonic solution extracts water from the cells (cryoosmosis). The cells may even become sufficiently dehydrated to resist further freezing. Large ice crystals may be formed, resulting in physical damage to membranes and other cell organelles, and also in disruptions at the molecular level caused by dehydration (Luyet, 1968). In the case of rapid cooling, crystalline nuclei are apparently formed in large numbers throughout the tissue and no exchange of water through membranes takes place (Luyet, 1968). However, some authors believe that extremely quick freezing leads to physical damage to the muscle (Hansen, 1980).

The "quick freezing", widely considered as the ideal commercial freezing conditions for good quality products requires that the whole piece of fish (either a specimen, fillet or block)

passes its thermal arrestment period in less than two hours and the fish should not be considered as frozen until its thermal centre reaches -18°C (Greenwood-Barton and Cole, 1966; U.K. Association of Frozen Food Producers, 1976).

Industrially, such "quick" freezing can be achieved in several ways, the most important being: air-blast freezers, which are chambers or continuous-flow tunnels, where previously cooled air is forced around the material to be frozen; plate freezers, where the material to be frozen is pressed in direct contact with freezing surfaces; and cryogenic freezers, where liquid nitrogen, carbon dioxide or fluorinated hydrocarbons are used, (Clucas, 1981).

Each of these techniques has its own advantages and disadvantages.

In the air-blast freezers, the effect of dehydration is more drastic and can easily reduce the quality of the products, but the method is easy to use whatever the size or shape of the material to be frozen. It is most used for freezing large carcasses, such as tuna, and irregularly shaped ones, such as lobsters.

Horizontal or vertical plate freezers reduce the effects of air dehydration, since the specimens are packed together between the freezing plates, but they can only be economically useful with regular sized and shaped materials. The majority of the freezing boats use plate freezers, although many of them have at least one air-blast freezer for odd specimens. The products obtained from plate freezers are always regularly shaped blocks which can be easily packed, and, as discussed below, packaging plays an important role in frozen fish quality.

Cryogenic freezing is less used than the above techniques because it is the most expensive and requires liquified gases that are often unavailable in remote regions.

As important as the freezing conditions in determining frozen fish quality are the conditions of cold storage after freezing. Many experiments have shown the need to have a storage temperature of at least -30°C for long term storage (see Table 1) (Cutting, 1977; Hansen, 1980; Anon. 1965). However, recently it has been pointed out that previous opinions of likely shelf life of fish products are probably too severe, owing to the use of expert panels instead of consumer taste panels (Torry Research Station, 1984).

Another important factor is temperature stability. When a cold store temperature rises, even a few degrees, its relative humidity decreases sharply (Clément, 1969). When the relative humidity of the air is lower than the vapour pressure of water in the frozen fish, it tends to give out water to the atmosphere leading to the dehydration damage processes often present in badly handled frozen fish. Good packaging, with materials which are very good water barriers, can overcame this problem (Anon., 1965).

Very little work has been carried out on the effects of temperature flutuations during frozen fish storage; however, Dyer and his Co-workers showed, on the basis of taste panel and protein extractability results, that with frozen fillets stored in cardboard cartons, a temperature rise up to -9°C after different periods of storage at -18°C induced considerable deterioration in quality (Dyer et al., 1957; Dyer, Fraser and MacCallum, 1957).

Table 1: Storage lives of frozen fish, in months

Sample	Storage temperatures		
	-18°C	-25°C	-30°C
Lean round fish			
cod, haddock	3-4	6-8	8-10
Lean flat-fish			
plaice, sole	3-4	7-10	
Fatty fish			
herring, mackerel, sardines, salmon	2-3	3-5	6
Shellfish			
lobsters (cooked)	2-3		
oysters, shrimps	3-4		

Source: Cutting, 1977

In addition to its importance relative to dehydration processes the temperature fluctuations in a cold store have another very important way of interfering with the quality of frozen fish, particularly when the temperature fluctuates in the range between -5°C and -10°C. Under such conditions, great variations can occur in the actual freezing status of the tissue solutes, and the resulting concentration or dilution of both enzymes and substrates, will play an important role in determining the rates of the autolytic processes (Dyer, 1968). Such autolytic processes would be taking place at a very slow rate under normal (much colder and steady) storage temperatures.

Deterioration increases logarithmically with temperature rise (below 0°C) and that explains why, even a short exposure at an higher temperature can greatly reduce the storage life of any frozen fish product (Love, 1968).

Thawing conditions can also be important, particularly in the case of fish frozen at sea, when freezing can sometimes occur before the onset of rigor mortis. In this case, any rigor changes must be slowly completed during thawing, otherwise, severe shrinkage and drip loss can occur (Stroud, 1969). Fish must never be overheated during thawing, or it will lose its subsequent keeping quality (Cutting, 1977).

Fish can be thawed in air, water, or dielectrically. Dielectric thawing, if controlled so that the fish temperature never exceeds 0°C, can be as good as thawing in air. Thawing in water may result in tougher muscle, where great moisture retention may add some "watery" taste to the cooked product (Lee, 1982).

2.2 Quality changes induced in fish by freezing, cold storage and thawing

Ideal conditions of freezing desirable for maintaining quality, i.e., quick freezing, are also the conditions which permit maximum bacterial survival, however there is no significant bacterial activity in seafoods below -5°C (Liston, 1980). So, although frozen seafoods should be regarded as potential health hazards if pathogenic microbes are present, microbial spoilage is not important in limiting the storage life nor the ultimate quality of a product which entered the process in good condition.

However, even when processed and stored under ideal conditions, frozen fish does not have an infinite shelf life, because of non-microbial spoilage processes, namely, autolysis, spontaneous chemical processes and physical processes. These changes result in two distinct kinds of problem which limit storage life: textural problems, i.e., the texture becomes firmer and ultimately inedibly tough, and flavour problems, i.e., strong off-flavours develop (Connell and Howgate, 1971).

The relative importance of each of these problems depends on many factors, but one of major importance is the lipid content of the particular fish species. In fatty fish, development of rancid off-flavours is the major problem, limiting the shelf-life before any textural changes can be detected, whereas in lean fish, the development of a tough texture can become a greater problem (Hansen, 1980).

The development of off-flavours during frozen storage of fish is a direct consequence of lipid deterioration, and the texture changes appear to be due to the process known as protein

denaturation; however, recent studies indicate that the two processes are related (Castell, 1971). These two major problems are discussed in detail below.

Surface desiccation can also become a problem, being responsible for the so-called "freezer burn", which can be recognised by the dry and spongy aspect of the outer exposed surfaces; however, this can now be completely prevented by correct packaging as mentioned above, and so, no longer constitutes a limitation in the shelf-life of frozen fish.

2.2.1 Lipid deterioration

The changes suffered by fish lipids during frozen storage are of two types, lipid hydrolysis and lipid autoxidation.

Fish flesh lipids consist predominantly of triglycerides and phospholipids with smaller proportions of free fatty acids, vitamins, colouring materials, sterols, etc. The hydrolytic cleavage of triglycerides and phospholipids gives rise to free fatty acids. This process, which can occur in an enzymic or non-enzymic way, is of great importance to the development of rancidity in dairy products, because of the high amounts of short chain fatty acids present, but not in fish lipids, where long chain highly unsaturated fatty acids predominate (see Table 2).

With fish lipids, which contain a high degree of polyunsaturated fatty acid chains, the most important cause of rancidity is lipid oxidation. Although a recent report indicates the presence of a lipoxygenase enzyme in fish skin (German and Kinsella, 1985), it is lipid autoxidation that is predominant in the development of rancid off-odours in fish products.

Because of its great importance in the keeping quality of

Table 2: Fatty acid profiles of some fats and oils (percentages)

FATTY ACID	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	16:1	18:1	20:1	18:2	20:4	20:5	22:5	22:6
Coconut oil			6	6	44	18	11	6		7		2				
Corn oil							13	4		29		54				
Olive oil	(also 2% of 18:3)						14	2	2	64		16				
Palm oil						1	48	4		38		9				
Butter	3	1	1	3	4	12	29	11	4	25		2				
Beef tallow						3	26	17	6	43		4				
Cod flesh						1	20	3	3	12	2	1	5	17	2	30
Whole herring (triglyceride)	(also 19% of 22:1)					6	13	1	14	16	14	1		7		3
Whole herring (phospholipid)						2	21	3	5	13	2	1	1	12	1	33
Dogfish flesh (triglyceride)						2	17	3	5	16	5	2	3	8	3	22
Dogfish flesh (phospholipid)						6	20	9	3	11	3	1	7	5	2	32
Queen crab (triglyceride)	(also 8% of 22:1)					4	14	2	10	24	12	1	2	11	1	9
Queen crab (phospholipid)						3	15	3	6	22	2	1	4	31	1	14
Shrimp flesh	(also 1% of 18:3)					3	16	3	6	19	2	2		22	1	16
American oyster (triglyceride)	(also 3% of 18:3)					4	23	1	5	8	1			14	1	14
American oyster (phospholipid)						1	5	3	3	5	3			16	1	20

Source: Brown and Morton, 1969 and Ackman, 1980.

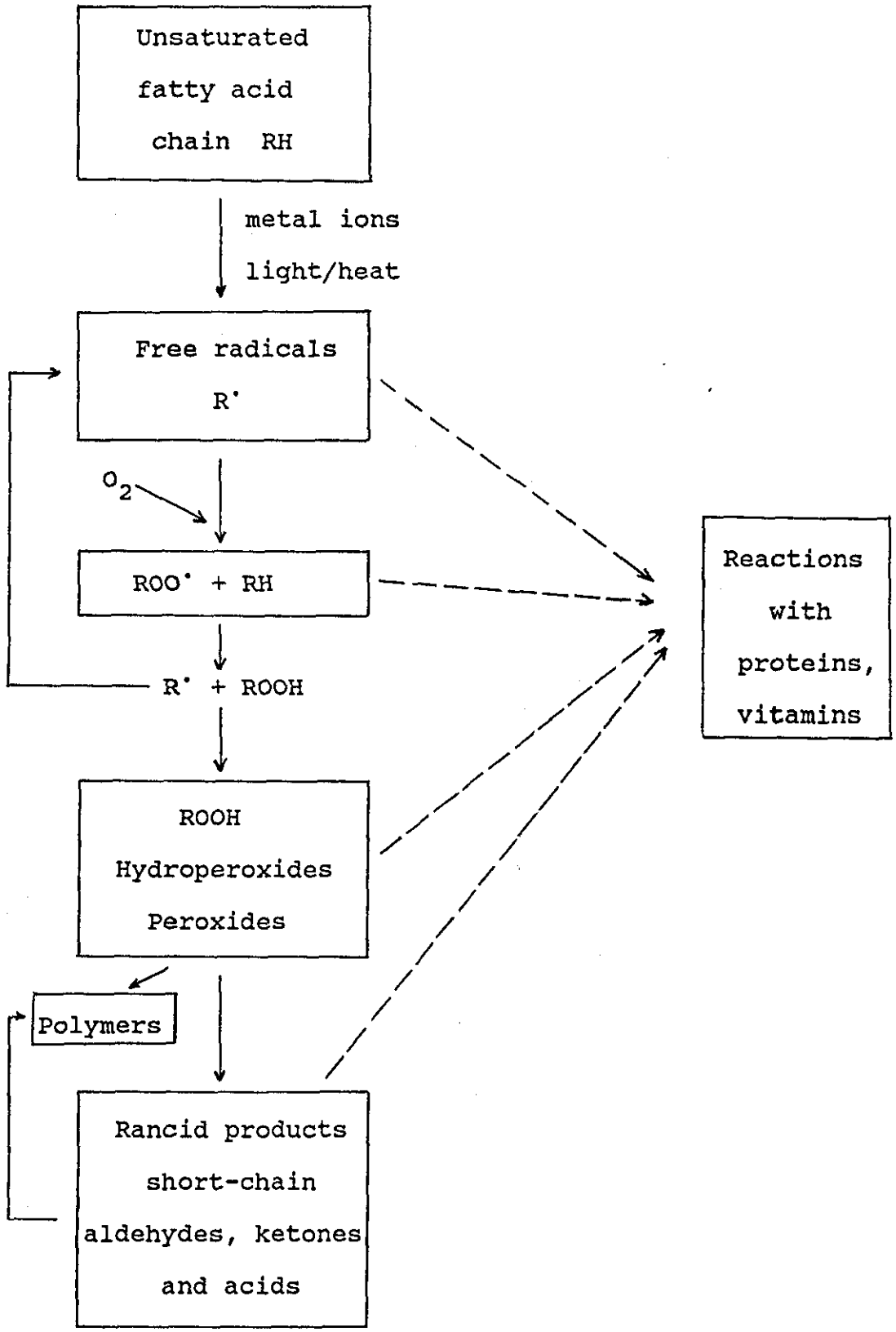
fish, particularly pelagic fatty fish, the subject of lipid autoxidation in fish has been intensively studied and its mechanism is at present, fairly well understood. An up-to-date general review on this subject can be found in Banasihan (1985).

Autoxidation is, by definition, the reaction of organic compounds with molecular oxygen in which the process is autocatalytic. In the case of lipid autoxidation, although in general the reaction rate increases with temperature rise, the reactions may proceed faster at lower temperatures, because interfering reactions competing for oxygen or producing antioxidants may be slowed down by the low temperature.

The mechanism of lipid autoxidation is known to be one of a free radical reaction, involving the production of intermediate lipid peroxides and ultimately unpleasant flavour products, such as short chain aldehydes, ketones, acids, etc. (Karel, 1984) (see Fig. 2). Final products and both peroxides and free radicals can react with proteins and vitamins, causing losses in nutritional value and further changes in food characteristics.

In the first stage of the lipid autoxidation process, leading to the production of intermediate hydroperoxides, three distinct stages can be defined: an initiation stage where initial free radicals are formed by homolytic cleavage of paired electron species, possibly involving singlet oxygen reaction with the alkene; a propagation stage where the initial free radicals abstract allylic hydrogen from the unsaturated fatty acid substrates and the new radicals formed react with oxygen to give peroxy radicals, which in turn react with the substrate to give hydroperoxides and more free radicals in a chain reaction; and a termination stage where radicals combine with each other,

Fig. 2: Overall scheme of lipid autoxidation



Adapted from: Karel, 1984.

producing paired electron species (See Fig. 3) (Hardy, 1980). The termination stage becomes predominant when the lipid material is subjected to very high temperatures or intense oxygen exposure for very long storage periods (Coultate, 1984).

Antioxidants, both synthetic butylated hydroxyanisole, (BHA) butylated hydroxytoluene (BHT) and propyl gallate (PG), or naturally occurring, such as tocopherols, work by blocking the propagation process. The antioxidant donates a hydrogen atom to a radical involved in the propagation process giving a new free radical product and leaving behind a low energy hindered antioxidant free radical that is unable to react with a fatty acid chain. These antioxidant free radicals are stabilised by resonance and thus become inactive in the continuation of the reaction chain. Antioxidants do not reduce the ultimate degree of rancidity, their action is to lengthen the induction period (Coultate, 1984).

Although the proportion of hydroperoxide initially formed is very small, this is an autocatalytic chain reaction and the subsequent breakdown of the hydroperoxide yields new free radical species that are able to propagate the chain (Coultate, 1984). Transition metal cations are important catalysts of hydroperoxide breakdown. Three possible pathways of hydroperoxide breakdown are summarised on Fig. 4, leading to rancid product formation.

Lipid autoxidation is a fairly well understood mechanism, and several ways of preventing it have been devised. By keeping the energy level to a minimum, i.e., avoiding any light and unnecessary heat or irradiation, the initiation stage of the process can be retarded; also at this stage, where the presence of metal ions is known to have a catalytic effect, complexing

Fig. 3: Lipid autoxidation - free radical mechanism of hydroperoxide formation

R-H = unsaturated fatty acid substrate

Initiation

Formation of initial radical In$^{\bullet}$ by homolytic cleavage of a paired electron species

Propagation

$$In^{\bullet} + R\text{-}H \longrightarrow R^{\bullet} + In\text{-}H$$

$$R^{\bullet} + O_2 \longrightarrow R\text{-}O\text{-}O^{\bullet}$$

$$R\text{-}H + R\text{-}O\text{-}O^{\bullet} \longrightarrow \underline{R\text{-}O\text{-}O\text{-}H} + R^{\bullet}$$

(the two steps above form a chain reaction)

$\underline{R\text{-}O\text{-}O\text{-}H}$ → Decomposition to give rancid products

Termination

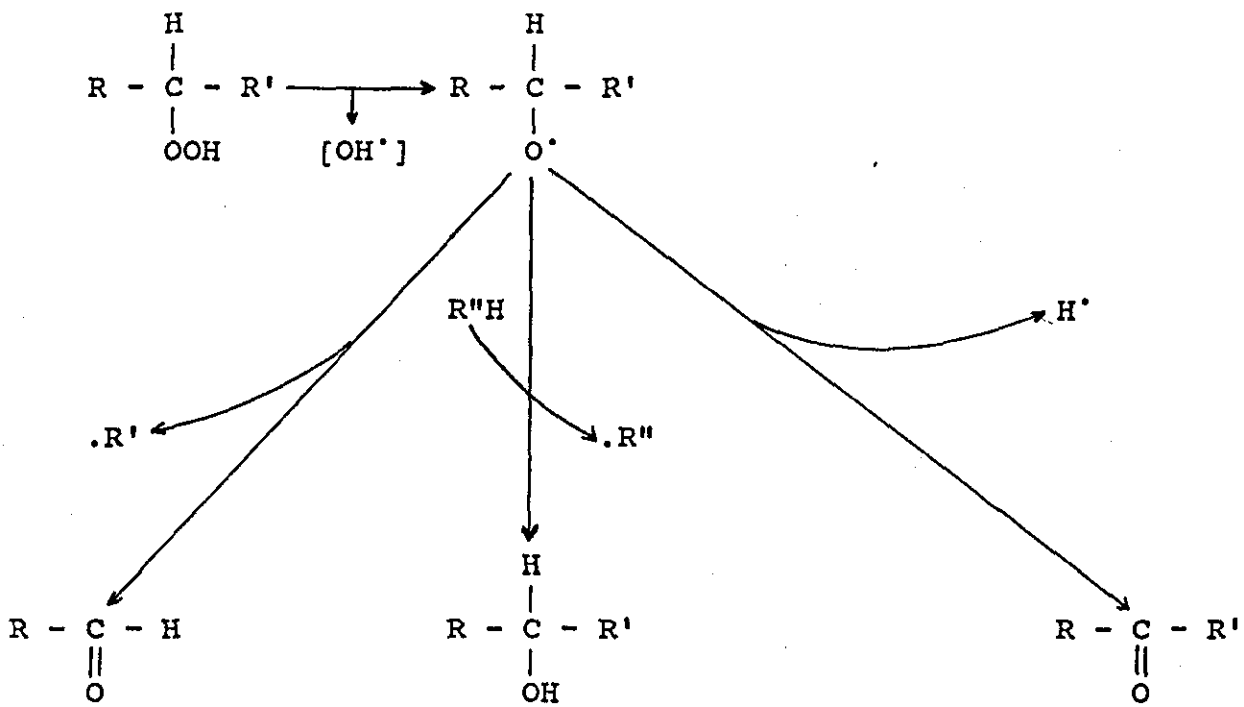
$$R^{\bullet} + R^{\bullet} \longrightarrow R\text{-}R$$

$$R^{\bullet} + R\text{-}O\text{-}O^{\bullet} \longrightarrow R\text{-}O\text{-}O\text{-}R$$

etc.

Adapted from: Hardy, 1980

Fig. 4: Lipid autoxidation - hydroperoxide breakdown



Aldehydes Secondary alcohols Ketones

Source: Coultate, 1984.

such ions by the addition of citric acid or phosphate, has proved to be a success. Later on in the process, the exclusion of oxygen is obviously important, and this can be achieved by adequate packaging or glazing. The addition of antioxidants, when possible (e.g. in fish minced products), has proved to be an important way of reducing the rates of oxidative rancidity development in frozen fish products.

It may appear, that the problem of lipid oxidation has been solved, however the rate of lipid autoxidation is influenced by the concerted action of so many variables that, in practice, no single sucessful method of controlling it has yet been devised and it is still the most important deterioration process limiting the shelf-life of frozen fatty fish and of some frozen lean fish.

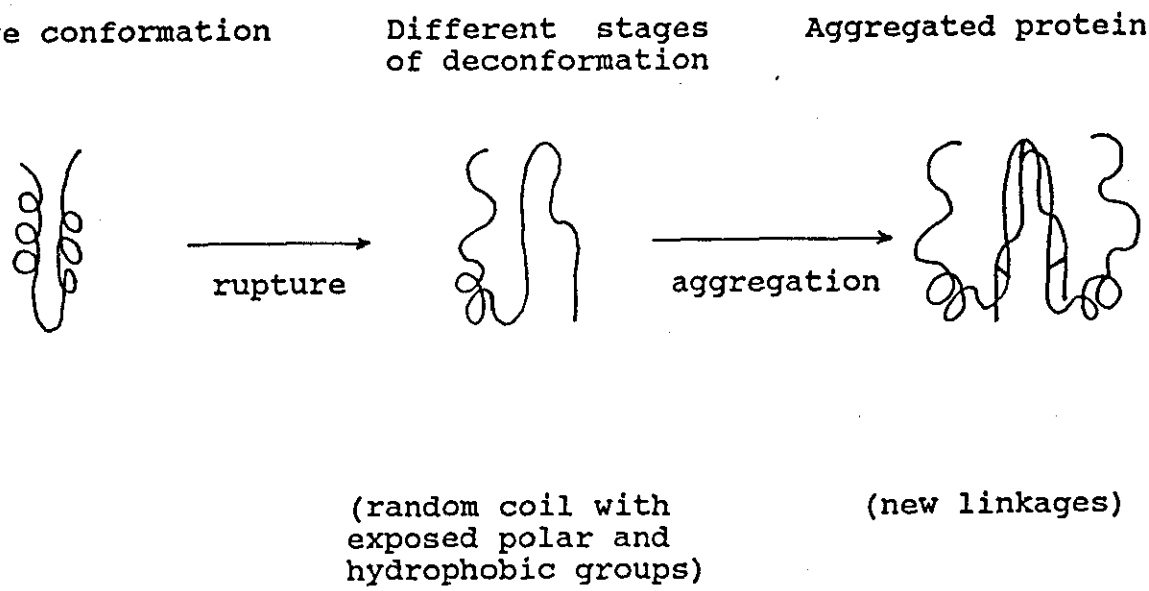
2.2.2 Protein denaturation

In its broadest sense, denaturation means any "deviation from the native state" and using a more strict thermodynamic approach, it can be defined as any increase in entropy - a change from an ordered to a more disordered state.

Proteins are highly complicated organised structures and an everyday practical definition of protein denaturation is "a change in protein structure that is not accompanied nor caused by any making or breaking of covalent bonds" (Feeney, 1980) (see Fig. 5). This definition does not however cover all examples of protein denaturation and the following broader definition is sometimes more useful: "a substantial change in protein properties that is not accompanied by cleavage of peptide bonds".

Before discussing protein denaturation in fish flesh in detail, fish muscle structure will be outlined and fish muscle proteins described.

Fig. 5: Protein denaturation



Adapted from: Sikorski, 1977.

2.2.2.1 Fish muscle structure

Fish muscle cells i.e. muscle fibres are practically indistinguishable from those of mammalian muscle and their detailed biochemistry seems to be very similar (Connell, 1960).

However, muscle fibres are shorter than in beef or rabbit, and, while in cattle the connective tissue is a very important factor in toughness because it is hardly softened by cooking, in fish, the amounts of connective tissue are much smaller and the collagen is very unstable being almost all converted to gelatin on cooking (Love, 1980).

In Fig. 6, the diagram of a typical white fish fillet shows the surface adjacent to the skeleton. Each block of muscle is separated from the adjacent by a thin sheet of connective tissue (myocomma). In fresh fish, muscle fibres are firmly attached to the myocommata (Murray and Burt, 1969).

Muscle fibres contain large numbers of sub-units, the myofibrils (see Fig. 7), and each myofibril is about 1000 filaments of the proteins actin and myosin, which, in association with the proteins tropomyosin and troponins, form the contractile mechanism of the muscle.

2.2.2.2 Fish muscle proteins

The adverse changes in textural characteristics that occur during frozen storage are, at present, considered to be the direct result of changes in the proteins (Connell, 1968) and this has been the incentive for an intensive study of fish muscle proteins and of the effects that freezing and frozen storage can have on them.

Fig. 6: Typical white fish fillet structure

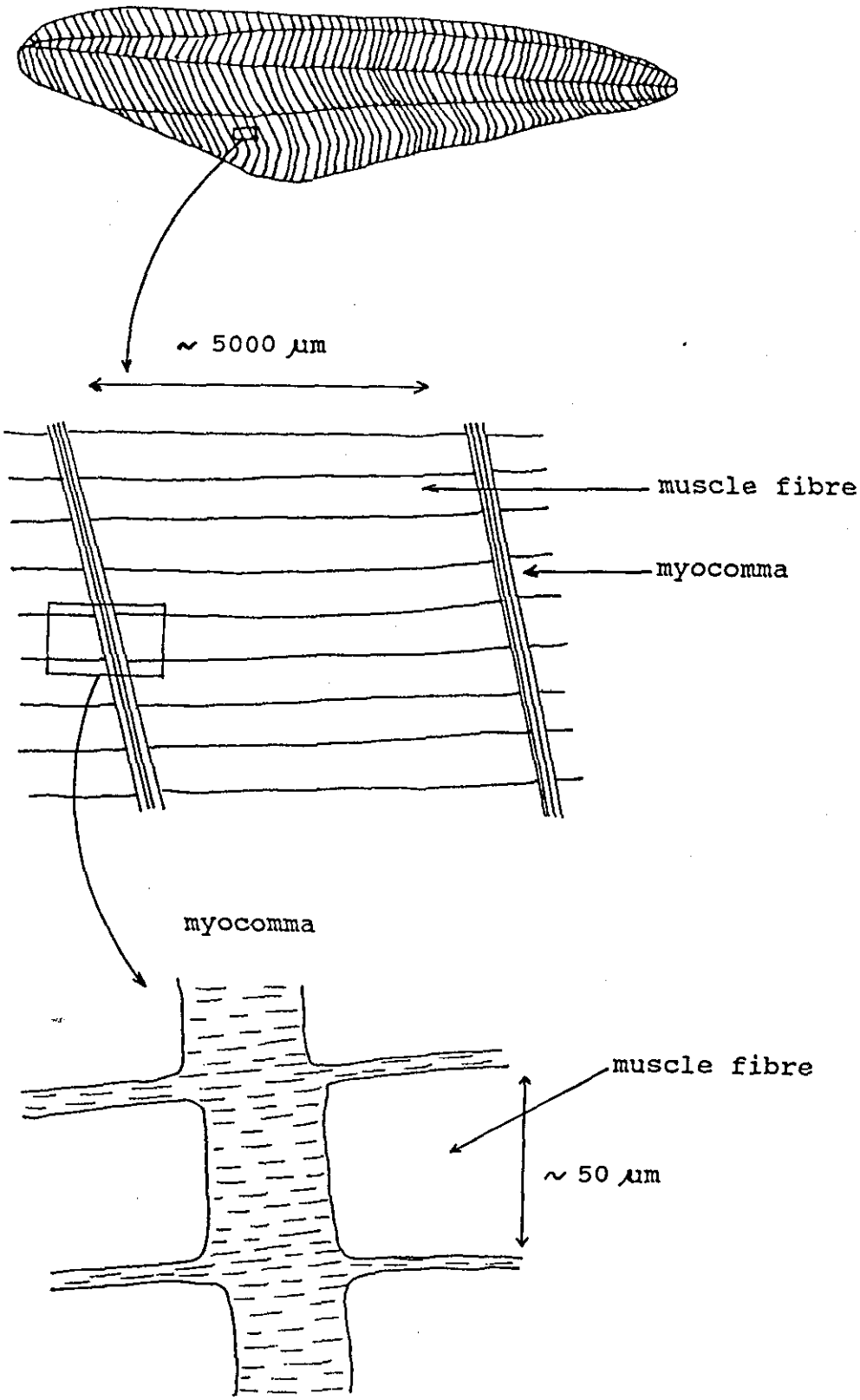
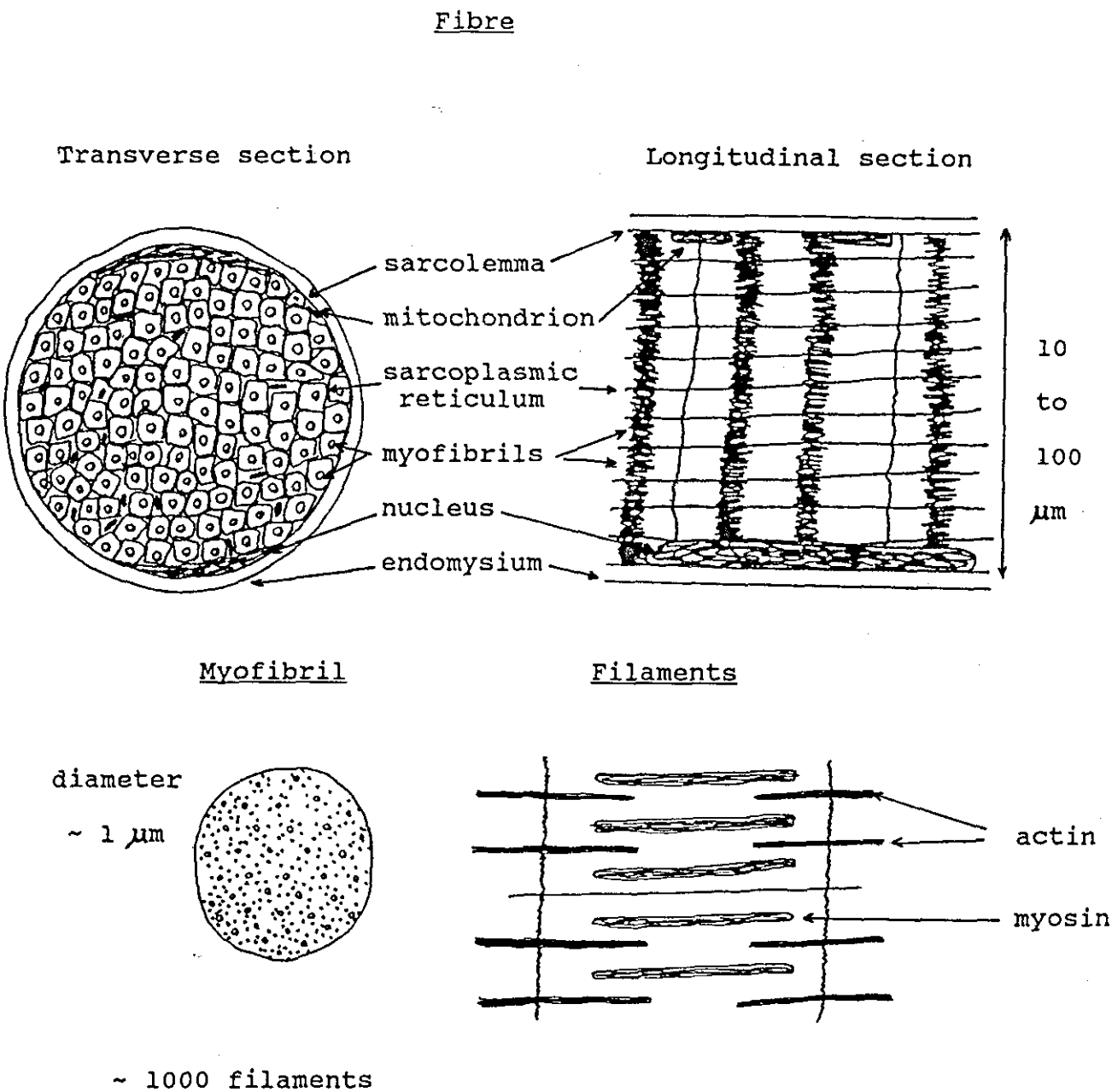


Fig. 7: Muscle fibre ultrastructure



The proteins in fish muscle (15-24% of total weight) are similar to those present in other vertebrates, but some differences do exist in relative amounts and in the properties of each protein (Matsumoto, 1980). In fact, fish proteins are definitely more unstable and undergo denaturation with greater ease than mammalian proteins (Connell, 1960).

Proteins can be classified into 3 different groups according to solubility and such classification is summarized in Table 3.

Myofibrillar proteins are the major proteins in fish muscle, and it has been known for a long time that denaturation of actomyosin, which is produced from actin and myosin post mortem, occurs during frozen storage. Until some years ago, it was generally held that the sarcoplasmic proteins did not suffer any significant alterations which might be responsible for textural changes (Partmann, 1977), but recently evidence has been gathered pointing out that the enzymes of the sarcoplasmic fluid also undergo denaturation during frozen storage (Matsumoto, 1980).

In practice, the protein denaturation in fish muscle is mainly manifested by a decrease in extractability of the myofibrillar fraction, due to the formation of aggregate structures.

In most fish species an increase in toughness, chewiness, rubberiness or stringiness is accompanied by a decrease in protein extractability and water holding capacity (Sikorski, 1980) and such facts clearly relate these textural changes with protein denaturation.

The results of experimental work led to the conclusion that denaturation and/or insolubilization of actomyosin and myosin during frozen storage is a result of aggregation caused by a

Table 3: Proteins of fish muscle

Group	Types of protein	Localization	Function	% of total protein
sarcoplasmic	myogens myoglobin	cell plasma	enzymes O_2 carriers	18-30
myofibrillar	myosin actin tropomyosin troponin	filaments	contractile mechanism	65-80
stroma	collagen elastin	myocommata cell membranes	connective tissue	3-5

Adapted from: Matsumoto, 1979.

progressive increase in intermolecular cross linkages due to formation of hydrogen bonds, ionic bonds, hydrophobic bonds and disulfide bonds (Matsumoto, 1980).

Several models of hypothetical mechanisms of such aggregation have been proposed and Fig. 8 summarizes one of the most recent and perhaps the most clear of them.

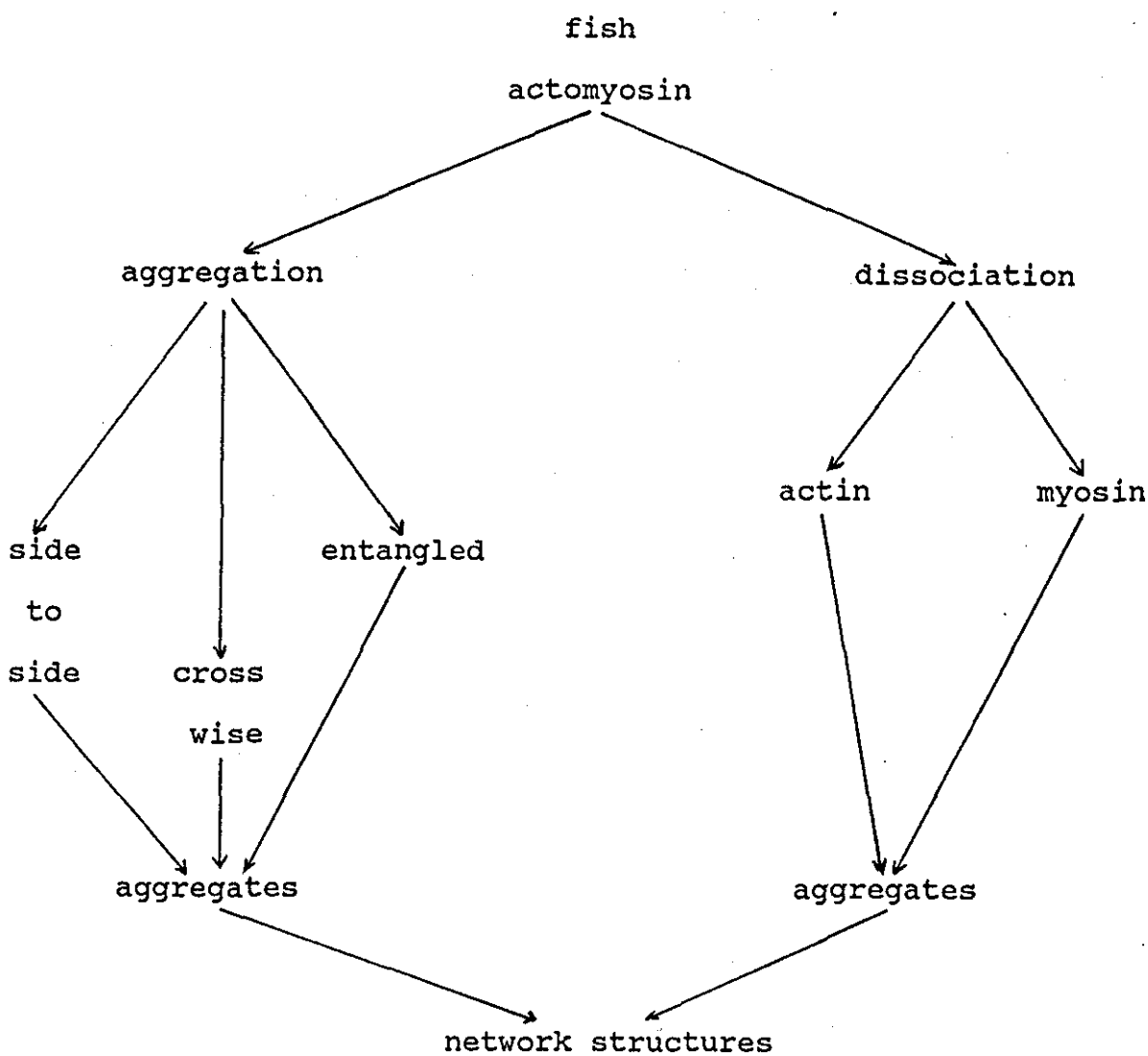
2.2.2.3 Factors which may cause protein denaturation

Protein denaturation occurring during frozen storage of fish muscle may be caused by a great number of interacting factors (see Table 4) and thus it is a highly complicated process, very difficult to prevent.

The changes in moisture occurring when both intracellular and intercellular water is frozen have long been considered as major causes of protein denaturation in frozen foods. Directly or indirectly, all these changes result from the formation of ice crystals. In the case of slow cooling, ice develops first in the intercellular spaces, the solute concentration in those spaces increases and the resulting hypertonic solution extracts water from the cells (cryoosmosis). When the cooling process is more rapid, crystalline nuclei develop through the tissue; in this case, no exchange of water occurs through the cell membranes (Luyet, 1968) but cellular water still leaves its native place to become part of the ice crystals.

The water in the muscle cells is attached to proteins by a large range of bonding energies, and the removal of any of it by freezing, causes damage to the protein (Love, 1968).

Fig. 8: Hypothetical mechanism of aggregation of fish actomyosin during frozen storage



Adapted from: Matsumoto, 1980.

Table 4: Factors which may cause protein denaturation during freezing and frozen storage of fish muscle

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1. Moisture related factors

   - Damage due to the formation of ice crystals
   - Damage due to dehydration (loss of hydration water molecules when they form ice crystals)
   - Damage due to increase in salt concentration

2. Lipid related factors

   - Interaction of intact lipids with proteins
   - Interaction of oxidised lipids with proteins
   - Interaction of free fatty acids with proteins

3. Trimethylmine oxide degradation

   - Interaction of formaldehyde with proteins

Adapted from: Shenouda, 1980.

The increase in solute concentration in the unfrozen phase is believed to affect dissociation and denaturation of proteins, because of the corresponding changes in ionic strength, pH and enzymic activity.

The effect of the inorganic salts is thought to be based on the capacity of different ions to hydration, leading to alterations of the water equilibria. The inorganic ions can also form cross-linking bridges between adjacent peptide chains, influence the activity of enzymes, and interfere with lipid oxidation and with the formation of lipid-protein complexes.

Furthermore, the increased salt concentration may directly lead to salting out, reducing the number of hydrophilic protein molecules associated with water. The proportional increase in the surface tension value favours the formation of new intra and intermolecular linkages (Sikorski, 1977).

It is thus clear, from the evidence above, why rapid freezing results in less protein denaturation than slow freezing (Connell, 1960 and 1968).

According to Connell, although protein-lipid interactions do occur, they do not appear to be a major cause of protein denaturation during the frozen storage of fish (Connell, 1968). But on the other hand, the products of lipid oxidation have been well known to produce aggregation of proteins (Partmann, 1977) and, in model systems, its effects were found to be even more severe than those of free fatty acids.

The mechanism of free fatty acid interference with proteins has been clarified with experiments on sodium dodecyl sulphate solubility. These indicate that the free fatty acids may bind polypeptide side chains via formation of intermolecular

hydrophobic-hydrophilic or hydrophobic-ionic linkages (Connell, 1975).

For some years, formaldehyde, originating from the enzymic breakdown of trimethylamine oxide in certain fish species (particularly in gadoid fish) has been considered of essential importance in protein alterations in such fish species (Partmann, 1977). In 1972, Babbit, Crawford and Law suggested that formaldehyde might interact rapidly with proteins through methylene cross linkages, in a manner similar to the cross linking observed with bovine fibrinogen, and, the same authors considered that such interaction could explain the undesirable textural changes that occur during frozen storage of Pacific hake (Merluccius productus).

However, more recent work has shown that the bulk of the proteins in cod muscle stored until it becomes extremely tough, are not extensively cross linked (Connell, 1975). This author thus regards the role of formaldehyde as a cross linking agent as questionable, but does not deny the possibility of other irreversible reactions between formaldehyde and proteins.

Owusu-Ansah and Hultin found that formaldehyde concentrations equivalent to those found in frozen stored fish muscle can cause polymerization of some proteins (unpublished results cited in Owusu-Ansah and Hultin, 1984) but still consider it premature to assign all of the changes in texture in frozen stored gadoid muscle to cross linking of proteins by formaldehyde (Owusu-Ansah, and Hultin, 1984).

It has also been suggested that the action of formaldehyde could result from its capacity to enhance the effects of free fatty acids (Childs, 1974).

Whatever its mechanism of action may be, formaldehyde is definitely involved in protein denaturation in the species where it is produced (Regenstein et al., 1979) and, hence the mechanism of trimethylamine oxide degradation will be briefly reviewed in the next section.

Other substances have been identified as possible causes in the protein denaturation process: oxygen, for instance, may encourage proteins to form S-S bonds during frozen storage (Matsumoto, 1980).

The rate and importance of protein denaturation in determining the shelf-life of frozen fish is dependent on many factors and the process is usually not controlled by any one of the possible factors, but by the concerted effect of many of them. Perhaps the most relevant amongst those factors are: fish species, pre-freezing history, storage temperature and rate of freezing.

2.2.2.4 Trimethylamine oxide degradation

Trimethylamine oxide (TMAO) is present in most species of marine fish, but it is particularly high in the gadoid family and elasmobranch order, where it apparently is a part of the osmoregulation system (Love, 1970).

Even in the members of these groups of fish, the TMAO content varies from species to species, varies within the same species with environmental conditions and varies within each individual from one organ to another (see Tables 5 and 6).

Marine microorganisms can convert TMAO to TMA and the

Table 5: TMAO content (mg of TMAO-N/100g) in different fish species

cod	110 - 151
haddock	60 - 81
hake	140 - 186
pollack	81 - 95
mackerel	41 - 55
flounder	45 - 101

Source: Regenstein et al., 1979.

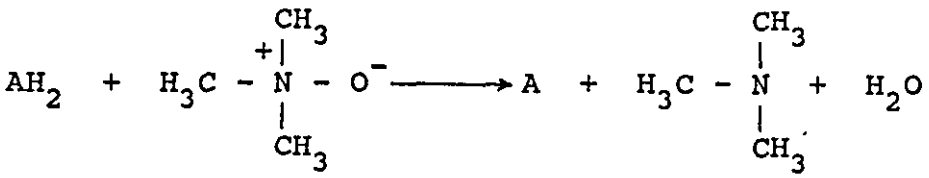
Table 6: TMAO content (mg of TMAO-N/100g) of various organs of cod

skin	42
superficial muscle	106
deep muscle	185
stomach	13
ovary	22

Source: Regenstein et al., 1979.

determination of TMA has been used as an indicator of fish freshness (Babbit, Crawford and Law, 1972).

Many Gram-negative psychotropic bacteria (such as Achromobacter and Pseudomonas species) possess the enzymic apparatus capable of the following:



Lactic and pyruvic acids can act as hydrogen sources and after the substrate activation by the enzyme triamineoxidase, a bacterial dehydrogenase can act on the substrate (Regenstein et al., 1979).

Because it is a bacterial process, the production of TMA loses its importance in the case of frozen storage of fish, since bacterial activity is virtually arrested at -5°C (Liston, 1980) and even in the case of chilled storage, the combination of 1°C, a 60% CO_2 atmosphere and ice containing potassium sorbate, can significantly reduce the importance of the process on the shelf life of, for instance, red hake and salmon (Regenstein et al., 1979).

However, another pathway of TMAO degradation can occur, the autolytic pathway, with the production of equimolar quantities of dimethylamine (DMA) and formaldehyde (Licciardello et al., 1981). During frozen storage, production of DMA is much larger than production of TMA, which is negligible in most cases (Hebard, Flick and Martin, 1982).

DMA and formaldehyde were first isolated from cod in 1945 (Dyer and Mounsey, 1945) and, since then, a considerable amount of work has been undertaken on the mechanism of their formation and also their importance in the fish spoilage process. A detailed review of such work will be found in Hebard et al. (1982).

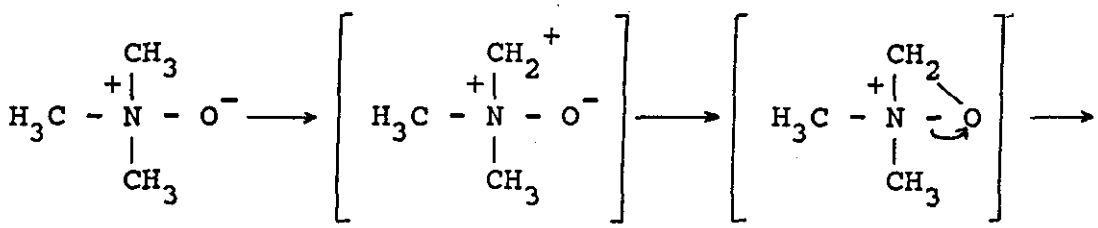
Referring only to the more recent conclusions of such investigations, an enzyme system is assumed to catalyse the breakdown of TMAO to DMA and formaldehyde (Tomioka, Ogushi and Endo, 1974). Such an enzyme system is known as trimethylamine N-oxide demethylase (TMAO-ase) (Racicot et al., 1984). A method of enzyme activity assay was devised (Tomioka et al., 1974) which permitted detailed studies of the enzymes properties.

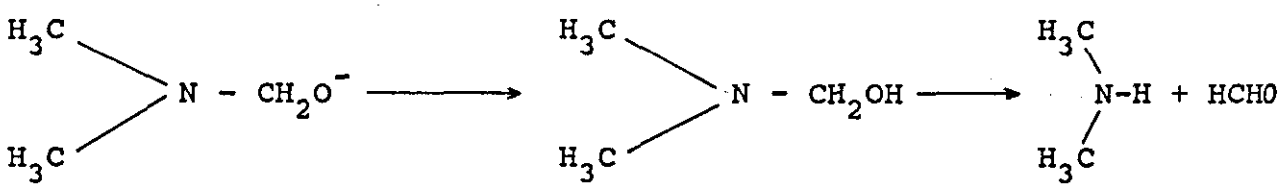
Various compounds have been reported to be activators or inibitors of the TMAO-ase system and from such influences, Tomioka and his coworkers deduced that this enzymic reaction is accompanied by some kind of oxidation-reduction system (Tomioka et al., 1974).

The same authors also found that the optimum pH of the enzyme reaction was 5.0; the enzyme was most stable at pH 6.2; that the enzyme was labile on heating at temperatures higher than 40°C, and that it was specific for tertiary amine N-oxides (Tomioka et al., 1974).

In 1975, Harada (as cited in Hebard et al., 1982) postulated the reaction mechanism shown in Fig. 9, with a general base acting as an electron donor. This mechanism, involving hydride ion abstraction in the first step, is highly improbable and a more likely mechanism involves proton transfer from a CH_3 group to the oxygen, followed by ^-OH loss giving $(CH_3)_2{}^+N{=}CH_2$, which

Fig. 9: Proposed reaction mechanism for the conversion of TMAO to DMA and formaldehyde





can cleave hydrolytically to give dimethylamine and formaldehyde. Since formaldehyde is an unstable compound and difficult to extract from fish, DMA can be measured instead to investigate the spoilage of frozen gadoid fish (Castell, 1971).

The problem with using DMA as a spoilage index of frozen fish is that it can be used only with gadoid fish, because other fish do not develop DMA in sufficient amounts during frozen storage (Castell, Neal and Smith, 1970).

Among gadoid fishes, DMA content is lowest in haddock and increasingly higher in cod, pollack, cusk and hake (Hiltz et al., 1976).

Seasonal variations in the DMA producing activity of the muscle of fish have been reported, and minced flesh is known to produce DMA more rapidly and in larger amounts than intact flesh (Hebard et al., 1982).

2.3 Frozen fish quality assessment

The ultimate judge for the quality of any foodstuff is the consumer and hence sensory taste panel testing is the most direct way of assessing frozen fish quality. However, such methods are time consuming and expensive to carry out and therefore many attempts have been made to develop objective methods that are equally effective. Because of differences inherent in fish species and because of the variable characteristics of fish muscle due to fishing grounds and season, no method has yet proved to be universally applicable.

2.3.1 Sensory assessment

There are broadly speaking, two kinds of sensory assessment methods: subjective and objective. In the former, hedonic classifications are used and personal opinion is allowed free rein (examples of this are consumer trials). In the latter, biases are deliberately minimized by the use of specially trained judges who concentrate on particular well defined attributes of the product, referring them to non-hedonic classification scales (Connell and Shewan, 1980).

Subjective sensory tests are undoubtedly those which ultimately count, because they come closest to assessing the consumer preferences; however, because of the difficulty and expense normally involved in arranging fully representative consumer tests, these are rarely feasible and instead, comparisons with the results of objective sensory tests are usually employed.

A variety of methods for the objective sensory analysis of foods have been described (Amerine, Pangborn and Roessler, 1965; American Society for Testing Materials, 1968) but four of them are most commonly used for fish: paired comparison, ranking, triangle testing and scoring of attributes.

It has been pointed out that these first three types give information about the judge's abilities rather than about the samples, and that they should be considered as a separate category of tests (Connell and Shewan, 1980).

Each of these methods has been useful for particular applications. For example Love (1966) used paired comparison between frozen and unfrozen fish, to detect increases in the toughness of fish stored in the frozen state. The ability of tasters to differentiate between different fish species has been measured using triangle and comparison tests on a number of occasions at Torry Research Station (Connell and Shewan, 1980).

However, scoring is by far the most commonly used method, particularly for assessing freshness of chilled fish. Coded samples are evaluated for the intensity of some specified characteristic and the panelist records his judgement on a graduated scale, whose intervals can be labelled with numbers or descriptive terms. From the scores, the size and direction of the differences between samples are evident and from the statistical analysis of the results the experimenter determines whether or not the differences are significant (Larmond, 1977).

For all forms of objective sensory assessment, the testing should be carried out by trained staff, under controlled conditions (Amerine et al., 1965). For instance, the frozen fish samples should be thawed and cooked under controlled conditions,

and presented at constant temperatures (according to Soudan (1968), 50°C is the ideal).

The descriptive terms in the scales must be as objective as possible and the inclusion of standards at various points in the scale can be used to minimize panel variability.

To use scoring effectively, all the panelists must be evaluating the same characteristic and the definition of the characteristic under test is often the greatest problem, since the panellists may not have the same interpretation particularly in the case of complex characteristics, such as, texture (Larmond, 1977).

Several widely different scoring systems are in active use, and an international agreement on universal systems would increase the usefulness of the results obtained. Among the more widely used systems is the Torry scheme, used widely in the U.K. for quality control of wet and frozen fish, both in the industry and in specifications. In France, a similar scheme has been developed (Soudan, 1957) which later became the basis of the more recent European Economic Community Scheme of Grading (EEC, 1970).

Scoring systems of a rather different kind are the so-called default tables, where quality faults capable of leading the product being declared below minimum standard are assessed. This is the basis of the Codex Alimentarius tables (Codex Alimentarius, 1979).

Still only at the research stage are the profiling methods, which probably require a development in the understanding of the sensory properties of fish products, before they can be improved sufficiently to be used by industry.

At present, the characteristics that appear to be most

important in the assessment of frozen fish quality are: freshness flavour, as a measure of the fish condition prior to freezing, cold storage flavour, as a measure of off-flavours due to frozen storage and texture changes, i.e., toughness on cooking.

2.3.2 Objective methods

No single objective method of assessing frozen fish quality can give results equal to those obtained by a well organised sensory panel, but several objective methods, some essentially chemical and biochemical in nature, others based on physical techniques, have been accepted as indicators of the eating quality of fish products.

The advantage of non-sensory or objective methods is their convenience, lower cost, and the fact that they can be standardized against reference standards (Connell and Shewan, 1980).

2.3.2.1 Chemical and biochemical methods

According to Gould and Peters (1971) the most accurate measure for frozen fish quality can be achieved by following the changes in the functional capacities of the fish muscle enzymes. Several enzyme activity based tests have been developed, such as for example, the aldolase activity test (Connell, 1966).

The most important weakness in these tests lies in the fact that the initial values of enzyme activity can vary greatly from species to species and within each species from individual to individual, and also with the biological condition of each specimen at the moment of death. Therefore, such tests always

require a very large sample in order to obtain reproducible results and even then, the results cannot be easily compared.

In the case of another particular enzyme - catalase - there is also the problem of the great sensitivity of the test to gill material or bacterial contamination (Gould and Peters, 1971).

In practice, enzyme activity tests have never been useful indicators of frozen fish quality and it is generally accepted that it is better to rely on a parameter that starts from zero and builds up during frozen storage, rather than the opposite.

The amount of total volatile basic nitrogen (TVBN) present in a fish sample has been used as a measure of protein degradation but it only becomes important when advanced stages of deterioration are achieved (Gould and Peters, 1971). However, because of its simplicity, this method is widely used.

Included in these values of TVBN are the amounts of TMA and DMA present in the fish flesh, and also the ammonia that may arise from several sources.

TVBN is often measured using the Conway Microdiffusion method, but it can now be measured using automated methods of nitrogen determination, such as the Tecator Kjeltec equipment. Recently, a specific gas sensor system has been developed (Storey, et al., 1984). Such a simple and inexpensive method is capable of some differentiation between TMA and DMA and may find application as a rapid method of estimating the freshness of a number of species of fish.

The magnitude of the total TVBN content depends to some extent on the method of measurement and on the method of recovering the bases from fish or fish extracts (Storey et al., 1984).

As an indicator of freshness prior to freezing, the presence of trimethylamine (TMA) in the frozen fish flesh is often used. Only bacterial enzymes can catalyse the degradation of trimethylamine oxide (TMAO) to TMA and since bacterial activity is halted at temperatures below -5°C, the presence of TMA in a frozen product will indicate pre-freezing bacterial activity (Hebard et al., 1982).

TMA is associated with fishy odour but it does not contribute to the odour entirely by itself. It is when TMA reacts with fat in the muscle of fish that the fishy odours are produced (Hebard et al, 1982). Many workers have conducted sensory tests along with TMA determination to show the correlation between the degree of spoilage and the TMA concentration, but there are many conflicting findings, indicating that the TMA determination should not be used alone as a spoilage index.

TMA can be measured by the microdiffusion method of Beatty and Gibbons (1937) but perhaps the most commonly used is the so-called picrate method (Dyer and Mounsey, 1945).

Recently, several gas chromatographic techniques have been devised but they involve expensive equipment and are still used only in the research fields (Dunn, Simenhoff and Wesson, 1976; Hobson-Frohock, 1979; Keay and Hardy, 1971; Miller et al., 1973; Mackie and Thomson, 1974; Ritskes, 1975; Scanlan et al., 1973; Tokunaga, Iida and Miwa, 1977).

As discussed before (Section 2.2.2.4) the members of the gadoid family possess an enzymic system capable of breaking down TMAO to give DMA and formaldehyde (Castell, Neal and Smith, 1970). This is strictly an autolytic process, which becomes important at frozen storage temperatures when bacterial spoilage

is inhibited.

The assessment of the amount of formaldehyde in a frozen sample is possible by means of a simple colour test (Connell, 1966) but, as formaldehyde is unstable and difficult to extract from fish, the presence of DMA is often preferred as an indicator of "frozen storage deterioration" in such species (Hebard et al., 1982).

A colorimetric estimation of DMA has also been devised (Dyer and Mounsey, 1945) but because this is a complicated and time consuming technique, relatively simple gas-liquid chromatographic methods were developed.

Most of the techniques referred to when dealing with TMA estimation make possible simultaneous determinations of DMA and TMA, providing information on both pre-freezing spoilage and deterioration during frozen storage (Castell, Smith and Dyer, 1974).

Although they seem very promising, these gas chromatographic techniques pose some problems that are still retarding their application on an industrial scale. The first of these is the high cost of the required equipment and also important are the problems inherent to the measurement of such polar, volatile compounds as TMA or DMA and the short life of the columns (Mackie and Thomson, 1974).

The extractable protein (EP) or soluble protein test is a useful measure of the changes occuring in proteins during frozen storage. It has been clearly shown that changes in texture are closely related with the myofibrillar fraction of proteins rather than with the sarcoplasmic proteins (Connell and Howgate, 1971). The solubility of the former in salt solutions decreases during

frozen storage, because of the aggregation of the denatured protein fractions.

The EP techniques consist briefly in measuring the nitrogen content of the salt extractable fraction of the fish. The results obtained may show great seasonal variation, and are very strongly affected by the storage temperature and also by the extraction procedure (Ravesi and Anderson, 1969). For these reasons, comparison of results obtained from different authors should be carried out only when the same techniques have been applied and the results should always be considered in relation to other methods (Quaranta and Pérez, 1983).

A recent approach to assessing protein alterations during frozen storage is the use of sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). This technique is used to analyse the nature of the various forms of cross linking, both inter and intramolecular, particularly in myofibrillar proteins (Laird and Mackie, 1981). Although a recent line of investigation, it appears to be providing promising results in relation to understanding the protein changes responsible for the development of toughness. However it is far from becoming an everyday test for looking at the quality of frozen fish.

In the case of fatty fish, lipid deterioration is the major cause of losses in eating quality, and rancidity measurements have the greatest importance in predicting the acceptability of such products.

Perhaps the most commonly used method of estimating rancidity is the peroxide value (PV) which measures directly the amount of hydroperoxides present in a sample. As hydroperoxides are intermediates and not products of lipid oxidation, this test can

give results before the thiobarbituric acid test (see below) becomes sensitive. However, after reaching a maximum at a moderate level of rancidity, the peroxide value tends to decrease, whereas the rancidity continues to increase. Hence, it is unreliable for highly rancid products and it has the severe inconvenience of requiring a previous lipid extraction.

The thiobarbituric acid (TBA) value measures the formation of a product of the oxidative lipid deterioration - malonaldehyde. Because of the mechanism of lipid oxidation itself, this measurement is not sensitive in early stages of rancidity, being only of value in those advanced stages where there is no doubt about the consumers rejection of the product. However it is a direct method which does not require lipid extraction and in those products where a certain degree of rancidity is acceptable, it can be of value (Banasihan, 1985).

For highly rancid products, the TBA value can, in the same way as the peroxide value, decrease after reaching a maximum (Banasihan, 1985).

Another way of measuring lipid degradation is by the so called acid value (AV), or as it is sometimes reported, the percentage free fatty acid (FFA), which is approximately equal to half the AV. The AV is a measure of the extent to which the glycerides and phospholipids have been hydrolysed under the action of lipase or phospholipase enzymes or non-enzymically.

There are two reasons why the test can be of value in frozen fish quality studies. Firstly, because this hydrolytic process is almost entirely non-bacterial, the measurement of such activity is indirectly an assessment of the frozen storage regime. Secondly, because free fatty acids are known to interact with

proteins the AV may be expected to follow closely other tests related with textural changes (Partmann, 1977). Again, it is a time-consuming test because it requires the extraction of lipid from the fish product.

2.3.2.2 Physical methods

The tests based on physical characteristics of the muscle are mainly related with the textural changes that occur during frozen storage. Amongst these, the most important ones are, the cell fragility test, the water holding capacity measurement and the objective measurements of texture, using mechanical devices, such as the "Texturometer" and the "Instron Universal Testing Equipment".

The cell fragility test consists of the optical measurement of the average state of subdivision of a small piece of muscle, homogenised under carefully standardised conditions (Love and Mackay, 1962). However the results obtained are so affected by the biological condition of the fish, state of bacterial spoilage and pH of the fish muscle at the moment of death, that they need very careful interpretation, and should normally be coupled with results of other tests (Love, 1983).

The water holding capacity is a measure of the amount of water held by the muscle under standardised conditions and during a determined period of time: the fluids which escape from the muscle are referred as "drip". The most widely accepted method of measuring water holding capacity of fish muscle is the so-called "centrifuge drip" method developed by Wierbicki, Kunkle and

Deatherage (1957) and which has been subject to several modifications.

Performed under ideal conditions, this method should lead to reliable results, but there are so many factors involved that this is not the case, especially with frozen fish (Gould and Peters, 1971).

Among the different equipment devised for the objective measurement of toughness of foodstuffs, is the "Texturometer", whose results are nowadays considered to correlate poorly with sensory assessments (Torry Research Station, 1984).

The "Instron Universal Testing Equipment" has gained worldwide acceptance as a useful instrument for the objective assessment of textural characteristics, particularly of meat. It can be set to measure the perpendicular force applyed to break apart one piece of fish muscle, in a way similar to a much simplified chewing process. Relatively little has been reported on such texture measurements of fish, but it is generally thought that fish muscle fibres are much too small for such measurements to be sucessful (Love, 1983).

However, some experiments have revealed a high correlation between shear force and sensory texture score, on cooked samples, providing that an adequate number of samples was analysed to minimise sampling variability (Licciardello et al., 1982). On the contrary, other authors (Racicot et al., 1984) working with the Instron using a Kramer Shear Press Cell, modified to six blades, on steamed samples, report no correlation between the results and the increase in toughness. As they say: "apparently, the sensory panel is measuring other attributes of mouth-feel that are not being analysed by the Instron test".

2.4 Previous work on the quality of frozen hake

The common name "hake" is used to describe a wide range of members of the Gadiformes, some of them so different from each other that they are included by some authorities in two distinct families, the Gadidae and the Merlucciidae (Moyle and Cech, 1982).

The gadoid hakes, genera Phycis, Urophycis, show the characteristics that define the family, namely a caudal fin, which is separate from the dorsal and anal ones, with the dorsal fin divided into two or three sections. The fins have no spines, the swimbladder is physoclistous (i.e., no duct between swim bladder and gut) and they have a chin barbel. The merluccid hakes, genus Merluccius, differ from the gadoid ones by having one or two dorsal fins, one anal fin, spines on the first dorsal fin and no chin barbel. Hakes of both families are found at moderate depths over much of the world, near coastlines (Watkin, 1976; Leal, 1984).

A few of the hake species are, in some countries as prized as cod or even preferred to it because of their delicately flavoured, slightly soft textured flesh.

The hakes now rank among the most important commercial fishes of the world because of their abundance and ease of capture with trawls.

The hake species in general, have a poor reputation for keeping quality in fresh and frozen storage, for the flesh tends to suffer from excessive softening during chill storage (Hiltz et al., 1976) and on the contrary to develop a tough texture during frozen storage.

Those species which are relatively large fish, as for example, Merluccius merluccius (average 75 cm long) have a very definite market among Portuguese, Spanish and French consumers, being commonly sold either frozen or in the fresh trade.

In the case of such species, problems of over-exploitation of the fishing grounds has decreased the supply and increased the costs, as happened with other lean, white-fleshed species, such as cod and haddock (Licciardello et al., 1981).

Other species, particularly those which are smaller fish such as Merluccius bilinearis (average 30cm long) and Urophycis chuss (red hake) have been underexploited, at least in some regions, and if these could be sucessfully processed, a potentially strong market might be created (Hiltz et al., 1976; Licciardello et al., 1982).

As happens with many gadoid fish species, all the hake species already studied undergo undesirable textural changes during frozen storage which limit their storage lives (Regenstein, Samson and Timberlake, 1981; Rivero, 1981; Babbitt et al., 1972; Hiltz et al., 1976). Most of the published work on hake species has been using filleted or minced fish, with the exception of the work by Rivero (1981), where Merluccius hubbsi was frozen as whole fish.

Among all gadiformes species studied, red hake suffers the fastest deterioration with cod and haddock the least susceptible. Silver hake (M. bilinearis) gives a spoilage pattern somewhat in between these two extremes (Hiltz et al., 1976).

During frozen storage, Hiltz et al. (1976) found that silver hake (M. bilinearis) keeps better than red hake (U. chuss,) showing no signs of deterioration for up to 6 months at -26°C. At

-10°C, rapid and extensive deterioration occurred, as indicated by changes in concentrations of DMA and free fatty acids and by the decrease in extractable protein. Sensory tests indicated the development of slightly rancid off-flavours, although textural toughening was the main factor in reducing acceptability.

Similar experiments carried out on M. hubbsi showed that the values of total volatile bases, TMA, TBA and PV gave no indication of significant differences between fillets stored at -19°C and -25°C. The DMA analysis also gave similar results up to 3 months storage, while for longer storage periods the fillets stored at the lower temperature had a lower DMA content, as expected. The protein extractability decreased relatively slowly when compared to data on other species and an almost linear correlation was found between the DMA formation and the decrease in protein extractability, during storage at -19°C. The freshness of the raw material (up to 9 days) seemed to have no influence on the quality of the frozen fillets, which had a storage life at -19°C of approximately one year (Rivero, 1981).

Frozen storage life of red hake fillet blocks was estimated by sensory evaluation to be 150 weeks at -28.8°C, 71 weeks at -20.5°C, 25 weeks at -15°C, 7.5 weeks at -12.2°C and 2.7 weeks at -6.6°C (Licciardello et al., 1982).

The same authors stated that DMA and formaldehyde contents and also shear force measurement correlate very well with sensory texture scores and could become useful indicators for predicting textural quality. On the contrary, centrifuged drip did not appear to be sufficently sensitive or reliable for assessing textural quality. At temperatures above -20.5°C, they found a good correlation between extractable protein and sensory texture

score but such correlation decreased with lower storage temperatures.

Also working with red hake, Lundstrom, Correia and Wilhelm (1981) studied the role of oxygen concentration on the production of DMA and formaldehyde. They concluded that DMA production was greater in fillets stored anaerobically than in fillets stored aerobically, due to lack of inhibition by oxygen, and that mincing the fillets and storing them anaerobically accelerated DMA production, due to increased contact between substrate and enzyme.

When studying the effect of heat and additives on the shelf-life of frozen red hake, Regenstein, Samson and Timberlake (1981) stated that the amount of DMA present does not directly reflect the changes in the texture of the material. Their work on the use of additives did not prove to have much success and although heating up to at least 60°C decreased the levels of DMA formation, the products thus obtained were not as good as unstored controls or even frozen controls stored at -45°C. With the present knowledge about the underlaying mechanism of texture change, these authors suggested that storing at lower temperatures is the best method of maintaining a high quality product over a reasonable period of time.

Because of the economic constraints of operating colder than normal storage facilities, the search has continued for an alternative method. Racicot et al. (1984) reviewed the different unsucessful trials on using several inhibitors and pointed out the feasibility of the use of oxidising agents. H_2O_2 proved to be effective in preventing textural toughening. The authors suggest that a combination of such an oxidising agent and lower

temperature would enhance the shelf-life of minced red hake blocks, and would be commercially feasible, because of the improved texture and whiter appearance. The problem still remains unsolved in the case of fillets, where the incorporation of H_2O_2 presents substantial difficulties.

During frozen storage at -20°C, Babbitt et al. (1972) observed the production of DMA and formaldehyde in M. productus. This formation was greatly accelerated in minced muscle and was not altered by exclusion of oxygen. The rapid decrease in the amount of total extractable protein corresponded to the increase in formaldehyde and DMA.

In an investigation to assess the significance of total volatile bases for evaluating changes in the quality of frozen M. hubbsi stored at -7°C, -15°C and -32°C, Almandos et al. (1984) concluded that the increases in total volatile bases correlate with increases in DMA content. The rate of increase of both indices was significant at -7°C and negligible at -32°C.

An overview of all the reported work on hake species reveals that much can still be done, with respect to understanding and minimising quality deterioration during frozen storage, in order to enhance the economic feasibility of hake as a frozen commodity.

2.5 Aims of the present work

Frozen hake is the most widely accepted frozen commodity among Portuguese consumers, but its quality varies from the highly prized big fish, to products of a very inferior quality, often small fish, where the major problem is often the development of a tough, dry and chewy texture.

Retailers show different attitudes concerning the handling and storage of the large and the small fish, the former normally being sold in specialised frozen food shops, the latter in every supermarket. The present work is based on the hypothesis that the quality differences observed can be attributed to the different storage conditions experienced by the products with particular regard to the storage temperature.

Hake is known as being a naturally soft fish species, unable to withstand the effects of freezing as well as other lean fish species, such as, for instance, cod. Because of the difficulties that such characteristics cause, some work has already been done on hake species, such as red hake (*Urophycis chuss*) and silver hake (*Merluccius bilinearis*). However, the reported results show great differences between species, which is understandable, considering that the common designation of hake is used to describe species so far appart that they are not even members of the same family (see Section 2.4).

Most of the research reported on frozen storage of such species is concerned with the quality of either fillets or minced products, where spoilage may follow different patterns to those for whole gutted fish.

The present work is mainly concerned with the quality changes

that occur during the frozen storage of whole Merluccius merluccius, because this is the most common hake species in Europe (normally frozen in blocks of whole gutted fish) and because there is no information available on changes during frozen storage of this species. The proposed aims can be divided into three areas:

1. Selection of the most appropriate methods to assess the quality of frozen whole hake (M. merluccius).

2. Elucidation of the storage factors responsible for loss of quality of whole hake (M. merluccius) during frozen storage at the high and fluctuating sub-zero temperatures as experienced during distribution and marketing of frozen fish in warm temperate countries such as Portugal.

3. Clarification of the mechanism of protein denaturation at the molecular level, in the species under study.

3. Experimental

3.1 Materials

3.1.1 Fish samples

The experiments were carried out on hake, Merluccius merluccius (L.), obtained from the Grimsby fishdocks. Three different batches of fish were used: one of them caught in February (fishing ground unknown), and the others in May and August, both caught in the Irish Sea and landed in Maillaig, West Scotland.

All the fish used, which had been iced since capture, were of good quality i.e., eyes with convex black pupil and translucent cornea, bright red gills, no bacterial slime and bright opalescent sheen. The fish had a fresh, "seaweedy" raw fish odour, firm texture, were elastic to finger touch, had bluish translucent flesh, and showed no reddening along the backbone and no discolorations. In the May batch, some fish showed physical damage and were discarded.

Specimens of total length between 35 and 50 cm were selected.

3.1.2 Equipment

The fish were frozen using a Jackstone laboratory horizontal plate freezer.

Accurate weighing of samples and reagents was achieved using a Mettler AE 163 4 place digital balance.

Homogenisation of samples was carried out using an Ystral top drive homogeniser.

Centrifuging was performed using either an MSE bench centrifuge or, when refrigeration was necessary, an MSE Europa 24 M centrifuge.

Measurements of pH were carried out using a Jencons PHM 4 pH/temperature meter.

All spectrophotometric measurements were carried out using a Spectroplus D spectrophotometer from MSE Scientific Instruments.

The objective measurements of texture were performed using an Instron food testing instrument, model 1140, equipped with a Warner-Bratzler meat shear.

The gas-liquid chromatography for amine detection was carried out on a Shimadzu GC R1A equipped with an FID detector, an automatic sampler AOC-7 and a data processor unit RPR-G1.

The gas-liquid chromatography for fatty acid profiling was carried out on a Perkin Elmer Sigma 3D chromatograph, equipped with a FID detector and using a Sigma 15 data processing station.

Nitrogen determinations for protein analysis were carried out using a Tecator Kjeltec Auto Analyser, model 1030, after digestion of the samples on a Tecator Digestion System 6.

3.2 Experimental procedures

3.2.1 Freezing technique

Freezing was carried out on the day the fish were received. The fish were packed in groups of five, in heat-sealed high density polythene bags and frozen in the plate freezer to a temperature of -20°C (about 1 hour). The temperature was monitored by placing thermocouples in the bodies of different fish in each batch.

3.2.2 Frozen storage

Immediately after leaving the plate freezer, the fish were transferred to chest or vertical freezers at the different experimental or control temperatures.

In the beginning of this work the lowest temperature freezer available for control storage was at -30°C, but this was replaced later on by a freezer at -60°C.

The experimental storage temperatures were -6°C, -11°C, both ±1°C, and a daily fluctuating temperature between -16°C and -4°C, (referred to in the results section as FT storage), obtained using a time controlled switch, 14 hours on and 10 hours off per day.

All the temperatures referred to above were measured by placing thermocouples in between closely packed bags of fish, and were not the air temperatures inside the freezers.

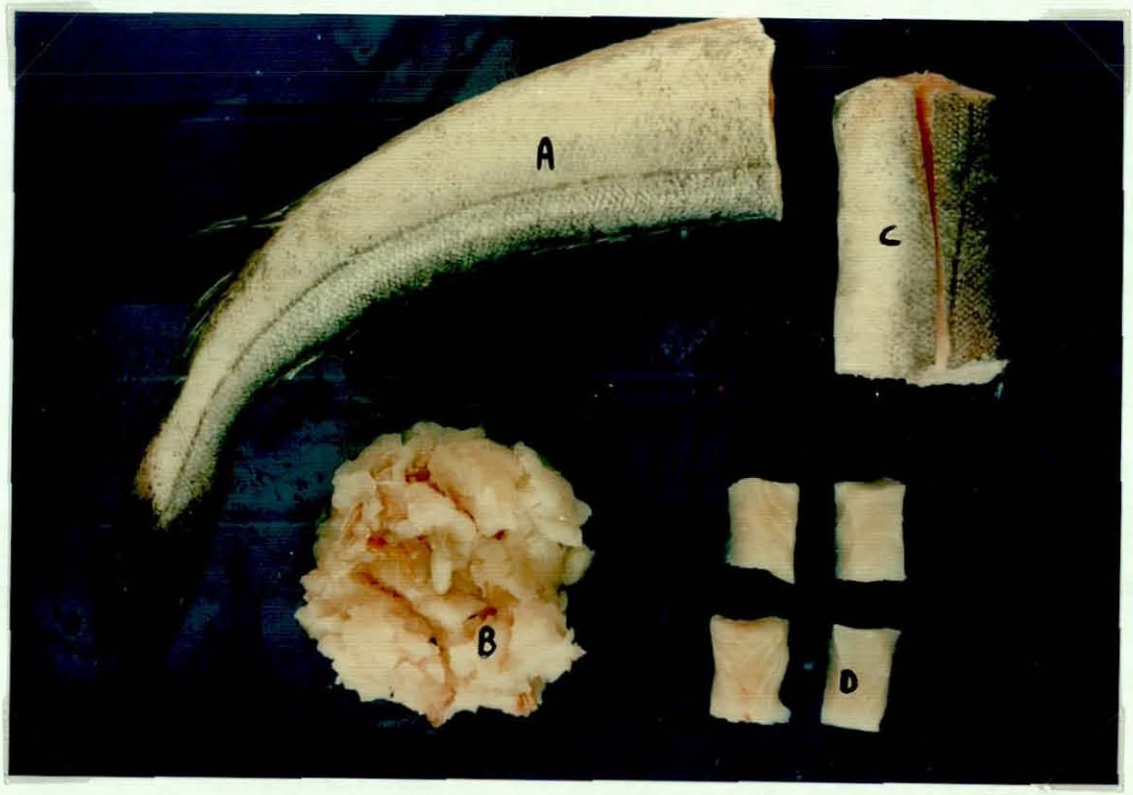
3.2.3 Sample preparation

After storage, the fish were thawed overnight inside a refrigerator at about 5°C. The region between the end of the belly cavity and the tail (A) was filleted, the fillets were chopped and the chopped material (B) kept in plastic containers, stored frozen until used (within three days) for analysis, (see Fig. 10).

The samples for sensory evaluation were 1 cm thick steaks cut from this same region while the fish was still frozen using a band saw and thawed at room temperature for approximately 3 hours.

From the dorsal region, along the belly cavity (C), blocks of fish flesh (2 cm x 1 cm x 0.5 cm) were cut (D) for objective textural measurements.

Fig. 10: Fish sample preparation



3.3 Sensory evaluation

Fish steaks, obtained as described on Section 3.2.3 were steamed for 15 minutes in Pyrex containers, kept warm and served to a trained taste panel of 6 to 12 members.

In order to try to overcome fish to fish variation, each sample consisted of two half steaks from different fish. One or two samples were presented accompanied by a control (identified for the May fish and not identified for the August fish).

Sample identification was by geometrical figures or three figure numbers.

Panellists were served an apple or a piece of white bread and recommended to use it, between each trial.

The criteria used for the judge selection, was their previous experience and the consistency of their judgements during the training runs.

Panellists were asked to assess the degree of toughness (as the difficulty in breaking down the fish flesh during the mastication process) on a 5 point non-hedonic scale, running from "tender" to "very tough" and also to assess the intensity of off-flavours (both off-tastes and off-odours) on a 5 point non-hedonic scale, running from "none" to "very strong" (see attached taste panel score sheets, Figs 11 and 12).

Fig. 11: Taste panel score sheet for toughness

Panellists name:..... Date:.....

TASTE PANEL SCORE SHEET FOR HAKE

For each of the samples, which consist of two pieces of flesh, please indicate your assessment of the degree of toughness (difficulty in breaking down the fish flesh during the mastication process) by writing the sample code against the most appropriate description.

(Please note that there is no limit on the number of sample codes that can be written against any description.)

4	VERY TOUGH
3	TOUGH
2	SLIGHTLY TOUGH
1	NEITHER TENDER NOR TOUGH
0	TENDER

Thank you for your assistance.

Fig. 12: Taste panel score sheet for off-flavours

Panellists name:..... Date:.....

TASTE PANEL SCORE SHEET FOR HAKE

For each of the samples, which consist of two pieces of flesh, please indicate your assessment of the intensity of off-flavours (both off-odours and off-tastes) by writing the sample code against the most appropriate description.

(Please note that there is no limit on the number of sample codes that can be written against any description.)

5	VERY STRONG
4	STRONG
3	MODERATE
2	SLIGHT
1	NONE

Thank you for your assistance.

3.4 Analytical procedures

3.4.1 Proximate analysis

The true protein was calculated as (total nitrogen - non-protein nitrogen) x 6.25, using results obtained as in Section 3.4.7. The lipid was determined by a standard Bligh and Dyer procedure (see Section 3.4.4). Moisture was determined by oven-drying according to the EEC method. Ash was determined at 500°C according to the EEC recommended method.

3.4.2 Fatty acid profiling

Methylation of lipid samples: Lipid samples were obtained as described below in Section 3.4.4 and methylated using boron trifluoride according to the British Standard Methods of Analysis (British Standards Institute, 1980).

Into a 50 ml pear shaped flask, 100-250 mg of lipid were accurately weighed, and anti-bumping granules and tetrahydrofuran (THF) (1ml) were added. The flask was fitted to a condenser over a water bath, and 0.5 M methanolic sodium hydroxide (4 ml) was added through the condenser.

The mixture was boiled under reflux until the fat droplets disappeared (5-10 min). After slight cooling, boron trifluoride solution was added (5ml) and the reflux continued for a further 2-3 minutes. After cooling again, petroleum ether was added (1 ml) and boiling continued for 1 minute. After removal from heat, saturated NaCl solution was added (5 ml), with slight shaking.

After removing the flask from the condensing apparatus, petroleum ether was added again (4 ml). After strong shaking, the

contents of the flask were transfered to a 250 ml separating funnel. The lower layers were run out from the funnel and the upper layer transfered into a 100 ml separating funnel.

The extraction was repeated twice more with petroleum ether (3 ml at each time), the extracts combined and washed with 2% potassium bicarbonate (4 ml). After drying with anhydrous sodium sulphate, the organic solution was filtered. The extract obtained was used directly for GC work or, when necessary, concentrated by evaporation of solute on a rotary evaporator.

GC conditions: Chromatography was performed applying 0.3 µl injections (using an SGE 0.5 ml stainless steel needle syringe) to a 25 m BP1 non-polar capillary column, 0.32 mm internal diameter. Column temperature was isothermal 210°C, detector (FID) temperature was 280°C. Carrier gas (helium) flow rate was approximately 0.9 ml/min.

3.4.3 pH

The pH of homogenates obtained for water uptake capacity measurement (Section 3.4.8) was measured using a digital pH meter with a glass electrode.

3.4.4 Peroxide value

Lipid was extracted according to the modified Bligh and Dyer method (Hanson and Olley, 1963) but using 0.01% BHT in the chloroform to avoid any oxidation during the extraction. A rotary evaporator and a vacuum oil pump were used to achieve total evaporation of the solvent.

The peroxide value was determined according to the standard procedure but, because of the relatively low lipid content of the fish only 0.1 g samples were used and the procedure modified accordingly.

The modified procedure was as follows:

Into a ground glass stoppered flask, fish lipid (W g, about 0.1 g), powdered potassium iodide (0.1 g) and solvent mixture (2 volumes of glacial acetic acid and 1 volume of chloroform) (2 ml) were added. The flask was allowed to remain in the dark at room temperature for 30 minutes. Potassium iodide solution (2 ml of 5% solution) and H_2O (5 ml) were added to the flask. The liberated iodine was titrated (using a microburette) with 0.002 M thiosulphate solution using 1% starch solution as indicator.

A blank was carried out without the lipid.

The peroxide value (PV) was calculated using the following equation:

$$PV = \frac{2 \times (S - B)}{W} \text{ mEq/kg of lipid}$$

where S = sample titre in ml,

B = blank titre in ml,

W = grams of lipid.

3.4.5 Thiobarbituric acid value

The method applied was based on that proposed by Sinhuber and Yu, modified by Tarladgis and further modified by Banasihan (1985).

Sample determination: To an homogenising vessel, chopped sample (10 g) and distilled H_2O (50 ml) were added. The mixture was homogenised for 30 seconds. In a round bottom, double-necked flask, the following was added to the homogenate: H_2O (46.5 ml), antioxidant mixture (0.3 g butylated hydroxyanisole (BHA) in 5.4 g of propylene glycol mixed with 0.3 g butylated hydroxytoluene (BHT) in 4 g of Tween 40) (5 drops), 0.2% ethylenediaminetetracetic acid disodium salt solution (EDTA) (1 ml), 4 M HCl (2.5 ml) and anti bumping granules (~ 20). The flask was wrapped with aluminium foil, placed on a heating mantle and connected for downward distillation under nitrogen. The heat was controlled in order to obtain 50 ml of distillate in 20 minutes. In a test tube with ground glass stopper, the distillate (5 ml) and thiobarbituric acid reagent (5 ml of 0.02 M TBA in 90% glacial acetic acid) was heated in a boiling water bath for 35 minutes. After cooling under tap water for 10 minutes the absorbance was read at 532 nm using a 1 cm cuvette.

Assuming a 70% recovery during the distillation process, the TBA value was calculated using the following equation:

TBA value = 5000 T x 100/70 mg of MA/kg of flesh

where T= concentration of malonaldehyde in mg/ml obtained from the calibration curve.

A calibration curve was obtained using solutions of 1,1,3,3-tetraethoxypropane equivalent to 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ug/ml of malonaldehyde (MA), which were reacted with the thiobarbituric acid reagent as described above. Absorbance was plotted against malonaldehyde concentration.

3.4.6 Free fatty acid

The free fatty acid determination was carried out according to the method described by Banasihan (1985), slightly modified in order to account for the very small samples of lipid used.

Lipid extraction was accomplished as described above in Section 3.4.4.

In a small beaker, the lipid sample (W g, about 0.1 g) was dissolved in a mixture of diethyl ether (2.5 ml) and ethanol (2.5 ml). Using a microburette, the acid in solution was titrated with 0.1 M NaOH, using phenolphthalein as indicator. A blank was carried out without the lipid.

Percentage free fatty acid (FFA) was calculated as follows:

$$\% \text{ FFA} = \frac{2.805 \times (S-B)}{W} \times 100$$

where:

S = sample titre in ml

B = blank titre in ml

W = grams of lipid.

This assumes that the average molecular mass of the fatty acids is that of oleic acid.

3.4.7 Extractable protein

Percentage extractable protein, i.e., the percentage of the protein that is soluble in an ice cold 5% solution of sodium chloride, was measured according to the method of Cowie and Little (1966).

Total nitrogen: To a Kjeldahl digestion tube, chopped flesh (W g, about 1 g), two Kjeltabs (3.5 g K_2SO_4; 0.4 g $CuSO_4.5H_2O$) and 98% sulphuric acid (10 ml) were added. After about 45 minutes of digestion the sample was diluted with 75 ml of distilled H_2O and 2.5 ml of silicone antifoaming agent was added. The digestion tube was connected to the distillation head and ammonia was determined.

In the determination, an alkali pump dispensed alkali into the sample tube and the ammonia produced was carried by steam into a receiver solution of boric acid and indicator (methyl red and bromo-cresol green) in the titration vessel. Hydrochloric acid solution (0.1 M) was continuously dispensed into the titration vessel until the acid-base equivalence point was reached (green indicator changes to red). The volume of acid required is read off the digital display.

The percentage of nitrogen was calculated as follows:

$$\% \text{ Nitrogen} = \frac{0.1401 \times (S - B)}{W}$$

where:

S = ml of 0.1 M HCl for sample

B = ml of 0.1 M HCl for blank

W = g of sample

Non-protein nitrogen: To an ice-cooled homogenising vessel, chopped sample (2 g) and 15% ice-cold trichloroacetic acid (50 ml) were added.

The mixture was homogenised for 30 seconds and filtered through N°1 Whatman filter paper. The filtrate (15 ml) was pipetted into a Kjeldahl digestion tube, digested, analysed and the percentage of nitrogen calculated as detailed above.

Soluble nitrogen: To an ice-cold homogenising vessel, chopped sample (1 g) and 5% ice cold NaCl solution in phosphate buffer, pH 7 (50 ml), were added. The mixture was homogenised for 30 seconds and the homogenate centrifuged at 4000 rpm for 30 minutes at 0°C. The supernatant (15 ml) was pipetted into a Kjeldal digestion tube, digested, analysed and the percentage of nitrogen calculated as detailed above.

The extractable protein nitrogen was calculated as a percentage of true protein nitrogen by using the expression:

$$\% \text{ Extractable protein (EP)} = \frac{\text{Soluble Nitrogen} - \text{Non protein nitrogen}}{\text{Total Nitrogen} - \text{Non protein nitrogen}} \times 6.25 \times 100$$

3.4.8 Water uptake capacity

Samples of chopped fish flesh (10 g) were homogenised with distilled H_2O (20 ml) for 30 seconds. The homogenate was filtered through N°1 Whatman filter paper and the filtrate collected after 30 minutes was measured.

$$\% \text{ Water uptake capacity} = \left(1 - \frac{\text{volume of filtrate}}{\text{volume of } H_2O \text{ added}}\right) \times 100$$

3.4.9 Water holding capacity

Water holding capacity was measured as "centrifuge drip" in thawed fish samples according to the method described by Del Valle and Gonzalez-Inigo, as modified by Hasan (1984).

Samples of fish flesh (10 g) were freed from skin and bones,

avoiding all possible structural damage and centrifuged at 3000 rpm for 30 minutes. The water released from the flesh was collected in a pre-weighed beaker using a Pasteur pipette and weighed.

$$\% \text{ Water holding capacity} = \left(1 - \frac{\text{weight of water}}{\text{weight of sample}}\right) \times 100$$

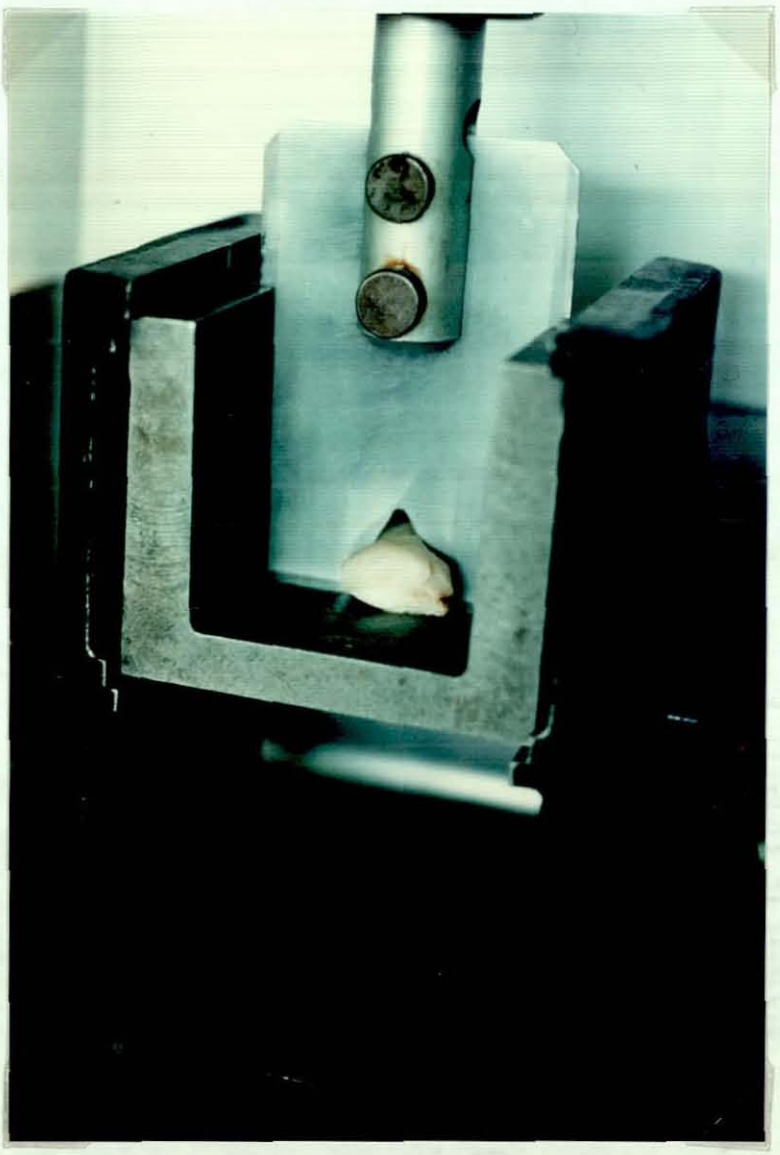
3.4.10 Instron objective texture measurements

An objective evaluation of texture was carried out using the "Instron testing machine" on the fish muscle sample blocks obtained as described on Section 3.2.3

The muscle samples (2 cm x 1 cm x 0.5 cm, 4 to 6 from each specimen) were steamed for 15 minutes in a Pyrex container, allowed to cool and tested in such a way that the machine blade always moved transversally through the muscle fibres (See Fig. 13).

The maximum shear force, required to move through each block, separating it into two fragments, was recorded and expressed in kg.

Fig.13: Fish sample being cut by the V-shaped lower edge of the descending Warner-Bratzler shear blade



3.4.11 Trimethylamine and dimethylamine determination

The determination of trimethylamine and dimethylamine was performed on perchloric acid extracts, prepared according to the method of Mackie and Thomson (1974). The extracts were steam distilled using a method based on that given by Keay and Hardy (1971).

In an Atomix blender, chopped fish flesh (50 g) was homogenised with 0.6 M perchloric acid (150 ml) for 30 seconds. The homogenate was filtered through a 2V Whatman filter paper. To an aliquot of the perchloric acid extract (50 ml) 20% NaOH was added (6.5 ml) and the mixture was steam distilled using a Markham apparatus, at such a rate that 40 ml of distillate were collected over 5 minutes. The distillate was collected into a graduated flask containing 1 M HCl (3 ml) and diluted with distilled water up to 50 ml.

The samples obtained were stored at -60°C in air-tight flasks. Immediately before analysis, the pH of the samples was adjusted to 12 by adding 1 M NaOH, and 2 µl injections were used.

The chromatography of the amines was carried out using a glass column (6 m long, i.d. 3mm) packed with a porous polymer column packing material based on 2,6-diphenyl-p-phenylene oxide (Phase-Sep Tenax-GC). The injection and column temperatures were kept constant at 120°C and the carrier gas (nitrogen) flow rate was 25 ml/min.

3.4.12 Rapid determination of volatile amines

The rapid determination of volatile amines was carried out according to the method described by Storey *et al.* (1984) utilising a solid state Taguchi gas sensor which is a sintered bulk semicondutor composed mainly of tin dioxide.

Fish acid extracts were prepared as described on Section 3.4.11 and the amines, released by injecting a 5 µl aliquot of extract into 30% NaOH (2ml) in the reaction chamber, were continuously flushed by an airstream that carried them over the gas sensor.

The sensor's resistance decreases due to the presence of amines and such change in resistance was measured using the peak reading digital display, described by Storey and Storey (1981).

The system was calibrated by means of injections of perchloric acid as a blank and standard solutions of TMA in perchloric acid.

3.4.13 Total viable count

The media and diluents were sterilised by autoclaving at 121°C, 1.0×10^5 Newtons/m^2, for fifteen minutes. The microbiological work was carried out in an aseptic manner.

Samples of 10 ± 0.1 g were weighed, added to Ringer solution (90 ml) to give a dilution of 10^{-1} and homogenised in the Stomacher for approximately 2 minutes. Succeeding dilutions were made by pipetting 1 ml of the blended material into Ringer solution (9 ml) to give a dilution of 10^{-2} and again 1 ml to 9 ml for sucessive serial dilutions to 10^{-7}.

Finally, 0.1 ml of each dilution was transfered to the surface of a "plate count agar" plate (with 1% NaCl added) and spread using a glass spreader. After absorption of the inoculum, the plates were incubated for 48 hours at 25°C.

Plates showing between 30 and 300 colonies per plate were counted and the colony forming units (cfu) calculated as:

$$\text{cfu/g} = \frac{\text{plate count x dilution factor}}{\text{size of inoculum}}$$

4. Results and discussion

4.1 Experimental design

Little information about the composition of Merluccius merluccius was found in the literature survey, hence before commencing on the storage trials, proximate analysis data were obtained, as a basis to understanding spoilage processes during frozen storage.

The information obtained during the literature survey was contradictory in many cases in respect of the value and reliability of the parameters used to assess frozen fish quality. Preliminary experimental work was therefore carried out, designed to indicate which of the cited tests would be most helpful in the present study, and simultaneously to get some background information on the characteristics of the species and its response to cold storage.

In this preliminary experimental work, hake was stored at -5°C and at -12°C, and controls were stored at -30°C. The following tests were carried out on stored fish at regular intervals until the -12°C stored ones became unnacceptable (according to the taste panel results): sensory evaluation, pH determination, peroxide value, thiobarbituric acid value, free fatty acid, extractable protein, water uptake capacity, water holding capacity, objective texture measurements using the Instron and DMA and TMA determination.

Two different experimental runs, using the selected tests, were carried out. In one of them, fish caught in May were stored at -6°C and -11°C and compared with controls obtained by storing

fish of the same batch at -60°C. Initial values were obtained directly after freezing. Because of the short storage lives expected under such conditions, samples were taken twice a week, whenever it was possible, in order to follow as closely as possible any changes occurring.

In the other run, fish caught in August were stored at -11°C and under a daily temperature fluctuation between -4°C and -16°C. Here again, sampling was carried out as often as possible. Controls were obtained as in the previous run. Initial values were obtained for unfrozen fish.

4.2 Chemical composition of hake

The results of the proximate analysis, carried out on hake caught in February are presented on Table 7, together with values for protein and lipid contents obtained for fish caught in May and in August. The detailed results are given in the Appendices (Sections 6.1, 6.2 and 6.3).

The results obtained are in line with those reported in the literature, although with some differences. Murray and Burt (1969) gave values of 0.4 to 1% fat, which are lower than those reported here, but since these authors do not indicate the experimental method used, such differences could be accounted for by a different method of extraction of the lipid from fish or even a different sampling technique. They reported that the crude protein content of M. merluccius flesh is 17.8 - 18.6% which is in line with the values reported in this study for true protein, i.e., the latter are about 2% less, corresponding to a non-protein nitrogen content of about 10% of the total nitrogen.

Rivero (1981) reported for Merluccius hubbsi a protein content of 16.4%, without stating whether it was crude or true protein. This author reports a large variation in the chemical composition between fish and marked seasonal variation.

The fish used in this study were caught in February, pre-spawning fish, in May, fish in spawning, and in August, post-spawning fish. The February fish were found to have significantly higher levels ($p < 0.1\%$) of both lipid and true protein, than the May fish, as might be expected.

The August fish had significantly higher ($p < 0.1\%$) lipid levels than the May fish, as expected for post-spawning fish, but

Table 7: Chemical composition of hake*

Month of catch	Nº of fish analysed	Moisture (%)	Lipid (%)	True Protein (%)	Ash (%)
Feb.	10	79.2 (0.9)	2.3 (0.6)	18.1 (0.8)	1.7 (0.8)
May	85 (lipid) 87 (protein)	-	1.5 (0.5)	16.4 (1.0)	-
Aug.	64	-	2.2 (0.8)	16.5 (1.2)	-

* The results are averages of the values obtained, the values in parentheses are standard deviations.

the true protein content did not differ significantly ($p>5\%$). This seasonal variation in composition has to be borne in mind when comparing results obtained from the May and August fish batches.

The production of rancid off-flavours does not appear to be a problem in respect of frozen hake quality, but, since lipid oxidation products can be involved in protein denaturation, and hence in texture changes, the fatty acid profile of hake lipid was determined, in order to assess its susceptibility to oxidation. The results showed the presence of large amounts of long chain highly unsaturated fatty acids, eg. 28.6% of 22:6 and 10.7% of 20:5, which are very susceptible to rancidity development (see Table 8).

Although the presence of rancid off-flavours appears unimportant in frozen hake storage, lipid oxidation products are likely to be produced (McGill, Hardy and Gunstone, 1977) and may be playing an important role in the mechanism of protein denaturation.

Table 8: Fatty acid profile of hake lipid*

Fatty acid	14:0	15:0	16:0	17:0	18:0	22:0	24:0	16:1	18:1
%	1.9 (0.5)	0.2 (0.2)	15.5 (0.2)	0.3 (0)	3.8 (0.1)	2.4 (0.1)	0.2 (0.3)	2.9 (0.1)	13.9 (0.7)

Table 8: (Continued)

Fatty acid	20:1	18:2	18:3	20:4	20:5	22:5	22:6	not identified
%	3.8 (0.4)	0.5 (0.9)	1.0 (0.1)	0.7 (0.2)	10.7 (0.1)	1.3 (0.1)	28.6 (0.3)	11.1

* The values given, which are averages of three replicates (standard deviations given in parentheses), are percentages of total fatty acid methyl esters determined by GC.

4.3 Quality assessment methods for frozen hake

4.3.1 Selection of quality assessment methods for storage experiments

From the literature reviewed there is no clear indication of which methods are most reliable and accurate for assessment of frozen fish quality in general and the quality of frozen hake in particular. Thus, an initial evaluation of methods was necessary, in order to obtain the most useful results from the storage trials in this work and to provide the basis for further investigations on the keeping quality of frozen hake.

The methods tested were: pH; peroxide value; thiobarbituric acid value; free fatty acid content; extractable protein; water uptake capacity; water holding capacity; shear force, using the Instron; DMA and TMA contents. The selection of methods for the present investigation was based on analysis of the results and comparison with sensory assessment results, for both off-flavours and texture.

The muscle pH values, shown in Table 9 did not show any significant changes with storage period. Hence pH was not monitored during the storage trials. However, since the initial values can interfere with the rate of textural changes and protein alterations (Kelly et al., 1966), the initial pH values of samples from each new batch were determined. These initial pH values were all in the range 6.5 to 6.6.

The results obtained for peroxide and thiobarbituric acid values are shown in Tables 10 and 11. Both peroxide value and thiobarbituric acid value results for the -5°C and -12°C stored

Table 9: pH values during storage at -5°C and -12°C*

Sample storage temperature	Time of storage (days)				
	0	10	18	25	32
- 5°C	6.4 (0.11)	6.5 (0.06)	7.2 (0.07)	6.5 (0.03)	6.8 (0.07)
- 12°C	6.4 (0.11)	-	6.6 (0.04)	-	6.5 (0.08)

* The values are means of determinations on three fish, standard deviations are given in parentheses.

Table 10: Peroxide values (mEq/kg) during storage at -5°C, -12°C and -30°C (controls)*

Sample storage temperature	Time of storage (days)			
	10	18	25	32
-5°C	1.6 (0.0)	1.0 (0.8)	1.0 (0.6)	4.0 (0.9)
-12°C		3.9 (1.3)		5.6 (1.2)
- 30°C	0.6	2.2	0.2	3.9

* For the samples stored at -5°C and -12°C, the values are means of determinations on 3 fish; standard deviations are given in parentheses. For the controls, the values were obtained on 1 fish.

Table 11: Thiobarbituric acid values (mg MA/kg) during storage at -5°C, -12°C and -30°C (controls)*

Sample storage temperature	Time of storage (days)			
	10	18	25	32
-5°C	8.0 (2.1)	4.3 (2.4)	1.5 (0.5)	2.7 (0.4)
-12°C	-	7.8 (2.6)	-	4.2 (1.3)
-30°C	8.0	8.4	3.1	2.8

* For the samples stored at -5°C and -12°C, the values are means of determinations on 3 fish; standard deviations are given in parentheses. For the controls, the values were obtained on 1 fish.

samples were not significantly different from those obtained for controls, at levels below those normally considered to indicate the onset of rancid off-flavours in lean fish.

It has been reported (Rivero, 1981) that M. hubbsi fillets stored at -19°C and -25°C show peroxide values higher than 40 after the third storage month, while remaining acceptable for much longer. Hence the relatively low peroxide values obtained in this experiment indicate that lipid oxidation is unlikely to be of significance in determining sample acceptability. Several authors have tried to use the TBA value to assess the development of rancidity in other hake species (Racicot et al., 1984; Hiltz et al., 1976; Licciardello et al., 1982; Rivero, 1981), with inconclusive results. All authors seem to agree that the PV and TBA values obtained were not indicative of perceptible rancidity in hake species and that textural toughening was the main factor in reducing acceptability. PV and TBA values were therefore not assessed in the main storage experiments.

As discussed in the literature survey, production of free fatty acids might be involved in the development of textural changes as a consequence of protein-fatty acid interactions, although the relative importance of such interactions may vary with the amounts of formaldehyde produced during frozen storage (Castell, 1971). As can be seen in Table 12, during storage the free fatty acid content showed a clear increase for the samples and controls, and so it was decided to retain this test in further work. Hiltz et al. (1976) measured lipid hydrolysis as acummulation of free fatty acids in silver hake (M. bilinearis) stored for two months at -10°C and also found a steady increase during the storage time.

Table 12: Free fatty acid contents (%) during storage at -5°C, -12°C and -30°C (controls)*

Sample storage temperature	Time of storage (days)		
	18	25	32
-5°C	31 (18)	50 (3.4)	82 (24)
-12°C	-	27.6 (9.7)	42.6 (6)
-30°C	8.6	11.8	32.2

* For the samples stored at -5°C and -12°C, the values are means of determinations on 3 fish, standard deviations are given in parentheses. For the controls, the values were obtained on 1 fish.

Practical and instrumental difficulties made some of the results on extractable protein unreliable (particularly those obtained during the middle of the storage period), but the results from the beginning of the experiment and those from the last weeks clearly indicated a decrease in extractable protein during storage as would be expected from the literature (Hiltz et al., 1976; Licciardello et al., 1982; Rivero, 1981). Therefore, it was decided to include extractable protein determination in the main storage trials.

The water uptake capacity method (WUC) appeared to be greatly affected by experimental conditions and the results (Table 13) showed no clear trends during storage. Water uptake capacity was therefore not assessed in the main storage experiments.

In the case of water holding capacity (WHC), measured by centrifuged drip, the results (Table 14) seemed to be a little more promising, but yet too much dependent on its practical execution. The fish muscle started to lose liquid as soon as it was cut and when working with large numbers of samples it was very difficult to avoid biases due to drip loss suffered before centrifuging took place. The changes during storage were small compared with the standard deviations. Although this is a widely used method, several authors have stated that so many factors are involved in determining the WHC that the test is of questionable value (Licciardello et al., 1982; Hasan, 1985). This is probably the reason why most of the work on related hake species did not use this test, and it was decided not to retain it for the main storage experiments.

The results obtained from the objective measurements of texture, as shear force, using the Instron do not show any

Table 13: Water uptake capacity (%) during storage at -5°C, -12°C and -30°C (controls)*

Sample storage temperature	Time of storage (days)		
	18	25	32
-5°C	86.7 (10.4)	48.7 (3.2)	69.2 (3.8)
-12°C	55.0 (8.7)	-	67.5 (4.3)
-30°C	50	54	65

* For the samples stored -5°C and -12°C, the values are means of determinations on 3 fish, standard deviations are given in parentheses. For the controls, the values were obtained on 1 fish.

Table 14: Water holding capacity (%) during storage at -5°C, -12°C and -30°C (controls)*

Sample storage temperature	Time of storage (days)			
	10	18	25	32
-5°C	-	95.3 (1.5)	92.6 (1.5)	90.6 (0.3)
-12°C	-	93.3 (2.1)	-	82.9 (0.6)
-30°C	97.1	93	89	92.5

* For the samples stored at -5°C and -12°C, the values are means of determinations on 3 fish, standard deviations are given in parentheses. For the controls, the values were obtained on 1 fish.

clear trend during the periods of storage (Tables 15 and 16). However, the few authors that have used this method on cooked fish muscle (Licciardello et al., 1981; Licciardello et al., 1982; Racicot et al., 1984 pointed out the need to use a very large number of samples to obtain reliable results. Since the cooked fish assessment relates more directly to consumer perception than uncooked fish assessment and since other authors always used cooked fish, it was decided to withdraw the uncooked fish trials, thus gaining more samples from each specimen for the cooked fish assessment. Some initial experiments gave promising results when using the larger sample size for cooked fish and it was therefore decided to include this assessment in the main storage trials.

No results were obtained on the DMA and TMA contents of the fish flesh during this preliminary series of experiments as the methods of determination by gas chromatography, and by use of a gas sensor, were still being developed, as discussed in the next Section. However, sample extracts were kept for future analysis, since it was initially decided, based on the literature review, to include DMA and TMA assessment in the main storage experiments. Most authors found good correlations between the increase in DMA content and the decrease in the extractable protein (Rivero, 1981; Racicot et al., 1984; Lundstrom et al., 1981; Licciardello et al., 1982; Hiltz et al., 1976; Babbit et al., 1972; Avdalov and Ripoll, 1981; Almandos et al., 1984), although Regenstein et al. (1981) noted that the amount of DMA does not directly reflect the changes in the texture of the material. In addition, the simultaneous use of DMA and TMA has been supported as one of the best ways of assessing deterioration

Table 15: Shear force (kg) for uncooked fish during storage at -5°C and -30°C (controls)*

Sample storage temperature	Time of storage (days)		
	10	18	25
-5°C	1.4 (0.2)	1.09 (0.2)	0.5 (0.1)
-30°C	1.4 (0)	3.27 (0.7)	0.6 (0)

* The values are means of determinations on 3 fish with 4 determinations on each fish; standard deviations are given in parentheses.

Table 16: Shear force (kg) for cooked fish during storage at -5°C, -12°C and -30°C (controls)*

Sample storage temperature	Time of storage (days)			
	10	18	25	32
-5°C	1.2 (0.3)	2.8 (1.1)	2.1 (0.4)	1.8 (0.3)
-12°C	-	2.6 (0.4)	2.6 (0.8)	-
-30°C	2.4 (0.3)	3.3 (0.7)	1.8 (0.2)	2 (0.6)

* The values are means of determinations on 3 fish with 4 determinations on each fish; standard deviations are given in parentheses.

in frozen gadoid fish, since DMA acts as an index of enzymatic deterioration during frozen storage and TMA as an index of pre-freezing quality (Castell et al., 1974).

However, as discussed in the next section, determination of volatile amines proved to be of little value for this hake species.

4.3.2 Volatile amines in frozen hake

4.3.2.1 Gas chromatographic determination of DMA and TMA

The gas chromatographic system developed for the determination of DMA and TMA involving the use of a Phase-Sep Tenax column, gave a good separation of both amines when standard aqueous solutions of several concentrations were analysed, although it was not possible to eliminate all tailing, especially in the case of the TMA peaks.

The attempt to find a suitable internal standard for this system was without success because most of the substances with other desired characteristics were not water-soluble (as happens with many alcohols and the other amines), and among those that were water soluble, those tested showed retention times too close to those of DMA and TMA, and it was preferred to work without any internal standard, but to carefully control the injection volumes. This system proved to be fairly sensitive, capable of quantitive detection at concentrations as low as 0.06 mg DMA-N/ml.

Although at pH 12 (the pH of the samples when injected) the amines are very volatile, a careful operation, using tightly

sealed autosample vials and avoiding any unnecessary exposure, was sufficient to obtain reproducible results.

During all experiments, under all experimental conditions tested, the hake samples never showed more than trace amounts of DMA and TMA, (i.e., less than 1 mg/ml TMA-N) similar to those found in the fresh fish. These results were confirmed by the Torry Research Station using their gas chromatographic procedure. Further confirmation was obtained using a gas sensor.

4.3.2.2 Volatile amines using a gas sensor

The rapid method of volatile amines determination using a gas sensor proved to be sensitive enough for the requirements of this work, and the results were fairly reproducible. In fact, although some variation in the results may be caused by the way the injection is performed, keeping the gas flow rate constant and using always a similar needle, an experienced and careful operator can obtain very consistent results. In order to avoid any air contamination, the use of compressed air as carrier is highly recommended.

The results obtained using this method show good agreement with those from the gas chromatography work, for the levels of volatile amines were low and appear not to increase significantly during the storage period (see Table 17).

Samples of cod, tested after 4 weeks of storage in an home freezer and prepared similarly, gave very high values, too large to be quantified without reducing the apparatus sensitivity.

The low TMA levels are not surprising since these relate to bacterial activity, as discussed in the next Section.

Table 17: Total volatile amines in hake, expressed in mg of TMA-N per ml of solution

Sample	Time of storage (weeks)		
	2	4	32
stored at -5°C		2.3×10^{-3}	
stored at -11°C	1.5×10^{-3}		1.7×10^{-2}
Cod, stored in a home freezer		$> 3.5 \times 10^{-2}$	

No information about DMA levels in this particular hake species has been reported previously but considering what happens in species of the same genus (Section 2.4), one might expect the production of high amounts of DMA, during frozen storage at such high sub-zero temperatures. However it should be emphasised that this work was performed on fish frozen and stored whole and not on minced flesh as in most of the related work, referred to in Section 2.4.

In 1974, Mackie and Thomson reported a similar finding when working with whole haddock stored at -15°C, i.e., they found no production of DMA while other gadoids, such as cod and saithe produced it, under the same conditions.

Such results point to the need for further study into the causes of textural deterioration in hake species, for, if it does become tough without the production of DMA and hence formaldehyde production, the mechanism of such deterioration is very far from being understood.

4.3.2.3 Bacterial activity and TMA levels

The total viable counts, performed to estimate the initial bacterial activity in the fish flesh, and the degree of survival of any bacteria present during the various storage conditions, showed very low values and confirmed the expectation that the frozen storage conditions used led in general to a decrease in the counts, leading probably to very low levels after long storage periods (see Table 18).

Hence, it is not surprising that TMA levels did not increase during storage, since the TMAO conversion is thought to be exclusively bacterial (see Section 2.2.2.4).

Table 18: Total viable counts on hake (cfu/g) *

Sample	Time of storage (days) 0	9	14	25
Before storage	2.9×10^5			
Stored at				
-6°C		4.7×10^5	2.7×10^3	-
Stored at				
-11°C		6.7×10^4	3.4×10^3	1.3×10^4
FT		6×10^4	9.3×10^2	6.6×10^3
Stored at				
-60°C		1×10^4	2.3×10^3	3.7×10^4

* The values are means of counts on 4 different fish, 3 plates from each.

4.4 The quality of frozen hake stored at -6°C, -11°C and with a daily fluctuating temperature

4.4.1 Sensory assessment

The sensory assessment scores for off-flavours are shown in Figs 14 and 15; score 1 indicates no off-flavours and score 4 indicates strong off-flavours. The data in Fig. 14 for the -6°C storage, -11°C storage and control have average coefficients of variation of 38% (standard deviation 9), 43% (s.d. 12) and 38% (s.d. 9), respectively. The data in Fig. 15 for fluctuating temperature storage, -11°C storage and control have average coefficients of variation of 44% (s.d. 16), 43% (s.d. 12) and 46% (s.d. 11), respectively.

The sensory off-flavours scores showed, in general, a decrease in quality with time in the fish stored under all the experimental conditions, and in particular for the fish stored at -6°C. When comparing fish from different batches although a similar trend in the results is noticeable, the actual scores do differ considerably illustrating the problems of comparing fish from different seasons, as discussed above.

Strong off-flavours did not develop under any of the storage conditions, providing some support for the idea that off-flavours are unimportant with regard to the acceptability of frozen hake; however, this needs to be further investigated using consumer taste panels in Portugal.

Although the off-flavour scores for the -60°C stored fish show some variation with time, the analysis given in Section 4.5 suggests that it is probably due to fish to fish variation.

Fig.14: Off-flavour sensory scores

May fish stored at -6°C, -11°C & control (-60°C)

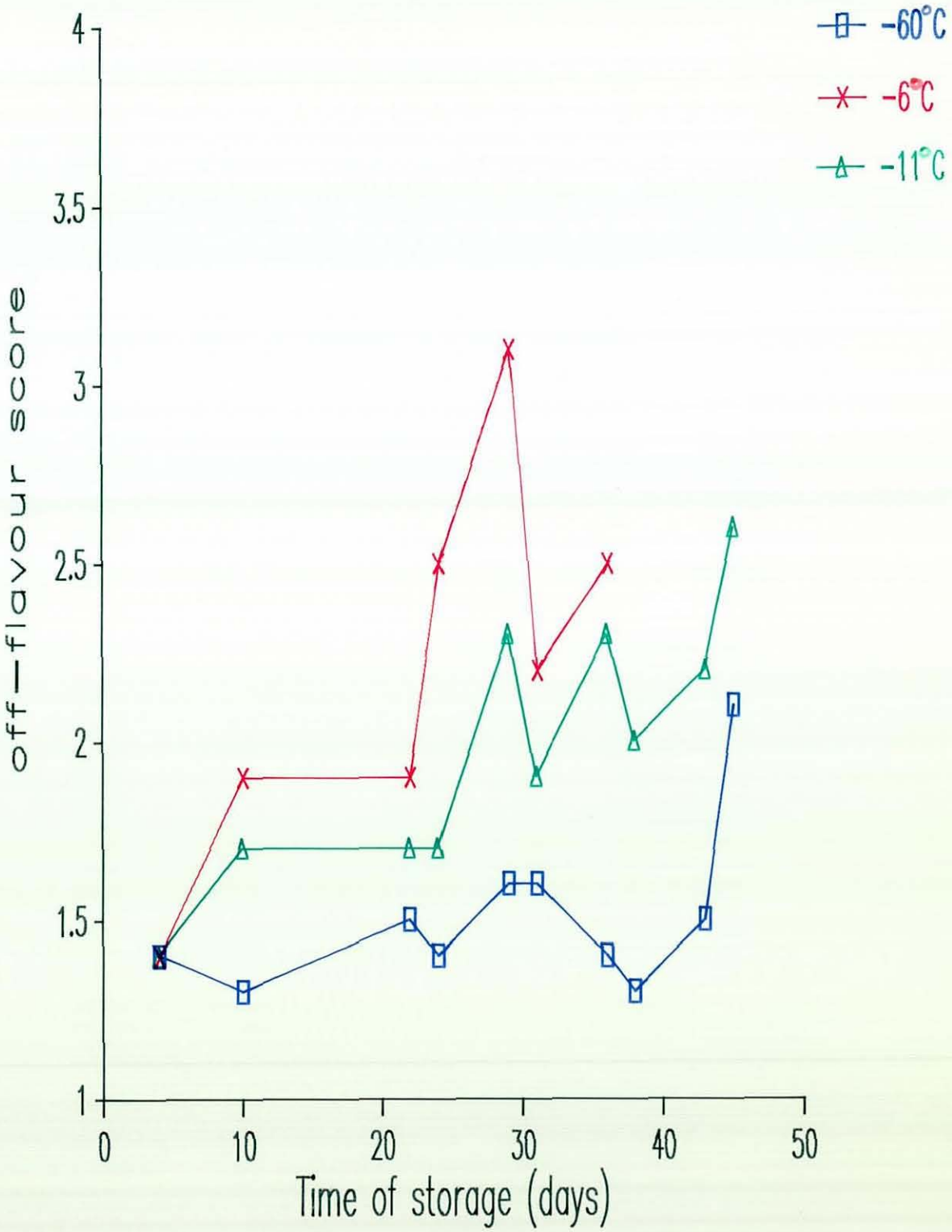
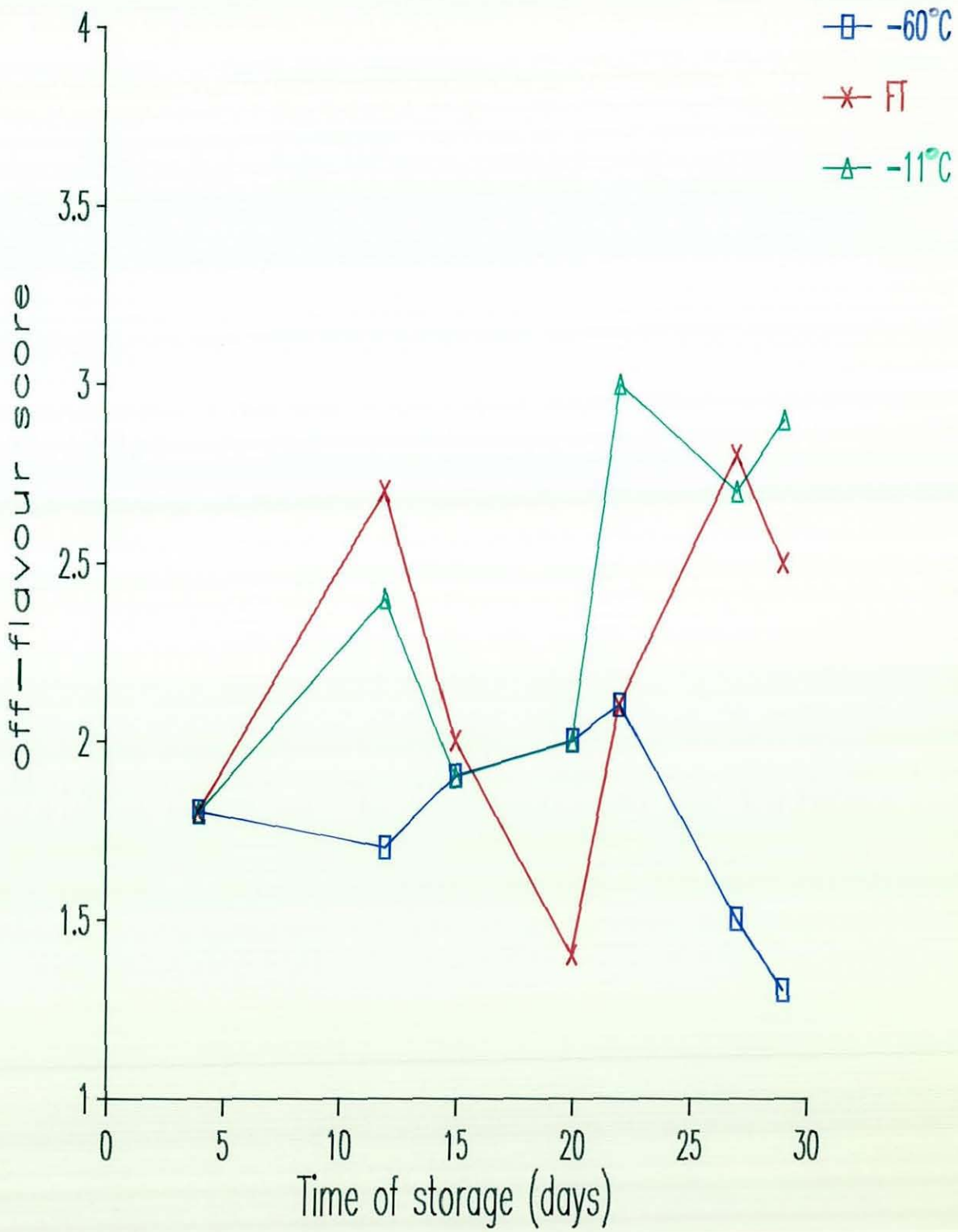


Fig.15: Off-flavour sensory scores

August fish stored at FT, -11°C & control (-60°C)



The results of the sensory assessment of toughness are shown in Figs 16 and 17; score 0 represents a soft texture and score 3 represents a tough texture. The data in Fig. 16 for the -6°C storage, -11°C storage and control have average coefficients of variation of 43% (s.d. 13), 39% (s.d. 12) and 82% (s.d. 31), respectively. The data in Fig. 17 for fluctuating temperature storage, -11°c storage and control have average coefficients of variation of 52% (s.d. 17), 63% (s.d. 25) and 62% (s.d. 20), respectively.

The results show a very definite pattern of increasing toughness in the fish stored at -6°C, -11°C and under the daily fluctuating temperature, whereas the controls show a comparatively steady behaviour during the storage periods (see also Section 4.5). The results of this sensory assessment of texture show a good agreement between the two different batches of fish.

The increase in toughness is more rapid for the -6°C stored fish than for those stored at -11°C. The fluctuating temperature fish did not differ appreciably from the -11°C stored fish.

4.4.2 Objective assessment

The shear force values obtained on cooked fish using the Instron are shown in Figs 18 and 19. The data for the -6°C storage have an average coefficient of variation of 28% (s.d. 7), the data for the -11°c storage have average coefficients of variation of 36% (s.d. 10) for the May fish and of 29% (s.d. 11) for the August fish, and the data for the FT storage have an average coefficient of variation of 36% (s.d. 15). Values were also obtained on fish stored at -60°C (the controls). No

Fig.16: Toughness sensory scores

May fish stored at -6° C, -11° C & control (-60° C)

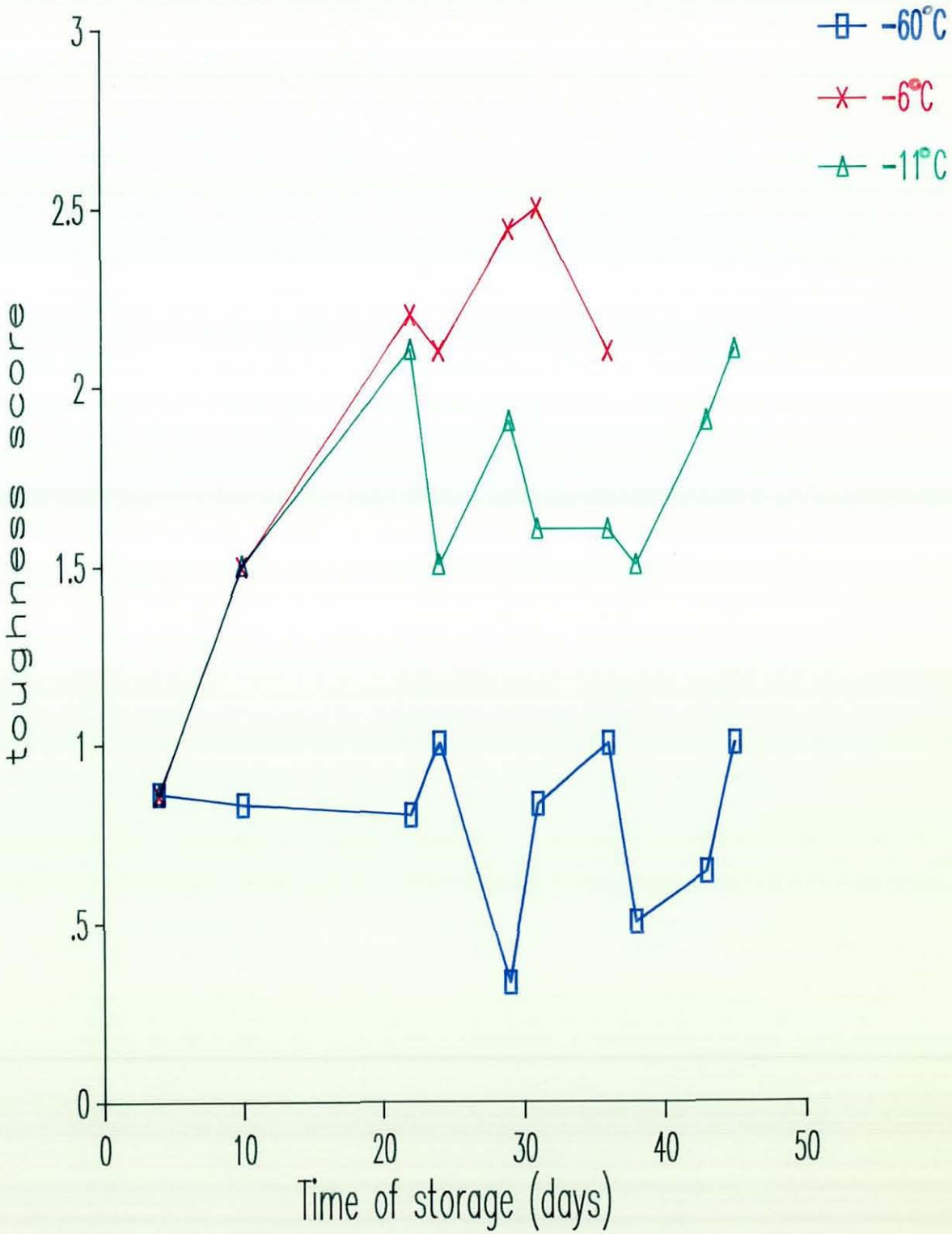


Fig.17: Toughness sensory scores

August fish stored at FT, -11°C & control (-60°C)

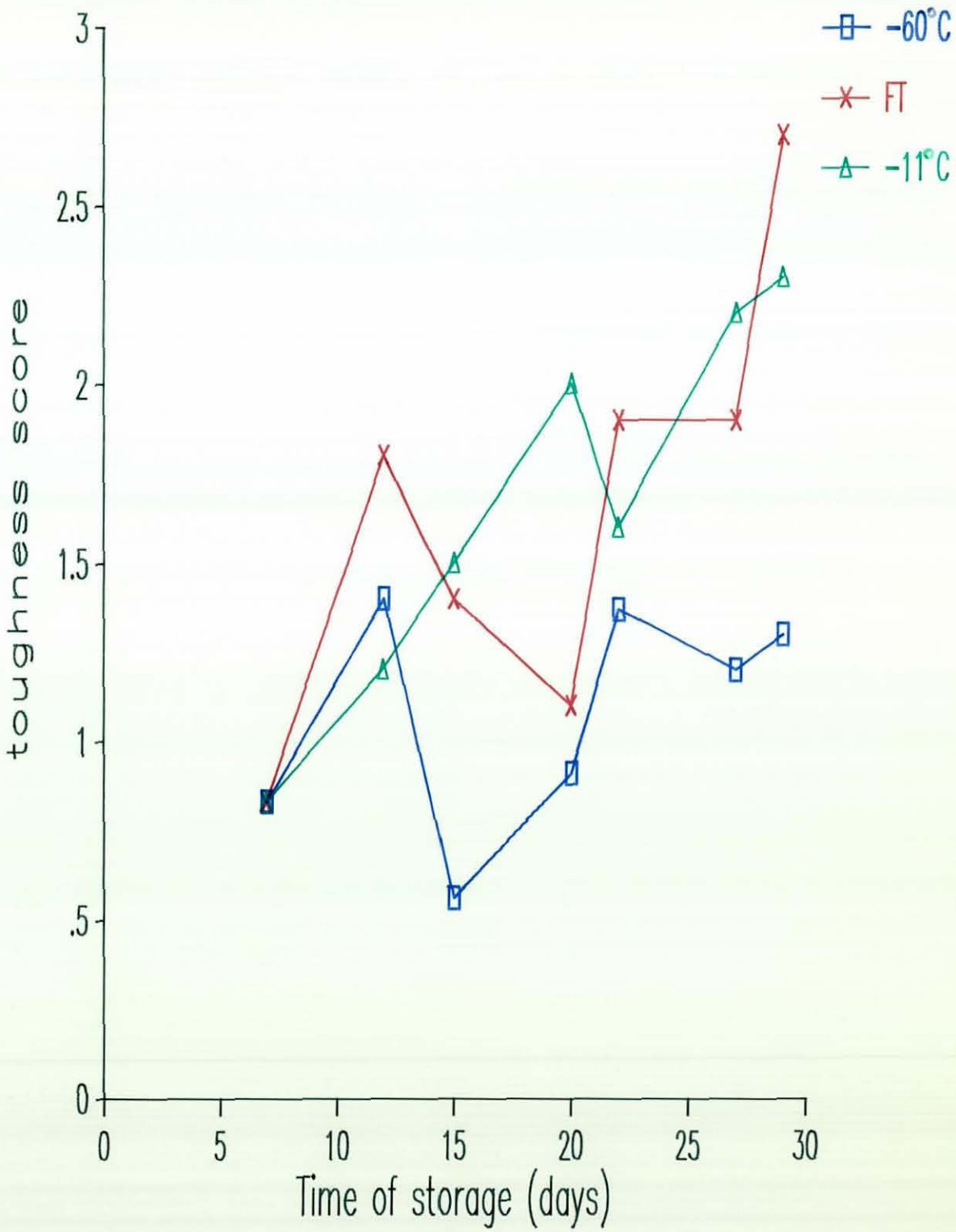


Fig.18: Shear force values

May fish stored at -6°C & -11°C

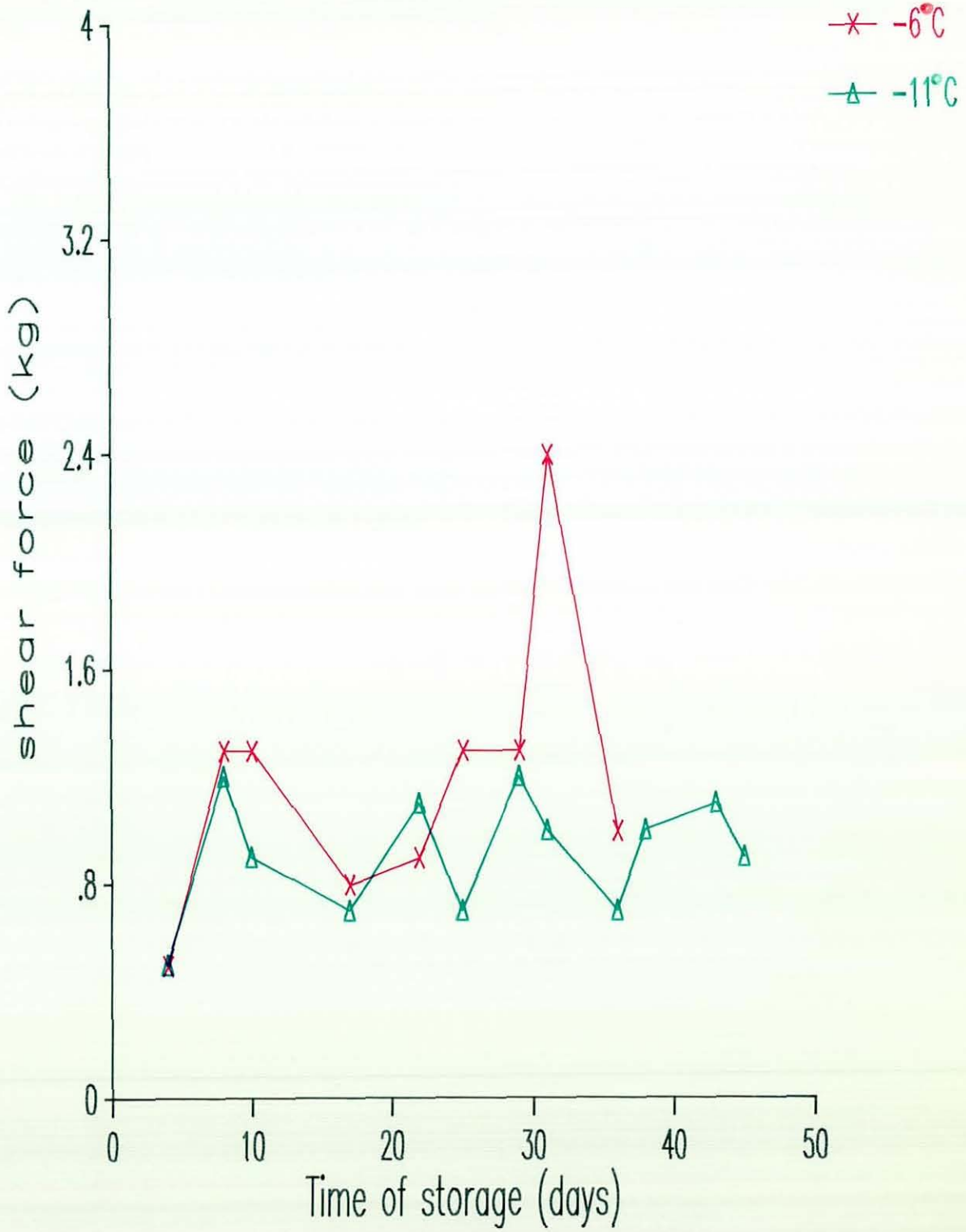
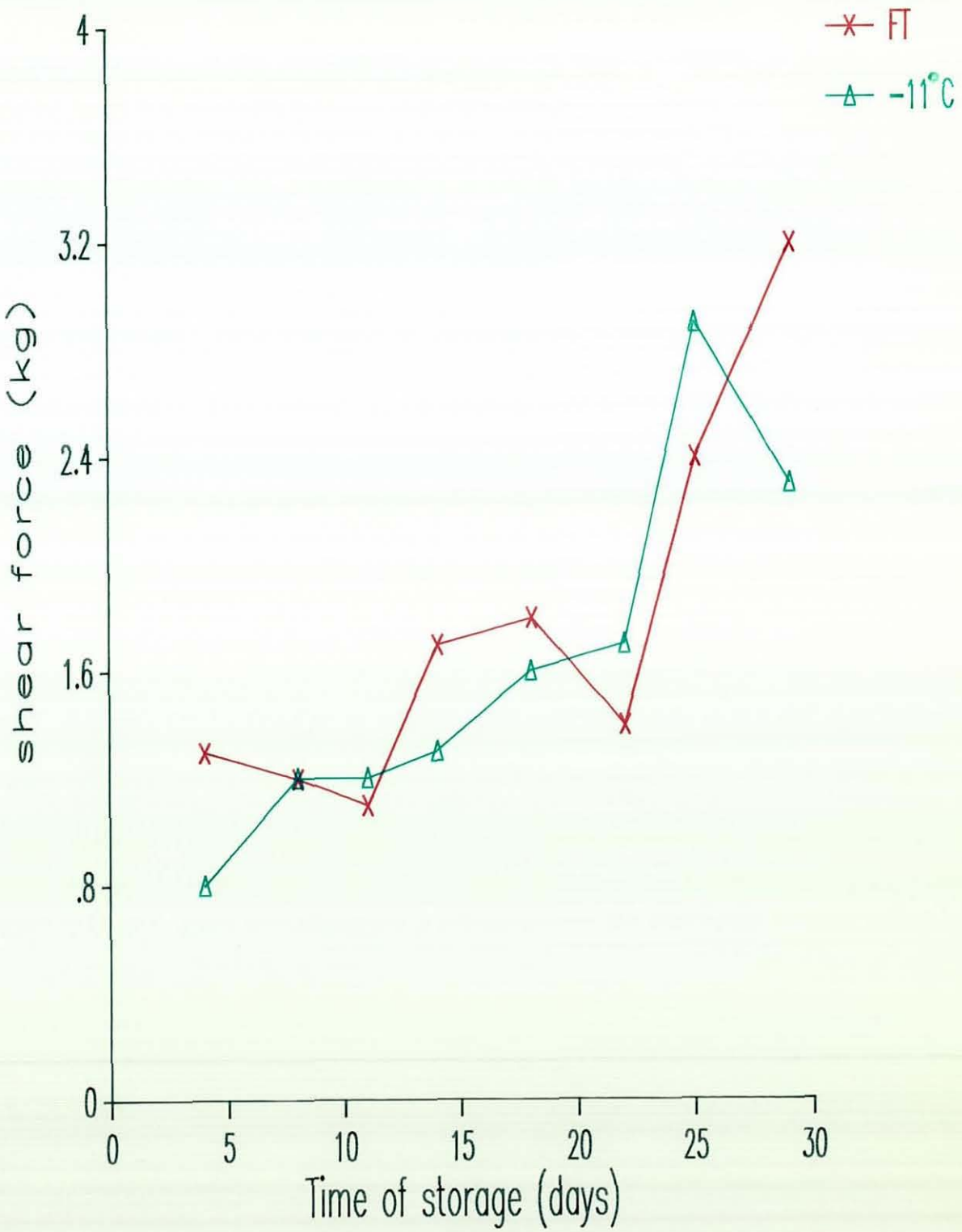


Fig.19: Shear force values

August fish stored at FT & -11°C



significant variation occurred with storage time (see Section 4.5); the average score was 0.67 (s.d. 0.11) for the May fish and 1.16 (s.d. 0.35) for the August fish.

The results indicate a clear dissimilarity between the two different batches of fish for both the -11°C and the control results. Possible seasonal factors leading to those differences are discussed in Section 4.5. It is therefore necessary to discuss the results from each batch of fish separately.

In the case of the fish caught in May, this assessment procedure did not produce any particular trend in the results either for the -11°C stored fish or even for the fish stored at -6°C, where the increase in toughness appears to be very evident from the sensory assessment results.

On the contrary, in the fish caught in August, there is a very clear pattern of increasing shear forces. This was very similar for both -11°c and FT stored fish and by comparing the values obtained at any time during storage with those at the beginning, some indication of the fish's textural quality could be obtained. This is discussed further in Section 4.5 when the correlation coefficients are considered.

The free fatty acid results are shown in Figs 20 and 21. The data have an average coefficient of variation of 24% (s.d. 8) for the -6°C stored fish, of 14% (s.d. 5) for the -11°C May fish, of 19% (s.d. 12) for the -11°C August fish and of 34% (s.d. 15) for the FT fish. Some values were also obtained on fish stored at -60°C (the controls). No significant variation with time occurred during frozen storage for the control fish (see Section 4.5); the May fish had an average value of 9.0% (s.d. 3.1) and the August

Fig.20: Percentage free fatty acids

May fish stored at -6° C & -11° C

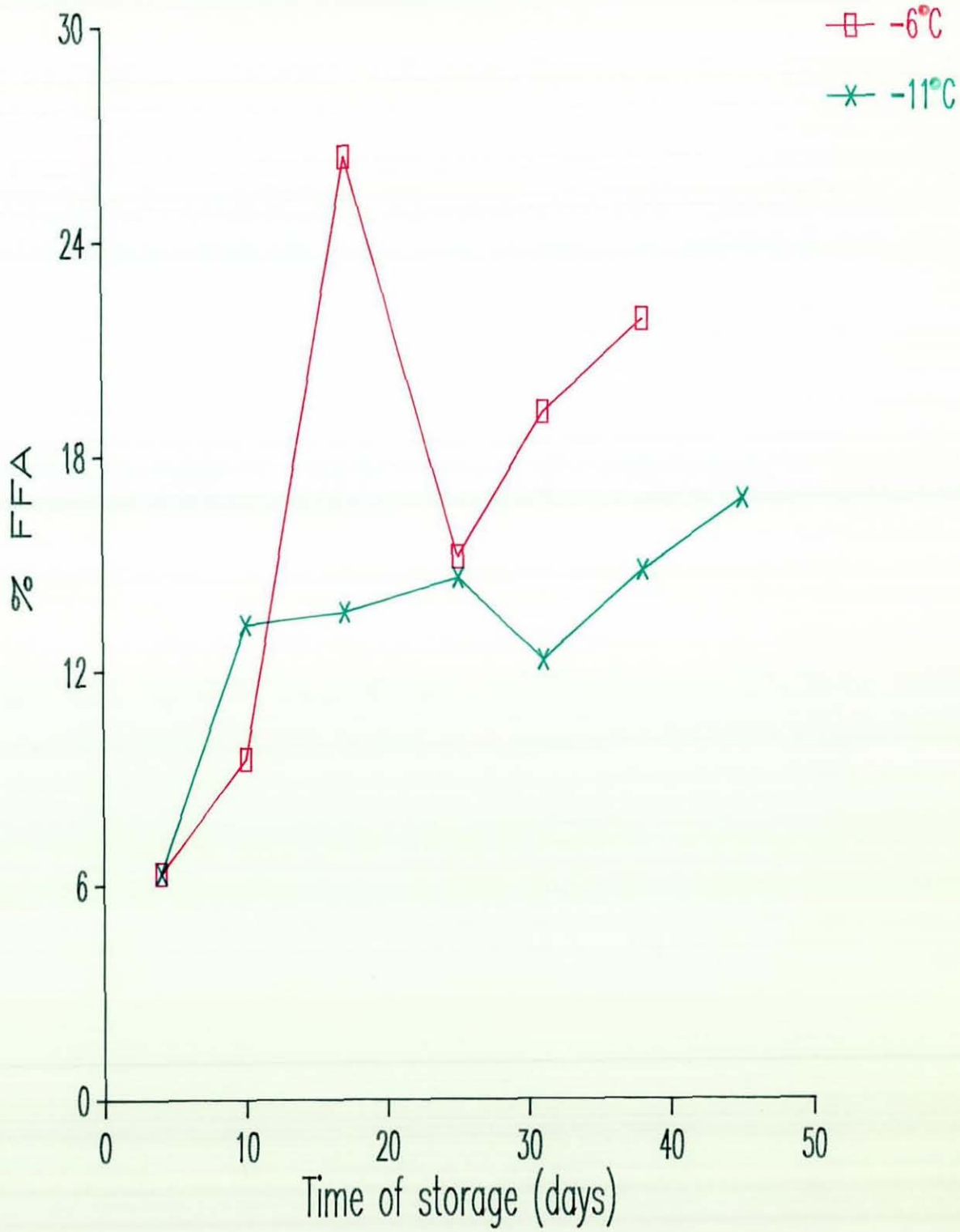
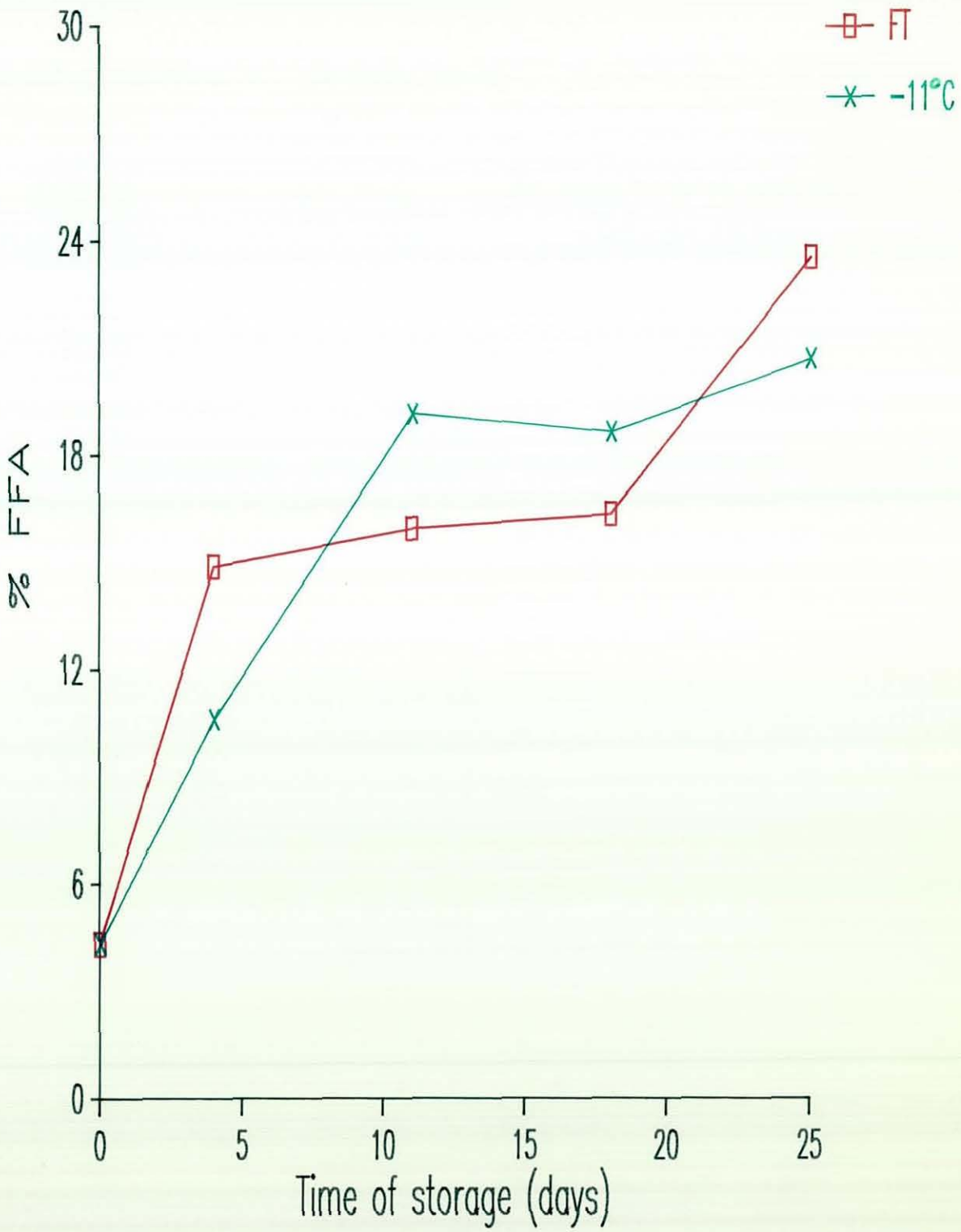


Fig.21: Percentage free fatty acids

August fish stored at FT & -11° C



fish had an average value of 12.6% (s.d. 5.6).

The -6°C stored fish attained higher values than the -11°c fish, but the difference is not significant. For the -11°C and the FT fish the values are very similar and no significant differences were found.

The percentage extractable protein results are shown in Figs 22 and 23. The data have an average coefficient of variation of 24% (s.d. 8) for the -6°C fish, of 15% (s.d. 11) for the -11°C May fish, of 14% (s.d. 10) for the -11°C August fish and of 29% (s.d. 16) for the FT fish. Some values were also obtained on fish stored at -60°C (the controls). No significant variation occurred during frozen storage for the control fish; the May fish had an average value of 74.2% (s.d. 6.0) and the August fish an average value of 64.8 (s.d. 12.4).

Under all the experimental conditions the sample results drop to levels lower than those of the controls but there is no significant difference between the results obtained for each of the experimental storage conditions (-6°C, -11°C and FT).

4.4.3 General discussion

The sensory assessment indicated a marked loss in quality under all the high sub-zero temperature conditions, when compared with the good quality maintained by the controls.

Although in this work only expert and not consumer taste panels were used, for the sake of this discussion and based on the author's experience, a score of 1.5-2 on the sensory toughness scale might be considered as a limit for acceptability by the Portuguese market.

Fig.22: Percentage extractable protein

May fish stored at -6°C & -11°C

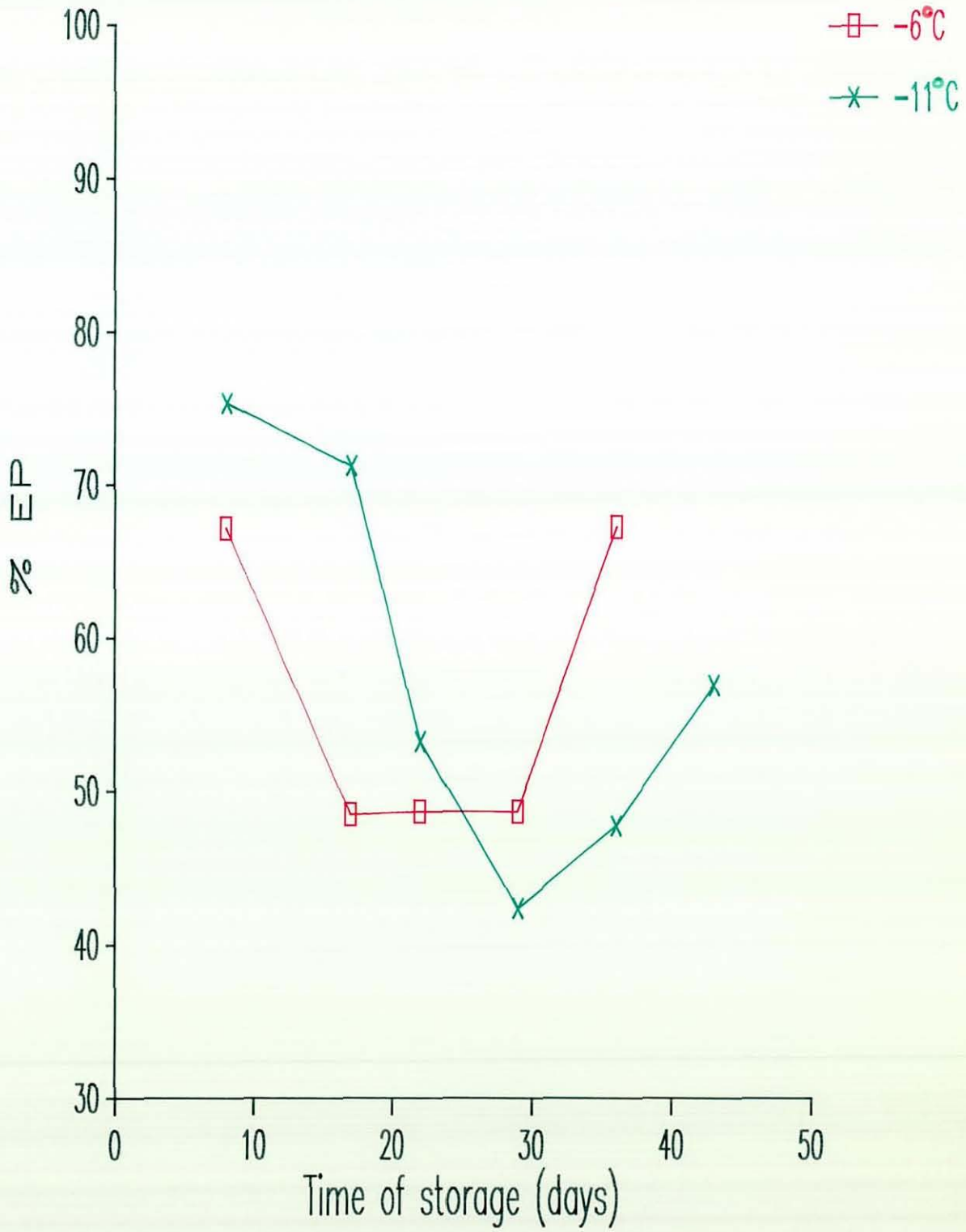
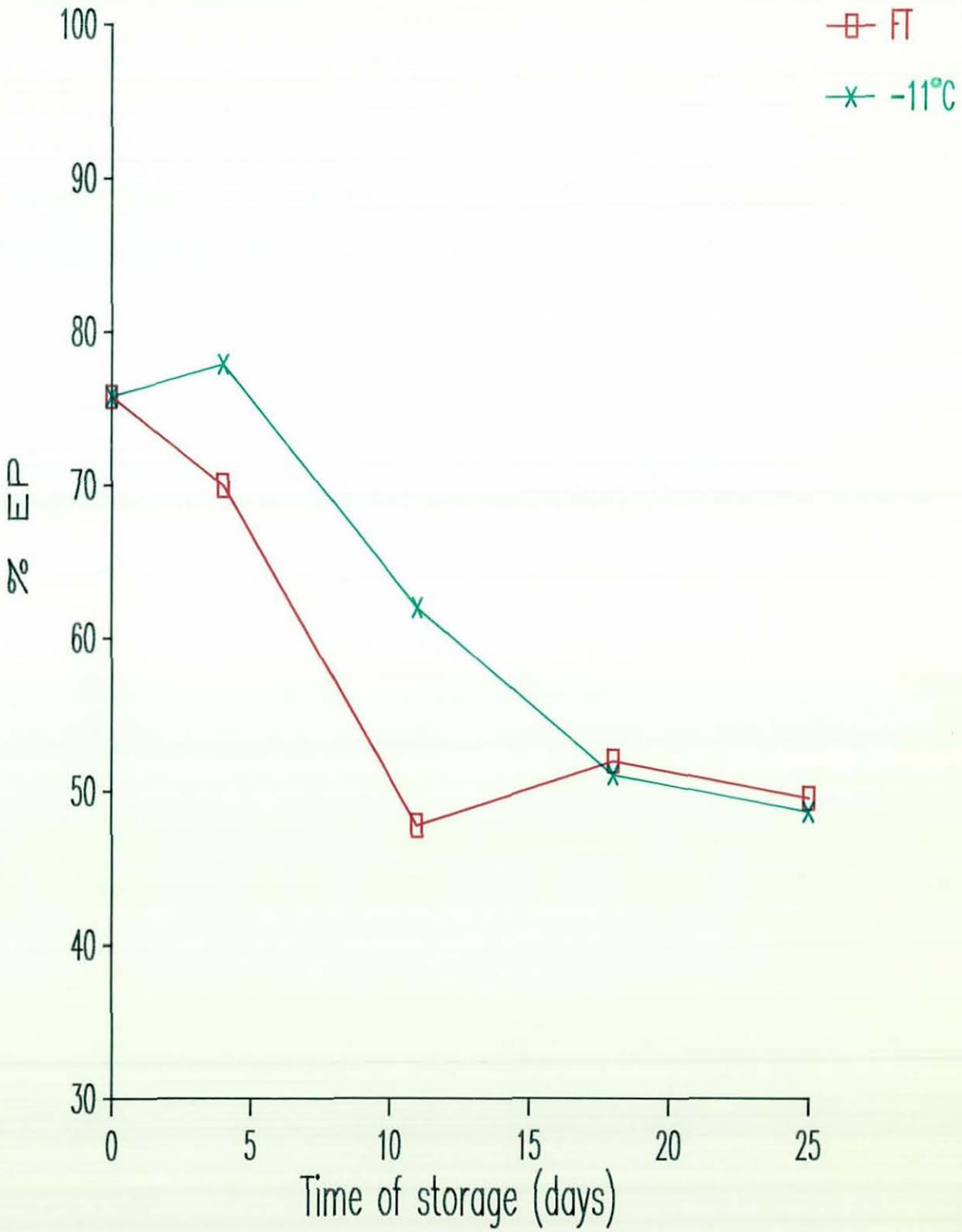


Fig.23: Percentage extractable protein

August fish stored at FT & -11°C



On this basis, if fish is stored under such conditions, then storage lives as short as 15 days are to be expected.

When comparing the results obtained from the fish stored at -6°C with those from the fish from the same batch stored at -11°C it is evident that although their good quality storage lives are very similar, the fish stored at -6°C continue to lose quality more rapidly than the -11°C fish. The more rapid changes for the -6°C stored fish are confirmed by some of the objective measurements. In the case of the -11°C stored fish and the fish from the same batch stored under fluctuating temperature, they both show the same pattern of deterioration as assessed by both sensory and objective methods.

Assuming an activation energy of 45.8 kJ/mole for cold storage deterioration (Olley, 1978), it can be calculated using the method of Olley, that the constant temperature equivalent to the fluctuating temperature regime (-4°C to -16°C) to which the fish were subjected is -11°C i.e. the same as the constant temperature regime used in these experiments. The limited results obtained in this investigation suggest that for this species of hake stored at high sub-zero temperatures, the fluctuating temperatures <u>per se</u> have little effect.

4.5 Analysis of the storage experiment results with regard to selection of quality assessment methods for frozen hake

One of the aims of this study was to select the most appropriate methods to assess the quality of frozen hake. After the preliminary selection reported in Section 4.3.2, the results obtained in the main storage experiments (results given in Section 4.4) were analysed with respect to establishing which objective tests reflect better the results of the sensory evaluation of texture.

The statistical comparison between each set of results and the taste panel texture scores was carried out. The linear correlation coefficients and significance are given in Table 19. The correlations of sensory toughness with storage time are also given in the same Table.

Analysis of the results presented in Table 19 shows that there is no clear correlation between the results of sensory evaluation of texture and off-flavours. In two cases there is a significant correlation ($p < 5\%$), while in the other the correlation is not significant ($p > 5\%$), thus indicating that off-flavours and texture changes are recognised as two different phenomena by the taste panel.

The significant correlations between the results of sensory assessment of toughness and shear force, in the August fish stored at -11°C and under fluctuating temperature conditions suggests that in these cases, both methods appear to be measuring the same characteristic. However, no significant correlations were found between sensory toughness and shear force for the May fish.

Table 19: Linear correlation coefficients for data obtained from the frozen hake storage experiments*

Methods compared	Storage temperatures					
	-6°C (May)	FT (August)	-11°C (May)	-11°C (August)	-60°C (May)	-60°C (August)
Sensory toughness scores/off-flavours	0.76 +	0.72	0.67 +	0.58	0.18	-0.33
Sensory toughness scores/ shear force	0.54	0.80 +	0.48	0.89 ++	-0.09	0.33
Sensory toughness scores/ FFA	0.83 +	0.89 +	0.93 ++	0.99 ++	0.42	0.85
Sensory toughness scores/ extractable protein	-0.80	-0.83	-0.79	-0.89 +	0.55	0.39
Sensory toughness scores/ time	0.88 ++	0.69	0.67 +	0.96 +++	-0.12	0.44

* Values that are significant at the 5% level are marked with "+", at the 1% level with "++" and at 0.1% level with "+++".

Sensory assessment of toughness can include aspects such as stringiness, chewiness and dryness, which are not necessarily measured in the shear force determinations, as was pointed out by Love (1980). It must also be remembered, as discussed above, that the August fish were post-spawning and in a very different nutritional state to the May fish, which were spawning. The seasonal variation in the relationship between sensory toughness and shear force obviously reduces the value of the latter as a quality assessment method.

The significant correlations found in each case between the sensory toughness and the free fatty acid content indicate that the latter has potential as an objective quality asessment method for frozen hake. It also raises the possibility that the production of the free fatty acids might be of importance with regard to protein denaturation during frozen storage of this species of hake.

When comparing sensory toughness scores and extractable protein results, although a significant correlation was found for the -11°C August fish, in the other cases the correlation was not significant, suggesting that the extractable protein method as applied in the present work was not a very good measure of the development of a tough texture.

Two secondary points arise from this data analysis. Firstly, it is seen that sensory toughness shows a significant correlation with storage time for the -6°C and the two -11°C storage conditions, and for the fluctuating temperature storage is only just outside the 5% significance level. These significant linear correlations indicate, but do not prove, that the process leading to tough texture does not require an induction time, but

continues at a steady rate over the storage period investigated.

Secondly, there are no significant correlations between any of the results for the control fish stored at -60°C. This supports the idea that although variation occurs in the results for fish during storage under those conditions, this variation does not reflect any quality alteration during storage and is probably due to natural differences between fish.

5. Conclusions and suggestions for further work

The conclusions of this work, and suggestions for further relevant work, presented in accordance with the three areas of the aims, can be summarized as follows:

1. With respect to selection of the most appropriate methods to assess the quality of frozen hake, sensory evaluation using an expert panel proved to be sensitive to changes in the toughness of hake flesh. No consumer testing was attempted in this study since hake is not commonly eaten in the U.K. Such consumer testing should be carried out in Portugal in order to obtain more reliable information on storage period in relation to acceptability.

   The use of the Instron to assess shear force for cooked fish flesh can be useful, provided that a large number of samples are used and that the results are always regarded on a comparative basis and never as absolute values. A more intensive study of the potential of this method in analysing fish flesh texture is necessary.

   Free fatty acid content appeared to be the best objective method for following quality changes in this species of hake. However, a more detailed study of this method needs to be carried out in order to assess its scope and limitations under different conditions and for different hake species.

2. The study of the storage factors responsible for loss of quality of hake during frozen storage at high and fluctuating sub-zero temperatures showed that hake has a good quality storage life of only 2 to 3 weeks under such conditions, and that storage temperature plays an important role in determining the storage life, but fluctuations in temperature appear to be unimportant.

Further investigation needs to be carried out in Portugal, where proper consumer taste panel work is possible (as mentioned above) to assess accurately the storage lives of the different frozen hake products, under both experimental and local retailers' conditions. A comparative study of the behaviour of the different hake species during frozen storage should also be carried out, since species variations may be the basis of many of the problems encountered.

3. The mechanism of protein denaturation in frozen hake is largely unresolved. Since in this species of hake the production of formaldehyde is negligible under the experimental conditions and lipid oxidation is slow, lipid hydrolysis products emerge as possible principal causes of protein denaturation, although moisture related factors may also be involved.

This aspect needs to be further studied and in particular the interactions between free fatty acids and proteins need to be investigated in detail using both real and model systems.

6. Appendices

6.1 Chemical composition of hake

Fish caught in February

Table 20: % True protein content

18.5	19.9	18.7	17.4	17.4
18.9	17.2	17.6	17.0	19.2

mean: 18.1 s.d.: 0.8

Table 21: % Lipid content

1.75	1.69	2.50	2.98	3.59
1.76	2.10	2.10	2.78	2.30

mean: 2.3 s.d.: 0.6

Table 22: % Moisture content

79.9	79.5	79.8	79.9	78.9
80.0	79.8	77.8	-	78.0

mean: 79.2 s.d.: 0.9

Table 23: % Ash content

2.13	3.27	0.96	1.13	1.29
2.99	1.25	1.21	1.26	1.29

mean: 1.7 s.d.: 0.8

6.2 Protein content

Table 24: Fish caught in May

% True protein content								
17.1	15.2	16.3	16.9	16.4	15.9	15.3	15.9	16.0
18.8	16.6	17.0	15.2	15.8	16.3	14.3	16.9	15.5
19.4	16.7	16.3	18.1	15.8	16.9	16.3	17.0	15.1
18.6	18.3	18.0	16.2	15.5	16.6	15.6	16.1	16.9
17.5	16.7	17.9	15.9	14.8	16.3	15.4	16.1	14.9
16.1	16.7	15.8	15.6	16.2	16.0	16.0	15.6	
15.8	16.6	16.2	14.8	16.4	17.1	14.8	16.6	
17.1	14.9	15.4	14.8	18.4	16.9	16.1	15.1	
16.7	17.7	16.1	16.8	16.8	17.1	15.4	15.6	
16.2	18.8	16.3	16.4	17.6	16.6	15.3	15.7	

mean : 16.4 s.d. : 1.0

Table 25: Fish caught in August

% True protein content						
13.9	14.9	16.4	15.9	16.6	15.9	16.4
15.1	16.9	18.2	15.8	16.6	15.8	17.1
16.1	15.3	17.8	15.6	16.1	16.3	13.9
15.9	15.2	18.4	14.8	15.9	16.1	15.7
15.9	17.0	17.6	15.8	16.7	15.4	15.9
16.1	14.7	17.7	17.1	16.8	15.3	
18.4	17.6	18.1	16.5	16.9	17.8	
17.2	17.8	17.2	18.7	19.6	18.7	
15.6	18.3	17.1	14.9	17.3	16.6	
17.0	16.9	16.8	17.8	17.1	17.6	

mean : 16.5 s.d. : 1.2

6.3 Lipid content

Table 26: Fish caught in May

% Lipid content								
1.1	1.4	1.1	1.2	1.2	1.12	1.68	1.15	1.91
1.5	1.2	1.0	1.1	1.8	1.29	0.79	1.40	1.12
1.2	1.4	0.7	3.3	1.26	1.69	0.62	1.49	1.04
2.2	1.1	1.1	2.9	2.21	3.01	0.88	1.22	1.73
1.0	1.1	1.3	1.3	1.64	1.62	0.75	1.11	1.11
1.29	1.48	3.09	1.91	1.85	1.41	1.05	1.60	1.36
1.07	1.97	1.62	1.85	1.37	1.03	2.84	2.21	1.07
1.77	2.03	1.81	1.23	1.46	1.92	2.41	1.34	
1.19	1.53	1.63	2.45	1.32	1.33	2.07	1.04	
1.70	1.49	1.79	1.18	1.30	1.46	1.12	0.96	
mean : 1.5						s.d. : 0.54		

Table 27: Fish caught in August

% Lipid content						
3.08	1.72	1.02	1.33	1.53	1.42	3.42
2.5	1.79	2.10	1.92	1.78	2.80	1.67
2.78	2.46	2.85	2.37	1.92	2.33	3.43
2.34	3.39	2.48	4.46	1.32	1.22	3.27
2.12	2.78	3.04	1.49	1.25	1.92	
1.22	2.24	2.64	2.23	3.25	2.27	
2.73	1.67	1.39	1.80	1.72	2.64	
2.41	1.41	2.05	1.65	4.27	1.38	
2.50	1.88	1.25	1.75	1.69	1.40	
1.22	1.81	3.74	1.29	4.28	1.88	
mean : 2.2					s.d. : 0.8	

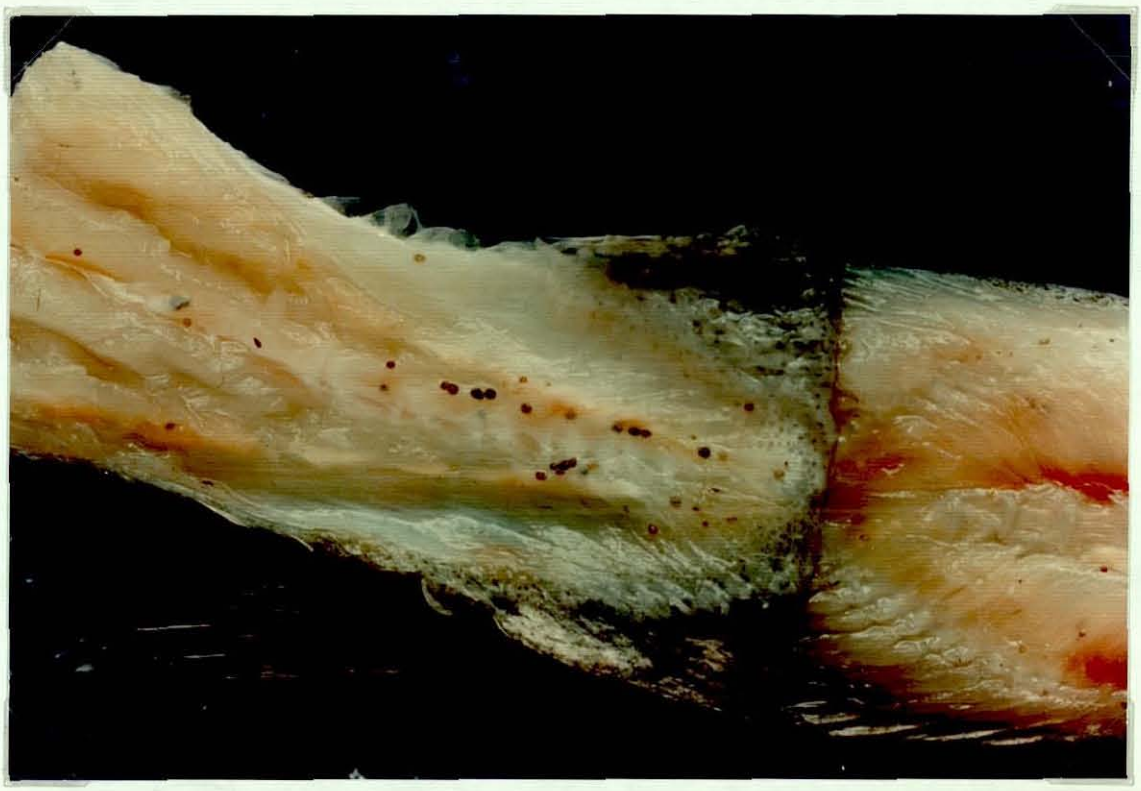
6.4 Nematodes in hake

During the present work, a nematode infestation became evident in almost every fish. When filleting the fish, white, thread-like objects with black oval (or spherical) formations at irregular intervals could be seen, spreading from the backbone towards the muscle, being particularly abundant near the tail end (Fig. 24).

These black formations have been identified as being encapsulated metacercariae of the digenean Prosorhynchoides gracilescens (R.).

This species has been found several times before in the spinal nerves of hake (Bray, 1985, personnal communication) and is not among the parasitic worms described as hazardous to health (Higashi, 1985).

Fig. 24: Hake sample showing nematode metacercariae



7. References

Ackman, R.G. (1980). Fish lipids. Part 1. In: Advances in Fish Science and Technology. Connell J.J., (Ed.). Farnham: Fishing News (Books)

Almandos, M.E., Giannini, D.H., Ciarlo, A.S. and Boeri, R.L. (1984). Relationship between the increase of total volatile bases and dimethylamine in frozen Patagonian hake (Merluccius hubbsi). Lebensmittel-Wissenschaft und Technologie, 17 (2), 111-113.

American Society for Testing Materials (1968). Basic Principles of Sensory Evaluation. Philadelphia: American Society for Testing and Materials.

Amerine, M.A., Pangborn, R.M. and Roessler, E.B. (1965). Principles of Sensory Evaluation of Food. London: Academic Press.

Anon. (1965). Quick freezing of Fish. Torry Advisory Note 27. Aberdeen: Torry Research Station.

Avdalov, N. and Ripoll, A. (1981). Handling, Quality and Yield of Fresh Hake. IIF-IIR Commissions, C-2, D-1, D-2 and D-3. Boston: 1981/4.

Babbit, J.K., Crawford, D.L. and Law, D.K. (1972). Decomposition of trimethylamine oxide and changes in protein extractability during frozen storage of minced and intact hake (Merluccius productus) muscle. Agricultural and Food Chemistry, 20 (5).

Banasihan, E.T. (1985). Control of rancidity development in minced fatty fish products. PhD thesis, University of Reading.

Beatty, S.A. and Gibbons, N.E. (1937). The measurement of spoilage in fish. Journal of the Biological Board of Canada, 3, 77.

British Standards Institute (1980). Preparation of methyl esters of fatty acids. BS 684: Section 2.34: 1980. ISO 5509 - 1978.

Bray, R.A. (1985). Personal communication. British Museum, England.

Brown, P. and Morton, I.D. (1969). Fats and fatty foods. In: Food Industries Manual, 20th edn. Woollen, A. (Ed). London: Leonard Hill.

Castell, C.H. (1971). Metal-catalysed lipid oxidation and changes of proteins in fish. Journal of the American Oil Chemists Society, 48 (11), 645-49.

Castell, C.H., Neal, W. and Smith, B. (1970). Formation of dimethylamine in stored frozen sea fish. Journal of Fisheries Research Board of Canada, 27, 1685-1690.

Castell, C.H., Smith, B. and Dyer, W.J. (1974). Simultaneous measurement of TMA and DMA in fish and their use for estimating quality of frozen stored gadoid fillets. Journal of Fisheries Research Board of Canada, 31, 383-389.

Childs, E.A. (1974). Interaction of free fatty acids with fish muscle in vitro. Journal of the Fisheries Research Board of Canada, 31, 111-113.

Clément, P. (1969). Conditions optimales d'entreposage des produits surgelés. Revue Génerale du Froid 9, Septembre.

Clucas, I. (1981). Fishing Handling, preservation and processing in the tropics: Part 1. Report of the Tropical Products Institute, G 144. London: Tropical Products Institute.

Codex Alimentarius. (1979). Draft Standards of frozen fillets for various kinds. FAO.

Connell, J.J. (1960). Mechanical properties of fish and fish products. In: Flow Properties of Blood. Oxford: Pergamon Press.

Connell, J.J. (1966). Changes in aldolase activity in cod and haddock during frozen storage. Journal of Food Science, 31, 313-315.

Connell, J.J. (1968). The effect of freezing and frozen storage on the proteins of fish muscle. In: Low Temperature Biology of Foodstuffs. Hawthorne J. and Rolfe E.J., (Ed.). Oxford: Pergamon Press.

Connell, J.J. (1975). The role of formaldehyde as a protein crosslinking agent acting during the frozen storage of cod. Journal of the Science of Food and Agriculture, 26, 1925--1929.

Connell, J.J. and Howgate, P.F. (1971). A critical appraisal of tests for evaluating quality loss in frozen stored fish. In: Fish Inspection and Quality Control. Kreuser, R., (Ed.). London: Fishing News.

Connell, J.J. and Shewan, J.M. (1980). Sensory and non-sensory assessment of fish. In: Advances in Fish Science and Technology. Connell, J.J., (Ed.), 56-64. Farnham: Fishing News (Books) Ltd.

Coultate, T. (1984). Food - the chemistry of its components. London: The Royal Society of Chemistry.

Cowie, W.P. and Little, W.T. (1966). The relationship between the toughness of cod stored at -29°C and its muscle protein solubility and pH. Journal of Food Technology, 1, 335-343.

Cutting, C.L. (1977). The influence of freezing practice on the quality of meat and fish. Australian Refrigeration, Air Conditioning and Heating, 31 (2), 25-8, 37-41.

Dunn, R., Simenhoff, M.L., Wesson, L.G. (1976). Gas chromatographic determination of free mono-, di- and trimethylamines in biological fluids. Analytical Chemistry, 48 (1).

Dyer, W.J. (1968). Deterioration and storage life of frozen fish. In: Low temperature biology of foodstuffs. Hawthorne J. and Rolfe, E.J.,(Ed.). Oxford: Pergamon Press.

Dyer , W.J., Fraser, D.I., Ellis, D.E. and MacCallum, W.A. (1957). Influence of intermittent short storage periods at 15°C as encountered during refrigerator car transportation, on the quality of frozen cod stored at 0°F. Journal of the Fisheries Research Board of Canada, 14 (4), 627-635.

Dyer , W.J., Fraser, D.I. and MacCallum, W.A. (1957). Further study of the influence of short storage periods, 3 days to 2 weeks at 15°F, on the quality of frozen cod stored at 0°F. Journal of the Fisheries Research board of Canada, 14 (6), 925-929.

Dyer, W.J. and Mounsey, Y.A. (1945). Amines in fish muscle. II. Development of trimethylamine and other amines. Journal of the Fisheries Research Board of Canada, 6 (5), 359-367.

EEC (1970). Determining common marketing standards for certain fresh or chilled fish. EEC regulation n°2445/70.

FAO (1984). Yearbook of Fishery Statistics 1982: Catches and Landings, Vol. 54. Rome: FAO.

Feeney, R.E. (1980). Overview on the chemical deteriorative changes of proteins and their consequences. In: Chemical deterioration of proteins. Whitaker J.R. and Fujinaki, M., (Ed.). Washington: American Chemical Society.

German, J.B. and Kinsella, J.E. (1985). Lipid oxidation in fish tissue-enzymatic initiation via lipoxygenase. Journal of Agriculture and Food Chemistry, 33, 680-683.

Gould, E. Peters , J.A. (1971). On testing the freshness of frozen fish. London: Fishing News.

Greenwood-Barton, L.H. and Cole, R.C. (1966). Problems Associated with the development of fisheries in Tropical Countries. IV - The preservation of the catch by more advanced processes. Tropical Science, 8 (2), 71-78.

Hansen, P. (1980). Fish Preservation Methods. In: Avances in Fish Science and Technology. Connell, J.J., (Ed.). Farnham: Fishing News (Books).

Hanson, S.W.F. and Olley, J. (1963). Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. Biochemical Journal, 89, 101-102.

Hardy, R. (1980). Fish lipids. Part 2. In: Advances in Fish Science and Technology. Connell J.J., (Ed.). 103-111. Farnham: Fishing News (Books).

Hasan, K.R. (1984). Influence of packaging materials on the quality of frozen mackerel during storage. C.N.A.A. MPhil Thesis (Humberside College of Higher Education).

Hebard, C.E., Flick, G.J. and Martin, R.E. (1982). Occurence and significance of TMAO and its derivatives in fish and shell fish. In: Chemistry and biochemistry of marine food products. Martin, R.E., (Ed.). London: The AVI Publishing Company, Incorporated.

Higashi, G.I. (1985). Foodborne parasites transmitted to man from fish and other aquatic foods. Food Technology, 69-74.

Hiltz, D.F., Lall, B.S., Lemon, D.W. and Dyer, W.J. (1976). Deteriorative changes during frozen storage in fillets and minced flesh of silver hake (Merluccius bilinearis) processed from round fish held in ice and refrigerated sea water. Journal of the Fisheries Research Board of Canada 33, 2560-2567.

Hobson-Frohock, A. (1979). The quantitative determination of trimethylamine in egg. Journal of Food Technology, 14, 441-447.

Karel, M. (1984). Chemical effects in food stored at room temperature. Journal of Chemical Education, 61 (4).

Keay, J. N. and Hardy, R. (1971). The separation of aliphatic amines in dilute aqueous solution by gas chromatography and application of this technique to the quantitative analysis of tri- and dimethylamine in fish. Journal of the Science of Food and Agriculture, 23 (1), 9-19.

Kelly, K., Jones, N.R., Love, R.M. and Olley, J. (1966). Texture and pH in fish muscle related to "cell fragility" measurements. Journal of Food Technology, 1, 9-15.

Laird, W.M. and Mackie, I.M. (1981). Protein changes during frozen storage of cod. Torry Memoir, 639.

Larmond, E. (1977). Laboratory methods for sensory evaluation of food. Ottawa: Research Branch Canada Department of Agriculture.

Leal, M.C. (1984). As Pescas portuguesas face à integração europeia. Estudos 22. Lisboa: Banco de Fomento Nacional.

Lee, C.M. (1982). Physical and biochemical changes in fish muscle under various freezing conditions. Quick Frozen Foods, October.

Licciardello, J.J., Ravesi, E.M., Lundstrum, R.C., Whilhem, K.A., Correia, F.F. and Allsup, M.G. (1981). Frozen storage stability of red hake block fillets. Refrigeration Science and Technology, 4, 377-382.

Licciardello, J.J., Ravesi, E.M., Lundstrum, R.C., Whilhem, K.A., Correia, F.F. and Allsup, M.G. (1982). Time-temperature tolerance and physical-chemical quality tests for frozen red hake. Journal of Food Quality, 5, 215-234.

Liston, J. (1980). Microbiology in fishery science. In: Advances in Fish Science and Technology. Connell, J.J., (Ed.). Farnham: Fishing News Book.

Love, R.M. (1966). The use of tasters for investigating cold storage deterioration in frozen fish. Journal of Food Technology, 1, 141-146.

Love, R.M. (1968). Ice formation in frozen muscle. In: Low Temperature Biology of Foodstuffs. Hawthorn and Rolfe, E.J., (Ed.). Oxford: Pergamon Press.

Love, R.M. (1980). Biological factors affecting processing and distribution . In: Advances in Fish Science and Technology. Connell J.J., (Ed.). Farnham: Fishing News (Books).

Love, R.M. (1983). Texture and fragility of fish muscle cells. Research at the Torry Research Station (Review paper). Journal of Texture Studies, 14, 323-352.

Love, R.M. and Mackay, E.M. (1962). Protein denaturation in frozen fish. V - Development of the cell fragility method for measuring cold storage changes in the muscle. Journal of Science of Food and Agriculture, 13, 200.

Lundstrom, R.C., Correia, F.F. and Wilhelm, K.A. (1981). Dimethylamine and formaldehyde production in fresh red hake (Urophycis chuss): The effect of packaging material oxygen permeability and cellular damage. Boston: IIF-I.I.R. Comissions, C-2, D-1, D-2, D-3.

Luyet, B.J. (1968). The formation of ice and the physical behaviour of the ice phase in aqueous solutions and in biological systems. In: Low Temperature Biology of Foodstuffs. Hawthorn and Rolfe, E.J., (Ed.). Oxford: Pergamon Press.

Mackie, J.M. and Thomson, B.W. (1974). Decomposition of trimethylamine oxide during iced and frozen-storage of whole and comminuted tissue of fish. In: Proceedings of the IV International Congress of Madrid, 1, 243-50.

Matsumoto,J.J. (1979). Denaturation of fish muscle proteins during frozen storage. In: Proteins at low temperatures. Fennema, O., (Ed.). Washington: American Chemical Society.

Matsumoto, J.J. (1980). Chemical deterioration of muscle proteins during frozen storage. In: Chemical deterioration of proteins. Whitaker, J.R. and Fugimaki, M., (Ed.). Washington: American Chemical Society.

McGill, A.S., Hardy, R. and Gunstone, F.D. (1977). Further analysis of volatile components of frozen cold stored cod and influence of these on flavour. Journal of Science of Food and Agriculture, 28, 200-205.

Miller, A, Scanlan, R.A., Libbery, L.M., Petropakis, H. and Anglemier, A.F. (1973). Quantitative determination of dimethyl- and trimethylamine in fish protein concentrate. Journal of Agricultural and Food Chemistry, 21 (3), 451-453.

Moyle, P.B. and Cech, J.J.Jr. (1982). Fishes, an introduction to ichthyology. New Jersey: Prentice-Hall.

Murray, J. and Burt, J.R. (1969). The composition of fish. Torry Advisory Notes 38. Aberdeen: Torry Research Station.

Olley, J. (1978). International Institute of Refrigeration, 1, July. Paris, International Institute of Refrigeration.

Owusu-Ansah, Y.J. and Hultin, H.O. (1984). Trimethylamine oxide prevents insolubilization of red hake muscle proteins during frozen storage. Journal of Agricultural and Food Chemistry 32, 1032-1035.

Partmann, W. (1977). Some aspects of protein changes in frozen foods. Zeitschrift Ernähmngswiss, 16, 167-175.

Quaranta, H.O., Pérez, S.S. (1983). Chemical Methods for measuring changes in freeze stored fish: a review. Food Chemistry, 11, 79-85.

Quintela, D.M. (1980). II Inquérito às instalações frigoríficas. Revista Portuguesa do Frio, 4 (14), 2-9.

Racicot, L.D., Lundstrom, R.C., Wilhelm, K.A., Ravesi, E,M.and Licciardello, J.J. (1984). Effect of oxidizing and reducing agents on trimethylamine N-oxide demethylase activity in red hake muscle. Journal of Agriculture and Food Chemistry, 32, 459-464.

Ravesi, E.M. and Anderson, M.L. (1969). Effect of varying the extraction procedure on the protein extractability of frozen-stored fish muscle. Fishery Industrial Research, 5, 175-180.

Regenstein, J.M., Samson, A. and Timberlake, A. (1981). Effect of heat and additives on the shelf-life of frozen red hake. IIF -IIR Commissions, C2, D1, D2, D3. Boston.

Regenstein, J.M., Schlosser, M.A., Samson, A. and Fey, M. (1979). Chemical changes of trimethylamine oxide during fresh and frozen storage of fish. Abstracts of Papers, American Chemical Society, 178 (1), AGFD 23.

Ritskes, T.M. (1975). The gas chromatographic determination of trimetylamine and dimetylamine in fish, fishery products and other foodstuffs. Journal of Food Technology, 10, 221-228.

Rivero, J.E. (1981). Quality of Frozen Hake. IIR-IIF Commissions, C2, D1, D2, D3. Boston. International Institute of Refrigeration.

Scanlan, R.A., Libbery, L.M., Petropakis, H. and Anglemier, A.F. (1973). Quantitative Determination of dimethylamine and trimethylamine in fish protein concentrate. Journal of Agricultural and Food Chemistry, 21 (3).

Shenouda, S.Y.K. (1980). Theories of protein denaturation during frozen storage of fish flesh. Advances in Food Research, 26, 275-311.

Sikorski, Z.E. (1977). Protein changes in muscle food due to freezing and frozen storage. IIR-IIF Commissions, C1, C2. Ettlingen. International Institute of Refrigeration.

Sikorski, Z.E. (1980). Structure and proteins of fish and Shellfish. In: Advances in Fish Science and Technology. Connell, J.J., (Ed.). Farnham: Fishing News Books.

Soudan, F. (1957). Organoleptic changes in frozen fish. Analles de la Nutrition et de l'Alimentation, 9 (1).

Soudan, F. (1968). Modifications des caracteres organoleptiques du poisson dues a la congelation. Révue Générale du Froid, 4.

Storey, R.M., Davis, H.K.T, Owen, D. and Moore, L. (1984). Rapid approximate estimation of volatile amines in fish. Journal of Food Technology, 19, 1-10.

Storey, J.G. and Storey, R.M. (1981). Peak reading digital display. Laboratory Practice, 30, 31.

Stroud, G.D. (1969). Rigor in fish: the effect on quality. Torry Advisory Note, 36. Aberdeen: Torry Research Station.

Tatterson, I. and Windsor, M.L. (1972). Cold storage properties of cooked fish (cod). Journal of Science of Food and Agriculture, 23, 1253-1259.

Teixeira, C. (1985). Personal communication. University of Aveiro, Portugal.

Tokunaga, T., Iida, H. and Miwa, K. (1977). The gas chromatographic analysis of amines in fish. Bulletin of the Japanese Society of Scientific Fisheries, 43 (2), 219-227.

Tomioka, K. Ogushi, J. and Endo, K. (1974). Studies on dimethylamine in food - II: concerning the enzymic formation of dimethylamine. Bulletin of the Japanese Society of Scientific Fisheries, 40 (10), 1021-1026.

Torry Research Station (1984). TRS Annual Report. On the handling and preservation of fish and fish products. Aberdeen: Torry Research Station.

U.K. Association of Frozen Food Producers (1976). U.K. A.F.F.P. code of pratice for the handling of quick frozen foods. London: U.K. Association of Frozen Food Producers.

Watkin, G. (1976). British food fish. London: The Worshipful Company of Fishmongers.

Wierbicki, E., Kunkle, L.E. and Deatherage, F.E. (1957). Changes in the water-holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. Food Technology, 11, 69-73.