Investigation into the effect of prior frozen storage (-20°C) on the quality of hot-smoked cod (Gadus morhua) and mackerel (Scomber scombrus)

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INVESTIGATION INTO THE EFFECT OF PRIOR FROZEN STORAGE (-20
°C) ON THE QUALITY OF HOT-SMOKED COD (GADUS MORRHUA) AND
MACKEREL (SCOMBER SCOMBRUS).

by

ANASTASIOS ZOTOS

A Master's Thesis
submitted in partial fulfilment of the requirements for the award of Master of Philosophy of Loughborough University of Technology, September 1991.

Humberside Polytechnic.
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To my wife, Stamatia

and

to my daughters, Maria and Elena
INVESTIGATION INTO THE EFFECT OF PRIOR FROZEN STORAGE (-20°C) ON THE QUALITY OF HOT-SMOKED COD (GADUS MORRHUA) AND MACKEREL (SCOMBER SCOMBRUS).

BY,

ANASTASIOS ZOTOS

ABSTRACT

Whole cod (Gadus morrhua) and mackerel (Scomber scombrus) were frozen using a Horizontal plate freezer and frozen stored at -20°C in bags and boxes. At suitable intervals (0, 11, 22 and 33 weeks) the fish were removed from the cold store and subsequently hot smoked (cod in fillets, mackerel in gutted form) using an AFOS-Torry Mini Kiln.

Both fish, frozen and smoked were analysed for their salt soluble protein (SSP), free amino acid content, available lysine, peroxide value, free fatty acid content, fatty acid profiles, thiamine content and texture using a Steven's Texture Analyser and organoleptically assessed by 10 panellists. Mackerel was also analysed for its histamine content. All smoked samples from both fish, despite their different previous histories and their different processed forms were assessed by the 10 panellists as moderately acceptable products in terms of their texture, flavour, colour and saltiness even after 33 weeks.

Protein in the cod samples was denatured on smoking to an extent which was independent of the extent of denaturation which had occurred during frozen storage, and was influenced by the free fatty acid content of the lipid, giving a moderately acceptable texture for up to 33 weeks. In mackerel the protein denaturation was only slightly influenced by the frozen storage history (24% drop in % SSP after 33 weeks frozen storage) and seemed to be affected by the free amino acids formed during frozen storage. After smoking the denaturation was extensive (above 80%) in all mackerel samples, with softer products than cod.

Very little lipid oxidation occurred in cod and off-flavours were not detected by the panellists. In the mackerel (0 and 11 weeks) frozen stored samples the lipid oxidation was slight, while in the 22 and 33 weeks frozen stored mackerel samples the lipid oxidation was quite extensive (PV of 108 meqO₂/kg lipid in the mackerel samples with 33 weeks frozen storage history Stored in boxes). However, no rancid flavour in the latter smoked mackerel samples was detected by the panellists.

Despite the increase of free amino acids content in both fish no significant difference was detected by the 10 panellists for the surface colour formation after smoking. The loss of available lysine in the smoked cod fillets was quite extensive (with an overall loss of 93% after 33 weeks and even for the fresh smoked fillets the loss was 35%). No significant loss of available lysine was found in the 0 and 11 weeks frozen stored mackerel samples, however, extensive losses were observed in the 22 and 33 weeks frozen stored mackerel samples after smoking (overall loss 74%).

Loss of thiamine appeared in both fish only after 22 weeks frozen storage. In cod fillets this may have been a result of the frozen storage history and thawing procedures while in mackerel samples thiamine loss may have resulted from the smoking (heating) process.

The histamine remained at low levels in all smoked mackerel samples even in those which had been frozen stored for 33 weeks and then smoked (9.4mg/100g) and would not be expected to cause symptoms of scombrototoxin poisoning.
# CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INTRODUCTION.</td>
<td>1</td>
</tr>
<tr>
<td>2. LITERATURE SURVEY</td>
<td>3</td>
</tr>
<tr>
<td>2.1. PRESERVATION OF FISH.</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1. FREEZING OF FISH.</td>
<td>7</td>
</tr>
<tr>
<td>2.1.1.1. Freezing time</td>
<td>8</td>
</tr>
<tr>
<td>2.1.1.2. Temperature changes during freezing</td>
<td>10</td>
</tr>
<tr>
<td>2.1.1.3. Methods of freezing</td>
<td>14</td>
</tr>
<tr>
<td>2.1.1.3.1. Air blast freezers</td>
<td>14</td>
</tr>
<tr>
<td>2.1.1.3.2. Plate freezers</td>
<td>15</td>
</tr>
<tr>
<td>2.1.1.3.3. Immersion or spray freezers</td>
<td>17</td>
</tr>
<tr>
<td>2.1.1.4. Thawing of fish</td>
<td>19</td>
</tr>
<tr>
<td>2.1.2. SMOKING OF FISH</td>
<td>20</td>
</tr>
<tr>
<td>2.1.2.1. Physical properties of smoke</td>
<td>21</td>
</tr>
<tr>
<td>2.1.2.2. Smoke constituents</td>
<td>22</td>
</tr>
<tr>
<td>2.1.2.3. Methods of smoking</td>
<td>25</td>
</tr>
<tr>
<td>2.2. QUALITY CHANGES IN FISH DURING PRESERVATION.</td>
<td>28</td>
</tr>
<tr>
<td>2.2.1. QUALITY CHANGES DURING FROZEN STORAGE</td>
<td>28</td>
</tr>
<tr>
<td>2.2.1.1. Protein denaturation</td>
<td>29</td>
</tr>
<tr>
<td>2.2.1.2. Lipid deterioration</td>
<td>34</td>
</tr>
<tr>
<td>2.2.1.3. Effect of packaging</td>
<td>41</td>
</tr>
<tr>
<td>2.2.2. QUALITY CHANGES DUE TO SMOKING</td>
<td>43</td>
</tr>
<tr>
<td>2.2.2.1. Process parameters</td>
<td>43</td>
</tr>
<tr>
<td>2.2.2.2. Effect of salting</td>
<td>44</td>
</tr>
<tr>
<td>2.2.2.3. Effect of smoking</td>
<td>47</td>
</tr>
<tr>
<td>2.2.2.3.1. Phenols</td>
<td>48</td>
</tr>
<tr>
<td>2.2.2.3.2. Furans</td>
<td>50</td>
</tr>
<tr>
<td>2.2.2.3.3. Carbonyl compounds</td>
<td>50</td>
</tr>
<tr>
<td>2.2.2.3.4. Polycyclic aromatic hydrocarbons</td>
<td>52</td>
</tr>
<tr>
<td>2.2.2.4. Effect of drying</td>
<td>54</td>
</tr>
<tr>
<td>2.2.2.5. Effect of heating</td>
<td>56</td>
</tr>
<tr>
<td>2.2.2.5.1. Protein quality</td>
<td>56</td>
</tr>
<tr>
<td>2.2.2.5.2. Lipid changes</td>
<td>58</td>
</tr>
<tr>
<td>2.2.2.5.3. Browning of lipids and proteins</td>
<td>59</td>
</tr>
<tr>
<td>2.2.2.6. Total effect of process parameters</td>
<td>62</td>
</tr>
<tr>
<td>2.2.3. NUTRITIONAL ASPECTS</td>
<td>64</td>
</tr>
<tr>
<td>2.2.4. TOXICOLOGICAL ASPECTS</td>
<td>67</td>
</tr>
</tbody>
</table>
2.3. ASSESSMENT OF QUALITY CHANGES DURING FROZEN STORAGE AND DURING SMOKING

2.3.1. Protein changes
2.3.2. Lipid changes
2.3.3. Nutrient changes
2.3.4. Texture changes
2.3.5. Sensory assessment

3. AIMS OF PRESENT WORK

4. EXPERIMENTAL

4.1. MATERIALS AND EQUIPMENT
4.1.1. Fish
4.1.2. Chemicals
4.1.3. Equipment

4.2. PROCESSES
4.2.1. Freezing and frozen storage
4.2.2. Smoking

4.3. ANALYTICAL PROCEDURES
4.3.1. Protein content
4.3.2. Lipid content
4.3.3. Moisture content
4.3.4. Salt content

4.4. ASSESSMENT OF CHANGES DURING FROZEN STORAGE AND DUE TO SMOKING
4.4.1. Salt soluble protein
4.4.2. Free amino acids
4.4.3. Peroxide value
4.4.4. Free fatty acids
4.4.5. Fatty acid profiles
4.4.6. Thiamine
4.4.7. Available lysine
4.4.8. Histamine
4.4.9. Texture (Steven's LFRA Texture Analyser)
4.4.10. Taste panel results

5. RESULTS AND DISCUSSION

5.1. CHEMICAL COMPOSITION OF FROZEN COD AND MACKEREL
5.1.1. Proximate analysis
5.1.2. Lipid FAME composition
5.1.3. Available lysine and thiamine
5.1.4. Histamine in mackerel
1. INTRODUCTION

Hard curing by salting and smoking permits lengthy preservation of fish and widens its acceptability by conferring traditional flavours that are relished as a condiment by many people, especially in Greece.

As transport has improved with industrialization, preferences have changed to milder cured products which do not keep very long and therefore require careful handling. Nevertheless hard cures will continue to be important.

Such a hard curing (salting and smoking or drying) process has been used in Greece since the sixth century (Cutting 1955) when dried, salted and smoked fish had become a common and popular dish. Today Greeks continue to hold fish in high esteem, especially fresh fish and many express a preference for hard cured fish. The main hard cured products of commercial interest are salted-dried sardines and anchovy and salted-smoked mackerel and herring. The fish smoking industry is very traditionally based and its development has been relatively slow. There are only a few smoking companies in existence in Greece and due to shortages of fish (mainly mackerel), fish are imported in a frozen condition from other European countries such as Denmark, France e.t.c. Thus, smoked products are now produced using frozen preserved fish.
It is known from the limited available data that the hard curing process affects the nutritive value and quality of the fish product, but there is no available data regarding how a twice preserved fish (such as frozen and hard smoked) is affected in terms of nutritive value and quality of the fish.

The main aim of this investigation is to evaluate the effect of smoking on protein and lipid quality of lean fish (cod) and fatty fish (mackerel) with different prior frozen storage times and to compare the sensory properties of such products.
2. LITERATURE SURVEY.

2.1. PRESERVATION OF FISH.

As soon as a fish is caught and dies, changes of various kinds begin to occur, leading to deterioration and eventual spoilage (Hobbs, 1982).

As part of the natural process by which organic matter is broken down and returned to the nitrogen cycle, fish flesh is rapidly invaded, digested and spoiled by the microorganisms which are abundant on the skin and in the intestines. Enzymes also contribute to this process, and oxidation by atmospheric oxygen is an additional process of deterioration, particularly in the case of fatty fish.

As a result, methods of preservation to prevent these processes must in any period have been essential to the utilization of fish as food.
The principal processes employed to check bacterial and other forms of spoilage are few in number, and include:

1. Moisture removal — dehydration and concentration.
3. Low temperature treatment, refrigeration (chill), modified atmosphere packaging and freezing.
4. Acidity control — fermentation and acidic additives.
5. Various chemical processing additives.
6. Irradiation.

(Karmas, 1975)

Some combinations of the above treatments have been also developed. Since all processed foods have to be stored until they are consumed, proper food packaging is an important co-processing aspect to the basic food processing methods.

Curing of fish, by drying, smoking and salting with common salt is a combination of the above basic principles of food processing, has its origin in prehistoric times and is bacteriostatic in varying degrees. The success of curing is due to the removal of biologically active water through drying and to the addition of chemicals through smoking and salting, which provide an inhibitory environment for microbial growth and also for enzymic and chemical reactions (Cutting, 1965).
The principal effect of heat treatment is in the denaturation of proteins, i.e. inactivation of microbial and other enzymes. Mild heat treatment (70 °C) frees the fish from human pathogens and most of the vegetative micro-organisms. Heat sterilization (120 °C) is the most effective process of fish preservation because of the destruction of all viable micro-organisms, but this has a severe effect on the nutritional and sensory quality of fish (Karmas, 1975).

Spoilage of low-acid fish is relatively rapid but the growth of fish spoilage organisms is greatly inhibited in a highly acidic environment, e.g. vinegar. Some other additives e.g. salts, sugars and certain spices and herbs have a similar effect (Karmas, 1975). Additionally fermentation enables preservation of fish if the process of decomposition is properly controlled e.g. Shiokara, Nuoc-mam etc.

Cooling by ice slows down the multiplication of micro-organisms and freeze-preservation inhibits microbial growth and slows down the rate of chemical and enzyme reactions. Rapid freezing is regarded as the most harmless method of food preservation in many respects.

Irradiation is the most recent method of food preservation but it is without any practical importance for finfish at the moment. It has been categorized as a "food additive" because high-energy irradiation produces new substances in irradiated fish via highly reactive free radicals (Karmas, 1975).
The situation regarding the preservation of fish catches according to the FAO Fishery Statistics in 1978 and 1987 is as shown in Table 1.

Table 1: Production and processed fishery commodities.

<table>
<thead>
<tr>
<th></th>
<th>1978</th>
<th>%</th>
<th>1987</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m.t.</td>
<td></td>
<td>m.t.</td>
<td></td>
</tr>
<tr>
<td>Total world catches including shell fish.</td>
<td>70,207,045</td>
<td>92,693,351</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish fresh, chilled.</td>
<td>206,436</td>
<td>0.3</td>
<td>317,977</td>
<td>0.34</td>
</tr>
<tr>
<td>Fish frozen.</td>
<td>9,677,221</td>
<td>13.8</td>
<td>13,284,338</td>
<td>14.3</td>
</tr>
<tr>
<td>Fish dried salted or in brine.</td>
<td>3,700,236</td>
<td>5.3</td>
<td>4,546,025</td>
<td>4.9</td>
</tr>
<tr>
<td>Fish smoked.</td>
<td>671,582</td>
<td>0.96</td>
<td>815,466</td>
<td>0.9</td>
</tr>
<tr>
<td>Fish canned.</td>
<td>2,881,658</td>
<td>4.1</td>
<td>3,189,418</td>
<td>3.4</td>
</tr>
</tbody>
</table>

m.t. = metric tonnes. (FAO 1987)

% = of each preservation in the total world catches.

From the Table 1 data, it can be observed that an increase of 32% of the total world catches is accompanied by an increase of all types of preservation, giving percentage increases of 54, 37, 23, 21 and 11 for fresh-chilled, frozen, dried-salted, smoked and canned respectively.
It is very common for combinations of the above methods of preservation to be used and usually the first preservation method is freezing.

In this project such a combination is investigated, in that freezing and smoking are used to preserve two different types of fish (lean and fatty).

2.1.1. FREEZING OF FISH

The purpose of freezing fish is to lower its temperature and thus, slow down spoilage so that when the product is thawed after cold storage, it is almost indistinguishable from fresh fish (FAO, 1977).

The need for freezing and cold storage arises when other methods of preservation of fish, such as chilling with ice, are unsuitable for the period of storage involved. Preservation of fish by chilling may only be suitable for a number of days or a week or two at the most, whereas good freezing and frozen storage will enable the fish to be preserved for months or even up to a year. Preserving fish by freezing has a number of applications, for example, if the fishing areas are distant from the port of landing and fishing trips last many days, freezing at sea is a means of keeping the catch in good condition. Additionally, if the consumer market is distant from the fishing port, freezing may again be necessary to preserve the fish during transportation and distribution. Freezing may also be considered advantageous to
overcome periods of glut and scarcity. The applications are to the advantage of the fisherman, the processor and the consumer since it results in the regulation of supplies, better fish quality and more stable prices. Preservation by freezing and cold storage is usually required if fish are to be exported (FAO, 1977).

The many advantages of freezing are therefore obvious and, in developing countries, the requirements for freezing is becoming more important due to the expansion of fisheries. Freezing and cold storage allows this valuable protein and lipid food to be distributed to a wider market.

2.1.1.1. Freezing time.

The freezing time is the time taken to lower the temperature of the product from its initial temperature to a given temperature at its thermal centre. The practice requires that the average temperature of the fish be reduced in the freezer to the intended storage temperature. The final temperature at the thermal centre is therefore selected to ensure that the average fish temperature has been reduced to this storage value. To ensure that the fish are frozen quickly, the temperature of the freezer must be lower than the storage temperature.

The variables, which affect freezing time, include: freezer type, freezer operating temperature, refrigeration system and operating condition, air speed (in an air blast
freezer), product temperature, product thickness, product shape, product contact area and density, product packaging and species of fish. The above factors will determine the overall heat transfer coefficient and hence the freezing time.

Plank's formula for calculating the freezing time of fish has been widely used in a variety of forms. There is, however, insufficient information available to make this calculation accurate. Calculated freezing times can be fairly accurate for uniformly shaped products such as blocks of fillets but, for other products with irregular shapes, calculation can only give a rough guide.

The more general form of Plank's equation for calculating freezing time is:

\[
\text{Freezing time} = \frac{L}{PD} + \frac{PD}{RD^2}
\]

Where:

- \( L \) = Heat to be extracted between the initial freezing point and final temperature (KJ / Kg)
- \( V \) = Specific volume of fish (m\(^3\) / Kg).
- \( O \) = Temperature difference between the initial freezing point of the fish and the refrigerating medium (°C).
- \( D \) = Thickness of product in direction of prevailing heat transfer (m)
- \( f \) = Surface coefficient of heat transfer (including effect of packaging) (KJ / h m\(^2\) °C).
- \( K \) = Thermal conductivity of frozen fish (KJ / h m °C).
- \( P \) & \( R \) = Constants which depend upon the shape.

(FAO, 1977).
Since fish is frozen from the outside inward, it is impossible to judge by the outward appearance whether the whole of it is frozen. The surface of the fish, which is close to the freezing medium such as the cold air or the cold metal, will very quickly be reduced to a temperature near to that of the freezer. The temperature inside the fish will, however, change more slowly.

The most suitable instrument for measuring freezing times is the thermocouple potentiometer. The critical point of this operation is that the temperature measurements must be taken at the points which are likely to freeze last. The centre of the fish or package is not necessarily the last part to freeze, this will happen only when freezing is carried out equally all sides. The thermocouple should therefore be inserted in the fish so that the temperature-sensitive point is likely to be in the part that will freeze last. (FAO, 1977).

2.1.1.2. Temperature changes during freezing.

Fish is composed of 60-80 percent water, depending upon the species, and the freezing process converts most of this water into ice. Freezing requires the removal of heat and the temperature falls in the manner shown in Fig. 1. During the first stage of cooling the temperature falls rapidly to just below 0 °C, the freezing point of water. In the second stage more heat requires to be extracted in the removal of latent heat, which allows the bulk of the water to form ice.
The temperature changes very little in this stage, which is known as the period of "thermal arrest".

Fig.1. Temperature-time graph for fish during freezing.

When about three quarters of the water is turned to ice, the temperature again begins to fall and during this third stage most of the remaining water freezes. A comparatively small amount of heat has to be removed during this third stage.

As the water in fish freezes out as pure crystals of ice, the remaining unfrozen water contains an increasing concentration of salts and other compounds which are naturally present in fish flesh. (FAO, 1977). The effect of the above is to depress the freezing point of the unfrozen water and
the result of this is that subsequent ice formation occurs over a range of temperature. The proportion of water in the muscle tissue of fish which is converted to ice at various temperatures is shown in Fig.2. The figure shows that even at a temperature of -30 °C, approximately 10% of the water in the fish muscle still remains in the unfrozen state.

Fig.2. Freezing of fish muscle. The percentage of water frozen at different temperatures.

The difference in quality between slow and quick freezing of foods has been extensively discussed but only in recent years has knowledge of the freezing process advanced sufficiently to explain these differences in freezing rates.
There was an early opinion that rapid freezing was unsatisfactory since sudden cooling was thought to disrupt and tear the muscle tissue. It was also thought that, since water expands on freezing, it might be reasonable to expect the cell walls to burst under the pressure (FAO, 1977). Another widely held view was that slow freezing resulted in the formation of large ice crystals which damaged the walls of the cells, which would result in a considerable loss of fluid when the fish was thawed. The smaller ice crystals which formed when fish is frozen quickly were thought to do little damage to the cell wall and, as a result, caused little fluid loss on thawing. This probably accounts for some of the differences in fish quality between slow and quick freezing but it has been shown that this still does not provide a full explanation. The walls of fish muscle cells are sufficiently elastic to accommodate the larger ice crystals without excessive damage. Also, most of the water in fish muscle is bound to the protein in the form of a gel and little fluid would be lost even if damage of the above nature did occur (FAO, 1977).

Slow freezing, however, does result in an inferior quality product and this is due to denaturation of the protein (FAO, 1977). This protein denaturation during freezing depends upon the temperature of the fish and as the temperature is reduced, below -20 °C, the rate of denaturation is reduced. A higher concentration of enzymes and other compounds in the unfrozen portion also results in an increase in the rate of denaturation. It has been demonstrated that the temperature
of protein denaturation is in the region of -1 °C to -2 °C.

The result of slow freezing is that a longer time is spent in this zone of maximum activity and this is the factor which accounts for the main difference in quality between slow and quick frozen fish. (FAO, 1977) (Section 2.2.1.).

2.1.1.3. Methods of freezing.

There are now many different types of freezer available for freezing fish and the three main factors which should be considered when selecting a freezer are financial, functional and feasibility.

There are three basic methods of freezing fish:

a. AIR BLAST FREEZERS.

b. PLATE FREEZERS.

c. IMMERSION OR SPRAY FREEZERS.

2.1.1.3.1. Air blast freezer.

The principal advantage of the air blast freezer is its versatility. It can successfully manage with a variety of irregularly shaped products, including single large fish.

There are many designs of blast freezer, but there are two basic types, a continuous freezer through which the product
moves during freezing. and the batch freezer in which the product remains stationary. In a continuous freezer the product is carried on trucks or on a conveyer, this type is most suited to mass production of standard products requiring similar freezing times. Continuous freezers can be further divided into two types, series flow when the air flows in line with the trucks or conveyer and cross flow when the air flows across the line of movement of the product. In a well designed and correctly operated blast freezer, the air speed over the fish should be uniform, thus ensuring uniform freezing.

A disadvantage for air blast freezers is that air has low heat capacity and is a poor conductor of heat, so that a fairly high air speed is necessary to effect freezing. However, the additional heat generated by powerful fans also has to be removed by the refrigeration system. An air speed of 3-6 m/s is usually adequate for economic freezing in many of these freezers (Graham, 1982; FAO, 1977).

2.1.1.3.2. Plate freezers.

Plate freezers along with air blast freezers are the types of freezer most commonly used for freezing fish in industrial countries. Plate freezers can be arranged with the plates horizontal to form a series of shelves to give "Horizontal Plate Freezers" or the plates may be arranged in a vertical plan to give "Vertical Plate Freezers". Plate freezers have their plates constructed from extruded sec-
tions of aluminum alloy arranged in such a manner as to al-
low the refrigerant to flow through the plate and thus
provide heat transfer surfaces on both sides. All plate
freezers are fitted with hydraulic systems which move the
plates to compact the produce and give higher density
products (blocks). They also improve the fish-to-plate area
for quicker freezing and assist with the release of the

Horizontal Plate Freezers.

The two main uses of this type of freezer are the freezing
of prepackaged cartons of fish and fish products for retail
sale and the formation of homogeneous rectangular blocks of
fish fillets for the preparation of fish portions. Horizon-
tal plate freezers are operated with a hot gas defrost and
have additional pipework to allow the cold refrigerant to be
discharged from the bottom of the freezer as the defrost
proceeds (Graham, 1982; FAO, 1977).

Vertical Plate Freezers.

The main advantage of using this type of freezer is that
fish can be frozen in bulk without any previous requirement
to package or arrange it. The plates form a bin with an open
top and fish are loaded directly into this space. This type
of freezer is therefore particularly suitable for bulk
freezing and it has also been extensively used for freezing
whole fish at sea.
Fish such as cod and haddock produce compact blocks with a block density of approximately 800 Kg / m³. With fatty fish such as mackerel and herring, it has been found advantageous to use wrappers and some water to fill the voids in the block. Fatty fish do not form blocks which are as firm and strong as blocks made from lean fish, especially during seasons when the oil content of the fish is high. Water added to the block helps to strengthen the block, protects the fish during subsequent handling and reduces the effects of dehydration and oxidation during cold storage. A wrapper that has been found suitable for this purpose is a single layer paper bag, coated internally with polyethylene and shaped to fit the space between the freezer plates. Fish frozen with wrappers will inevitably require a longer freezing time due to the insulating properties of the wrapping material (Graham, 1982; FAO, 1977).

2.1.1.3.3. Immersion or spray freezers.

The liquid for immersion freezing must have a freezing point well below that of water, e.g. use of refrigerated brine. A brine made from a solution of common salt in water cannot cool fish much below -18 °C, but fish immersed in it are frozen quickly because excellent contact is made between them and the refrigerant.

Unfortunately, salt absorbed by the fish during freezing, can accelerate deterioration during cold storage and can make the thawed product taste too salty. The method is used
in some countries for freezing large individual fish such as salmon and tuna for subsequent canning.

Other liquids with a low freezing point have been used for fish: e.g. refrigerated solution of salt, or sugar and salt, used mainly for immersion freezing of shrimp, but contamination of the product with the immersion liquid can lower its acceptability (Graham, 1982; FAO, 1977).

Liquid Freon, Carbon Dioxide, Nitrogen.

A purified form of dichlorodifluoromethane, known as liquid Freon Freezant, can be used in direct contact with fish. The product is first immersed in liquid Freon to harden the surface quickly and prevent products sticking together, it is then sprayed with refrigerant to complete the freezing. Some products such as fillets require to be sprayed first and immersed afterwards in order to maintain their shape. Recent environmental awareness has resulted in the suggested removal of dichlorodifluoromethane from use as a refrigerant.

Using liquid nitrogen or carbon dioxide, the product meets a stream of cold gas, which partially freezes and then further spraying completes the freezing process. These methods have many advantages e.g. short freezing time, compact equipment and a low product weight loss, but against these must be weighed the high cost of freezing, equipment & operation. Immersion freezing is generally used for high
value products such as shrimp and scampi (Graham, 1982; FAO, 1977).

2.1.1.4. Thawing of fish.

Ideally fish should be thawed in the shortest possible time, where thawing time is the time taken to raise the temperature above -1 °C throughout the fish, immediately after removal from the cold store. The process should have no noticeable effect on quality and the conditions should not encourage the growth of bacteria on the fish or the surroundings. Proper attention to thawing can help to avoid product losses resulting from damaged fish, drip formation and evaporation. These factors emphasize the need for compact automatic thawing equipment (Jason, 1982).

Methods of thawing can be divided into two groups: those in which heat is conducted into the flesh from the surface and those in which heat is generated more or less uniformly throughout the flesh. In the first group, heat is applied to the surface of the fish by exposing the fish to still or moving moist air, by immersing them in or spraying them with water or by allowing water vapour to condense on them. Methods in the second group include dielectric heating, electrical resistance heating and microwave heating. High rates of thawing can be obtained by these second methods, but a limit is set by the risk of uneven heating and sometimes local cooking if power is fed into the fish too rapidly. (Jason, 1982).
2.1.2. SMOKING OF FISH.

The process of smoking commonly involves four main stages: salting, drying, smoking and heat treatment, all of which increase the shelf-life of the product by suppressing the growth and development of microorganisms.

It is known that the preservation effects of wood smoke on foods result from partial dehydration and deposition of chemicals with antimicrobial and antioxidant activity (Chen and Issenberg, 1972). Storey (1986) described smoking as a destructive distillation process involving the partial combustion of wood in comminuted form or otherwise.

Traditionally, hard woods are recommended for smoking, especially oak and hickory, because they contain more lignin which under pyrolysis and on oxidation, liberate phenols which are responsible for imparting a desirable flavour. Soft wood, e.g. spruce and pine have a high cellulose content, which produces acetic acid and other undesirable organic acids when burnt. These acids are known to impart an acid flavour and bitterness to the product (Clifford et al 1980).

In some modern societies, fish are smoked to impart a desirable flavour rather than for a preservative effect and a large proportion of this type of smoked fish are frozen after smoking. However, traditional methods of smoking are still important whereby fish are exposed to wood smoke gen-
erated by heating sawdust to an appropriate high temperature (Moini & Storey, 1980).

The preservative effect and the long storage life of the traditional smoked products is due to the combined effect of hard salting, smoking, drying and heating. Such a product has a high salt concentration, about 15% and much more smoking, drying and heating than the mild cured fish (Burgess et al 1965).

2.1.2.1. Physical properties of smoke.

When wood is heated and undergoes destructive distillation, it gives off volatile compounds some of which condense in the cool zone above the fire to form a stable aerosol. It is these tarry droplets (particles) which constitute the visible smoke and the vapours which constitute the invisible smoke (mainly volatile compounds). The deposition and absorption of smoke by the foodstuff results in the characteristic colour, flavour and preservative properties of smoked foods.

The chemical composition of particles and vapours is similar, but the particles contain a greater proportion of high molecular weight components. The partition of mass concentration between particles and vapours as well as their chemical composition, depends upon the nature of the sawdust and the conditions of combustion.
The quantity of smoke deposited, which occurs under the influence of forces of gravity, turbulence, diffusion and a force arising from the effects of any temperature gradient, known as a radiometer force is a complex function of the composition and concentration of the smoke, the environmental conditions and the nature of the surface (Cutting, 1965).

2.1.2.2. Smoke constituents.

The three major constituents of wood are cellulose, hemicellulose and lignin. The natural abundance of each polymer varies with the type of wood and with the method of its estimation but they can be considered to exist in the ratio of 2:1:1 respectively (Gilbert & Knowles, 1975).

Each of these above constituents appears to give different products due to pyrolysis. The breakdown of celluloses produce acetic acid as a main pyrolysis product and occasionally small amounts of furans and phenols. Hemicelluloses, which are the least heat-stable of the wood components, give various breakdown products dependent upon the source, with hardwoods being richer in pentosans and softwoods in hexosans. Lignins, are important wood flavour producing components, and produce compounds such as phenols and phenolic ethers (Gilbert & Knowles, 1975).

The above is a very general description of the behaviour of each wood component but during thermal degradation wood be-
haves as if it were a mixture of its three major components. Thus, wood smoke, which is used for smoking of foods, contains a tremendous number of compounds formed by pyrolysis of the wood components mixture. More than 300 substances have been detected and many more may exist (Maga, 1987).

The most important classes of chemical compounds detected in smoke are: phenols, carbonyls, acids, furans, alcohols, esters, lactones and polycyclic aromatic hydrocarbons (Table 2). Some of the above compounds have desirable effects on foods such as flavouring, preservation and colouring and some have undesirable effects such as contamination with toxic components and destruction of some essential amino acids (Hamm, 1977).

It is generally accepted that the typical aroma of smoked foods seems to be due to phenols, whilst carbonyls and acids possibly contribute little to the flavour of smoked foods. The antioxidative and bacteriostatic effect of curing smoke is caused mainly by the presence of certain phenols. The characteristic golden-brown colour seems to be an effect of carbonyl compounds which are thought to undergo non-enzymic browning with the amino groups of protein. Acidic compounds also influence colour formation by causing surface hydrolysis of proteins creating brown pigments, which inhibit the penetration of carbonylic and other smoke components. Furthermore, smoke contains toxic components mainly polycyclic aromatic hydrocarbons, such as benzo(a)-pyrene, which has carcinogenic properties (Hamm, 1977).
Table 2: Compounds identified in wood smoke.

<table>
<thead>
<tr>
<th>Category</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acids (47) including</td>
<td>Alcohols (22) including</td>
</tr>
<tr>
<td>formic (methanoic)</td>
<td>methyl (methanol)</td>
</tr>
<tr>
<td>acetic (ethanoic)</td>
<td>ethyl (ethanol)</td>
</tr>
<tr>
<td>glycolic (hydroxyethanoic)</td>
<td>propyl (1-propanol)</td>
</tr>
<tr>
<td>Lactones (16) including,</td>
<td>Phenols (78) including</td>
</tr>
<tr>
<td>γ-butyrolactone</td>
<td>phenol</td>
</tr>
<tr>
<td>β-angelica lactone</td>
<td>α-cresol (2-methylphenol)</td>
</tr>
<tr>
<td>γ-crotonolactone</td>
<td>m-cresol (3-methylphenol)</td>
</tr>
<tr>
<td>Carbonyls (130) including,</td>
<td>Miscellaneous (50) including</td>
</tr>
<tr>
<td>methanal</td>
<td>methylamine</td>
</tr>
<tr>
<td>ethanal</td>
<td>dimethylamine</td>
</tr>
<tr>
<td>hydroxyethanal</td>
<td>trimethylamine</td>
</tr>
<tr>
<td>Furans (45) including,</td>
<td></td>
</tr>
<tr>
<td>furfuryl alcohol</td>
<td></td>
</tr>
<tr>
<td>2-furfuryl methyl alcohol</td>
<td></td>
</tr>
<tr>
<td>furan</td>
<td></td>
</tr>
</tbody>
</table>

(Maga, 1987).
2.1.2.3. Methods of smoking.

There are two distinct types of smoking process and these vary in the amount of heat to which the fish are exposed.

The first type is "Cold smoking" in which the normal smoke temperature is not more than about 30 °C and the smoked fish are not even partly cooked, while the second type is "Hot smoking" in which the fish are cooked at different temperatures, from 60 to 100 °C and sometimes above 100 °C. This heat treatment results in partial sterilization, although subsequent spoilage of the cooked flesh is still rapid, thus preservation of these products at low temperature is a necessity if the products are not extensively dried or/salted (Cutting, 1965). A more extensive salting treatment is usually used in cold smoking and because of the heating stage, hot smoked products are expected to become more dried.

Smoking takes place in a smoking kiln, which can be one of two types, a traditional kiln with natural convection or a mechanical kiln with forced convection and temperature control.

The traditional kiln is simply a chimney and requires considerable practice and skill to produce satisfactory results. The main problem is to control the process in such a way as to secure reasonable uniformity of product. Obviously the temperature, relative humidity, flow rate of air
and smoke intensity above an open fire are subject to wide fluctuations and they depend upon a number of factors, such as outside weather conditions, size and construction of kiln, type and moisture content of sawdust etc. The above disadvantages and difficulties become greater for hot smoking products.

The mechanical kiln, in which the smoke is moved over the fish by means of a fan, has obvious advantages compared with the traditional chimney kiln. The temperature of the smoke can be controlled since the smoke is generated outside the kiln in the smoke producer part of the kiln. The speed of the smoke can be controlled and the smoke can be much more regularly distributed, so that all fish in the kiln are smoked to the same approximate extent at the same time (Storey, 1982).

An alternative to traditional methods of smoking has been developed. This is the liquid smoke treatment, and its introduction in the 1930s was a technical break-through, which was to replace the laborious and time-consuming procedure of hanging or layering in the smoke kiln (Cutting, 1965).

Smoke consists of water-soluble and-insoluble compounds and is divided into a tarry and an aqueous phase. If freshly developed smoke is condensed in water, the solution is a bright yellow colour. During storage it becomes increasingly dark with the formation of brown-coloured condensation or polymerization products. These products, accompanied by PAH,
precipitate from the aqueous solution. Precipitation may be prevented by the addition of acids (acetic or citric), organic solvents (ethyl alcohol or glycol) or detergents (Toth & Potthast, 1984).

The production of liquid smoke is described in numerous patent specifications. The most usual method of producing liquid smoke is to pyrolyze hardwood by smouldering sawdust and to capture the wood smoke in water. The smoke condensation is carried out until a given concentration has been reached (Toth & Potthast, 1984).
2.2. QUALITY CHANGES IN FISH DURING PRESERVATION.

The quality changes in smoked fish differ from those of frozen fish, therefore each preservation process will be considered separately.

2.2.1. QUALITY CHANGES DURING FROZEN STORAGE.

In general, the temperature history of frozen fish includes the following phases: unfrozen storage, freezing, frozen storage, thawing and storage in the thawed phase (Lee & Jiang, 1985). During each of the above phases the fish may change in ways which will affect its acceptability, these changes being of three types: spoilage due to microbiological action; changes due to the action of endogenous enzymes; and chemical or physical changes such as oxidation or loss of flavour components through leaching. All these types of changes can occur during the unfrozen storage phase, whilst during the frozen storage only the latter two can occur. The rate of each type of deteriorative change depends upon the temperature, whilst its extent depends upon the length of the phase.

As far as frozen storage is concerned there are two stages of commercial importance, firstly, storage in a holding store at a temperature of usually -20 to -30 °C and secondly, storage in a retail cabinet at a temperature -15 to -20 °C (Connell & Howgate, 1968)
2.2.1.1. Protein denaturation.

Apart from water, proteins are by far the major constituents of fish flesh. They determine to a very large extent the physical properties of flesh and modifications in the amount, nature or types of these proteins are reflected in modifications of physical properties. Like other skeletal muscle proteins, those of fish flesh can be divided into the classical solubility groups.

Connell (1964) described them as follows:

<table>
<thead>
<tr>
<th>Ionic strength at which soluble</th>
<th>Name of group</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Equal to or greater than 0</td>
<td>&quot;Myogen&quot;</td>
<td>Mainly sarcoplasm (muscle cell juice)</td>
</tr>
<tr>
<td></td>
<td>Easily soluble proteins</td>
<td></td>
</tr>
<tr>
<td>2. Greater than about 0.3</td>
<td>&quot;Structural&quot;</td>
<td>Mainly myofibrils (contractile elements of cell)</td>
</tr>
<tr>
<td></td>
<td>Less soluble proteins</td>
<td></td>
</tr>
<tr>
<td>3. Insoluble</td>
<td>&quot;Stroma&quot;</td>
<td>Mainly connective tissue.</td>
</tr>
</tbody>
</table>

The easily soluble proteins comprise the bulk of the enzymes in the muscle. They are, therefore, particularly relevant to studies of biochemical changes occurring during post-morten
handling of fish. Their importance is illustrated by the work of Connell (1964) who showed how certain enzymes play a part in producing compounds responsible for taste, smell and colour and, through autolytic softening, the physical characteristics of the fish. The amount of this group is about 20-22 percent of the total protein content of fish muscle and the space occupied by the sarcoplasm in live muscle is probably of similar proportions (Connell, 1964).

The sarcoplasmic proteins as a whole are more stable than the structural ones and in the muscle they survive drastic treatments. They also play a minor role in determining properties such as textural eating quality.

Therefore, as far as proteins are concerned, the major technological problems are connected more with the structural or myofibrillar proteins rather than the sarcoplasmic ones. This group of proteins is much simpler in composition than the sarcoplasmic group and comprises two main proteins: actin and myosin. According to Connell (1964) the amounts of these proteins (actin & myosin) are probably 15-25 percent and 50-60 percent of the total muscle proteins respectively. These two proteins can be accurately estimated as the actomyosin complex, which amounts to 67-75 percent of the total muscle proteins (Dyer et al 1950).
Another myofibrillar protein, tropomyosin, has been isolated from fish muscle but its amount is small (3-5 percent of the total muscle protein) and there is no evidence that it has any technological significance.

The actomyosin complex is of prime technological importance, as it is well-known that the toughening process occurring during frozen storage of fish is attended by a progressive diminution in the amount of actomyosin that can be extracted (Ironside & Love, 1958). This may be illustrated by freeze dried fish, which is notable for its comparatively tough and dry texture.

The insoluble proteins or stroma, which comprises the connective tissue and cell walls, has received less attention. This group is present only in a very small amount, 2-3 percent, in fish and it appears that the principal determinant of the physical properties of fish flesh is the muscle contents rather than the surrounding connective tissue.

When fish is held in the frozen state it undergoes deterioration changes in both texture and flavour. The rates of deterioration are generally faster than for mammalian flesh but they vary markedly from one species to another. It is known, for example, that gadoids develop toughness more rapidly than flatfish (Sikorski et al, 1976) and the fatty species, such as herring and mackerel, produce rancid flavours more rapidly than white fish species (Ackman, 1980).
Although a great deal of research has been carried out, there is no completely satisfactory explanation for the development of toughness in fish flesh during frozen storage. It is known, however, that changes in the properties of the myofibrillar proteins are reflected in changes in texture and as toughness develops the solubility of this group of proteins progressively decreases. This solubility in salt solution is used as an index of frozen storage deterioration. It has been suggested that this loss of solubility is due to the various reactions of formaldehyde with proteins. Under certain conditions formaldehyde forms covalent methylene crosslinks between protein chains with a resultant increase in molecular weight and polymers that are insoluble in hydrogen-bond breaking solvents. These conditions are high protein and formaldehyde concentrations, high temperatures and prolonged reaction times. Otherwise there is a tendency for formaldehyde to bind rapidly reversibly or irreversibly to single amino acid residues. The first type of reaction could readily account for the toughening observed during frozen storage (Connell, 1975).

Mackie & Thomson (1974) attributed the accelerated denaturation of the proteins, during frozen storage of cod, to the increased concentration of formaldehyde in the fish flesh.

King et al. (1962) and Anderson & Steinberg (1964) suggested the interaction of lipids with proteins as a possible source of denaturation of frozen storage. This was proved by Jarenback & Liljemark (1975) using electron microscopy. Connell
(1968) & Matsumoto (1979) did not accept this interaction as the only essential factor. However, Andou et al. (1980) stated that free fatty acids (produced by the hydrolysis of lipid) of small molecular mass cause protein insolubility during frozen storage.

Jiang et al. (1987) studied the effect of free amino acids on protein denaturation and possible interactions between these free amino acids and myofibrillar proteins during frozen storage. They used two mixtures of free amino acids, the first consisting of glycine, proline and glutamic acid and the second histidine, lysine and taurine. They observed that after freezing the solubility of actomyosin with the first mixture was significantly higher than that of the second mixture and of a control made up without adding free amino acids. They also observed that during frozen storage, the free amino acid content decreased and the solubilities of the control and the sample with histidine, lysine and taurine were significantly lower than the sample with glycine, proline and glutamic acid. Jiang et al. (1987) concluded that some free amino acids such as histidine, lysine and taurine accelerate the denaturation of protein and some others such as glycine, proline and glutamic acid have a preventive effect on the denaturation of protein.

Additional factors which cause fish muscle toughness and loss of extractable protein (protein denaturation) during frozen storage include the frozen storage temperature (Dyer, 1951), temperature fluctuation (Love, 1983), moisture
changes (Hultin, 1986) and enzymatic degradations (Amano & Yamada, 1964; Regenstein et al, 1981).

2.2.1.2. Lipid deterioration.

Fish lipids are known to be very complex mixtures, incorporating a wide range of components (Lovern, 1962). Ackman (1980) classified the lipids into the two categories of polar and neutral, the former category consisting mainly of phospholipids and the latter of triglycerides. Other components of the neutral division of fish lipid includes sterols, free fatty acids, wax esters and glyceryl ethers, but whereas free fatty acids, triglycerides, phospholipids and sterols occur in all fish oils, wax esters and glyceryl ethers only occur in a few species i.e. wax esters may be found in the lipid of cod.

Lean fish (0.5-1.5% lipid) contains 85-95% polar lipid, mostly phosphatidyl ethanolamine and phosphatidyl choline, whereas fatty fish (2-25% lipid) contains mainly neutral lipid 75-85%.

Stansby (1981) stated that in contrast to the generally accepted idea that oils from one species of fish varied only a little, if at all, in fatty acid profile, there are in fact tremendous variations. Such variation occurs from fish to fish of the same catch, from batches of fish caught in the general area at different times of the year, from batches of fish caught at the same time but in different geographical
locations and even from fish caught in one geographical location at one date from one year to another. These variations in fatty acid profile may be very large i.e. It was found by Stansby (1981) that the composition of menhaden oil was: 14:0, from 6.7 to 16.3%, 16:1, from 11.2 to 17.9%, 18:1, from 10.7 to 23.4%, 20:5, from 10.2 to 14.1% and 22:6, from 3.3 to 10.6%.

Table 3, demonstrates the fatty acid profiles of cod & mackerel.

It has been observed that the two common "plant" fatty acids 18:2n-6 (linoleic) and 18:3n-3 (linolenic) do not exist in most fish oils to the extent of more than 1 or 2%.

There is much interest in the beneficial effects of fish oil consumption. Evidence for the effectiveness of dietary n-3 PUFAs in delaying, preventing and ameliorating many diseases such as thrombosis, cardiac arrhythmia, arthritis, inflammation etc. (in humans and animals) by reducing eicosanoid production from arachidonic acid, is accumulating from epidemiological, clinical and nutritional studies. This evidence illustrates the importance of fish and fish products in the diet, and the importance of prevention of fish lipid deterioration (Kinsella, 1986 and 1988).
Table 3: Typical fatty acid profiles of a lean (Gadus morrhua) and fatty (Scomber scombrus) fish.

<table>
<thead>
<tr>
<th>Saturated</th>
<th>Cod (G. morrhua)</th>
<th>% of total</th>
<th>Mackeral (S. scombrus)</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.4</td>
<td></td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td>-</td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>19.6</td>
<td></td>
<td>17.6</td>
<td></td>
</tr>
<tr>
<td>17:0</td>
<td>-</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>3.8</td>
<td></td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>19:0</td>
<td>-</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>20:0</td>
<td>-</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>24.8</td>
<td></td>
<td>29.6</td>
<td></td>
</tr>
<tr>
<td>Monounsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:1n-9</td>
<td>-</td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>16:1n-7</td>
<td>3.5</td>
<td></td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>18:1n-9</td>
<td>13.8</td>
<td></td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>18:1n-7</td>
<td></td>
<td></td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>18:1n-5</td>
<td>-</td>
<td></td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>20:1n-9</td>
<td>3.0</td>
<td></td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>20:1n-7</td>
<td>-</td>
<td></td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>22:1n-11</td>
<td>1.0</td>
<td></td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>22:1n-9</td>
<td>-</td>
<td></td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>21.3</td>
<td></td>
<td>43.1</td>
<td></td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:2n-4</td>
<td>-</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>16:3n-3</td>
<td>-</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>16:4n-3</td>
<td>-</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0.7</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.1</td>
<td></td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>18:4n-3</td>
<td>0.4</td>
<td></td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>20:4n-6</td>
<td>2.5</td>
<td></td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>20:4n-3</td>
<td>-</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>20:5n-3</td>
<td>17.0</td>
<td></td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>22:5n-6</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.3</td>
<td></td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>22:6n-3</td>
<td>29.8</td>
<td></td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>51.8</td>
<td></td>
<td>25.3</td>
<td></td>
</tr>
</tbody>
</table>

Fish lipids are subject to two major changes: hydrolysis and oxidation, both of which may proceed via enzymic and non-enzymic mechanisms. The effect of these changes results in the development of unpleasant odours, colour, flavours and texture, the latter as a result of the interaction between oxidized unsaturated lipids and proteins to form insoluble lipid-protein complexes.

Hydrolysis of triglycerides and phospholipids is normally a stepwise process leading to the production of free fatty acids. It mainly occurs through catalysis by lipolytic enzymes (lipases) with small contributions from bacterial or chemical hydrolytic mechanisms (Olley & Lovern, 1960). In mackerel (Scomber scombrus) species the breakdown of its muscle lipids in frozen storage were found to follow the known process of lipid hydrolysis (Ackman, 1980). In lean fish, it was found that both triglycerides and phospholipids were hydrolyzed over 12 months of storage, however, in fatty fish, the free fatty acids recovered were mostly from triglycerides (Hardy, 1980).

Lovern (1962) noted that lipid hydrolysis in salted cod (Gadus morhua) ceased when the lipids contained 50% of free fatty acids. The author suggested that the reaction products may inhibit the enzyme activity. Hardy et al. (1979) studied lipid antioxidative changes in cold stored (-10 °C) cod. The analysis of lipid fractions indicated that the neutral lipid tended to remain unchanged, but the phospholipids were hydrolyzed and free fatty acids were generated. The phos-
Phospholipids showed an increase in the C18 acids with a decrease in the C16 and C20 components and in the free fatty acids the proportion of the C22 acids increased primarily at the expense of the C18 component.

The autoxidation of lipid involves free radical attack on unsaturated lipids, including free fatty acids, triglycerides and phospholipids, and has a variety of known effects on fish quality. The free radicals and hydroperoxides are unstable and may react with other fish constituents as demonstrated in Fig. 3.

The following simplified scheme shows the various steps in autoxidation.

<table>
<thead>
<tr>
<th>STAGE OF AUTOXIDATION</th>
<th>RADICAL CHAIN REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation</td>
<td>RH (\rightarrow) R.</td>
</tr>
<tr>
<td>Propagation</td>
<td>R. + O(_2) (\rightarrow) ROO.</td>
</tr>
<tr>
<td></td>
<td>ROO. + RH (\rightarrow) ROOH + R.</td>
</tr>
<tr>
<td>Termination</td>
<td>ROO. + R. (\rightarrow) ROOR</td>
</tr>
<tr>
<td></td>
<td>R. + R. (\rightarrow) R - R</td>
</tr>
<tr>
<td></td>
<td>ROO. + ROO. (\rightarrow) ROOR + O(_2)</td>
</tr>
</tbody>
</table>

Interaction of radicals to produce non-initiating and non-propagating species.


Where, ROO. is a lipid peroxy radical, R. is a lipid radical and RH is an unsaturated lipid (Khayat & Schwall, 1983).
**Fig. 3.1** Reactions of polyunsaturated fatty acids leading to quality and nutritional changes in food.

Unsaturated lipid

\[
1 \xrightleftharpoons[O_2]{A \to B} \text{Hydroperoxides} \xrightarrow{C \text{ Derivatives}} \text{Vitamins} \xrightarrow{\text{Changed. nutrients}} \text{NUTRITIVE VALUE}
\]

\[
\text{TEXTURE} \quad \text{PROTEIN} \quad \text{Cross linked protein} \quad \text{FUNCTIONALITY}
\]

\[
\text{SAFETY} \quad \text{HYDROGENATED} \quad \text{TASTE}
\]

\[
\text{Scission} \quad \text{Volatiles compounds} \quad \text{AROMA}
\]

\[
\text{Rearrangements} \quad \text{Amino compounds} \quad \text{COLOUR}
\]

**Pro-oxidant catalysts**

1. Lipoxygenase
2. Haemoproteins
3. Trace metals

**Antioxidants**

A. Superoxide dismutase
   Glucose oxidase
   Catalase

B. True antioxidants (e.g. tocopherols)
   Synergists
   Flavonoids
   Phenolic compounds (e.g. herbs and spices)
   Metal binding compounds (e.g. proteins)

C. Maillard reaction products

The lipid peroxides also break down to produce many types of secondary reaction products. Such products contribute to the oxidized flavour of fish lipids. Secondary products include carbonyl compounds, alcohols, acids, hydrocarbons, lactones and esters. Extensive autoxidation of fishery products is usually an adequate indicator to prevent these of being marketed or consumed by humans.

The following factors play an important role in oxidative reactions in fish tissue.

1. Nature of fat, e.g. the type of fatty acids, degree of unsaturation and proportion of phospholipids.

2. Distribution of fat in the body, e.g. potential contact of fat with accelerators or inhibitors of rancidity.

3. Presence or absence of other chemical compounds in the tissue which may act as accelerators or inhibitors of rancidity reactions, e.g. metal ions such as Fe$^{2+}$, Cu$^{2+}$ and Fe$^{3+}$ each of them appearing to have a different effect on lipid oxidation.

4. External factors, such as frozen temperature, light, exposure to oxygen (packaging).
Khayat & Schwall (1983) studied the changes in the fatty acid profile of jack mackerel by gas-liquid chromatography. They reported that polyunsaturated fatty acids decreased during storage at 5 °C. The most predominant unsaturated fatty acid (C22:6) decreased the most, while saturated and monoenic fatty acids showed comparatively little decrease.

2.2.1.3. Effect of packaging.

The importance of adequate packaging of frozen fish is in order to prevent loss of moisture vapour from the product and penetration of oxygen into the product (Bramsnaes, 1969).

It can be assumed that the relative humidity within the voids of a sealed packaged product will be 100% and if such a product is stored at a constant temperature no dehydration can occur (Storey, 1980). If, however, the temperature of the product fluctuates and the product is not totally enclosed by the container, moisture will migrate to the inside surface of the package forming ice crystals and dehydration will take place (Bramsnaes, 1969; Storey, 1980).

According to Dyer (1951) dehydration results in protein in-extractability and irreversible denaturation and oxidation of the lipid fraction, resulting in flavour deterioration and possible deterioration of the protein and texture. Sensitive products may suffer from this dehydration so that vacuum packaging may be preferable. The storage life of
peeled shrimp in normal foil bags was only 6 months, while vacuum packing more than doubled the storage life (Bramsnaes, 1969).

Fatty fish benefit considerably by being vacuum packed. Bramsnaes (1969) found that the storage life of frozen (at \(-20\) °C) eviscerated trout was extended from 5 to 15 months by vacuum packing.

The optimum requirements of a package for frozen seafood are:

1. Flexible enough to fit the contour of the fish and leave little or no air space.
2. Should not become easily damaged when cold.
3. Should not deteriorate in cold storage.
4. Puncture resistant.
5. Moisture vapour proof.
6. Impervious to oxygen.
7. Easily filled.

It should be also practical and convenient to handle during storage and transportation.

Many organic films as well as laminates have been developed with varying degrees of permeability to water vapour and to oxygen and some have proved satisfactory for fish products (Dyer & Peters, 1969). Materials which have been used for packaging include metals, paperboard, wood and plastics.
Plastics are relatively new compared to others, but they are widely used and new applications are still being developed. There are 50 commercially available plastics (Hasan, 1984).

2.2.2. QUALITY CHANGES DUE TO SMOKING.

The major changes due to smoking are in flavour, colour and texture of the fish product. The effect of each stage of the smoking process on quality of smoked fish will be considered.

2.2.2.1. Process parameters.

The process of "smoking" is the combination of salting, smoking, drying and heating each of which has its own preservative and other quality effects in the product.

The flavour of some products is quite characteristic of the local smoking process, reproducible within limits and relished by many people.

Smoking controls in various way the deterioration caused by micro-organisms, proteolytic enzymes, oxidation (particularly of lipids), and various non-enzymic reactions (Cutting, 1962). In general, products may be divided into hard cures, suitable for long-term storage and mild cures, which are slightly dried and contain only sufficient salt or smoke for flavouring (Cutting, 1962).
Although the effects of smoking are a combination of the whole process, they are discussed separately for the purposes of this work.

2.2.2.2. Effect of salting.

Most fish are salted before smoking. Formerly salting was an essential part of the preservation process in the smoking cure. However, the salt concentration in the product has to be between 8 & 10% before there is appreciable inhibition of moulds and bacteria (Shewan, 1954). Today the main purpose of adding salt is to impart flavour, since the amount present in modern smoked products does not have much effect on keeping quality (Storey, 1982).

Fish can be salted in three basic ways, as follows:

During salting some water is removed from the fish and the flesh becomes salty. The amount of water lost and the degree of saltiness required vary according to the product (Storey, 1982). According to Cutting (1962) there is little overall weight loss after dry-salting (pickling), and no appreciable change in weight in a 70-80% saturated brine solution (brining), while a gain in weight has been observed in weaker brines. Brining is also a much quicker method of imparting salt than pickling, according to the same author, probably because the area of contact with the flesh is so much greater in the case of a liquid and it has been widely
adopted for effecting the mild treatments used today.

The uptake of salt during brining is a complex process which depends upon many factors, including:

1. The time of immersion in the brine.
2. The size and shape of the prepared fish.
3. Presence or absence of skin.
4. The ratio of brine to fish.
5. Whether or not the fish has been filleted or split.
6. How well the brine is stirred.

Storey (1982).

Penetration occurs more quickly through a cut surface than it can through skin. House & Cutting (1956) observed that "block filleted" (without skin) haddock (i.e. one fillet per fish) required only 4 minutes in 80% brine to absorb the required 2-3% of salt, while larger haddocks split for fin-nans or "single filleted" (skin on one side) required 10-15 minutes depending upon the size of the fish. Cutting (1962) also reported that fatty herring require 20-30 minutes brining, which is at least twice as long as the time required for haddocks of similar size.

Deng (1977) studied the effect of freezing and frozen storage on salt penetration of mullet muscle. It was observed that a sharp increase of salt penetration occurred in muscle that had been stored frozen for one week and thawed, when compared to fresh mullet fillets. According to Deng
the sharp increase may be due to cell wall damage of mullet flesh after freezing, resulting in a higher rate of diffusion when thawed muscle was immersed in the brine.

Obviously, with all the interchange of fluid and equilibration of constituents between fish and brine, some loss of water soluble constituents, such as minerals and vitamins of the B group, would be expected in any salting process (Cutting, 1962). The same author also noted that salting may slightly decrease the total nitrogen content of fish, presumably by leaching out of proteinaceous material. This effect according to Munro & Morison (1965) is more pronounced in heavily salted fish than in those treated with a mild salt cure. The same authors have demonstrated that salting and smoking had no effect on the biological value of cod protein.

Petrichenko & Dolzhenko (1981) found that brining reduces the original amino acid content by 9.8% in silver carp and 14.5% in big head carp.

In addition, in the case of fatty fish, oil is lost and undergoes hydrolysis and oxidation. The autolysis that occurs during pickle salting results from the activity of proteolytic enzymes and micro-organisms, which is an essential feature of the ripening process (Cutting, 1962). According to the same author technological and accidental losses during salting depend upon the freshness of the raw material.
2.2.2.3. Effect of smoking.

Smoking as the main part of the cure, provides the desirable and attractive colour, aroma and flavour of smoked products and contributes to preservation by contributing effective antimicrobial and antioxidant agents. These versatile effects of smoking alone have been attributed to wood smoke constituents.

Numerous researchers have devoted a great deal of time and effort in attempting to identify, and in a few cases quantify, the compounds that are present in wood smoke (Maga, 1987). It should be noted, however, that just because a compound has been identified as being present in smoke does not necessarily mean that it makes any contribution to the overall properties or to certain specific sensory properties of the resulting product. Thus, the individual compounds that are responsible for the characteristic flavour, colour and aroma of wood smoke have not been positively identified. Additionally even the general nature of the compounds responsible for the characteristic flavour associated with different wood sources has not been resolved. In general, flavour chemists currently conclude that the overall characteristic flavour of wood smoke is probably due to the distribution of compounds from several compound classes, each of which contributes certain portions to the overall sensory properties of wood smoke (Maga, 1987).
Historically, numerous researchers including Knowles & Gilbert (1975) have concluded that phenols, as a compound class, are the primary contributors to wood smoke flavour. However, the exact meaning of "phenolic fraction" was in dispute for some time, as smoke actually contains other compound classes which also can have potent sensory properties.

2.2.2.3.1. Phenols.

Lustre & Issenberg (1970) demonstrated that not all phenolic compounds produced in wood smoke are responsible for the flavour of smoked foods. These include the compounds 2,6-dimethoxy-4-vinylphenol, 2,6-dimethoxy-4-cis (and trans-) propenylphenol, propiosyrigone, coniferaldehyde and sinapaldehyde. Lustre & Issenberg (1970) also concluded that because these phenolic structures contain carbonyl groups, they can react with meat proteins to form other compounds. However, Maga (1987) found sinapaldehyde [3-(4'-hydroxy-3'-5'-dimethoxyphenol)-2-propenal] in a variety of smoked foods in the untreated state.

Wasserman (1966) pointed out that in addition, to the type of phenols, the actual amount of compound present and the system in which they were present was important. He demonstrated that the most important flavour index phenol was 4-methylguaiacol and that certain phenols had lower flavour and odour thresholds in a water system, whereas others were more sensitive in an oil system. Issenberg & Lustre (1971) also demonstrated that for some phenols, the
percent recovery was lower in water than from a lipid system, whereas for some compounds e.g. guaiacol it was essentially the same.

Toth & Potthast (1984) reported that phenols of medium volatility are the most important. The flavour properties of phenol fractions according to the same authors were characterised as follows:

1. The low-boiling fraction (60-90 °C), composed primarily of phenol, cresols, guaiacol and ethyl-methyl guaiacol, had a hot and bitter taste.

2. The medium fraction that distilled over at (91-132 °C) and contained cis- and trans-isoeugenol, syringol and methylsyringol, had a pure and characteristic smoke flavour.

3. The high-boiling fraction (133-200 °C) had an acid, chemical property that was judged of poor quality.

However, apart from the responsibility of phenols for smoked flavour, it has been pointed out that phenolic fraction of wood smoke possesses the highest antimicrobial and antioxidant inhibiting ability. Within this fraction, phenols of lower-boiling points have a more effective antimicrobial activity (Olsen, 1976). The other fractions, e.g. terpenes, show no antibacterial effect. What is more, there are some indications that the terpene fraction is not only bacteriostatically inactive, but it acts an antagonist to the lower-
boiling point phenolic fraction, lowering its bacteriostatic effect (Olsen, 1976).

Investigations have shown that the phenolic fraction is also mainly responsible for the antioxidant effect, whereas other fractions, e.g. hydrocarbons, may even demonstrate opposite prooxidative activity (Picielna, 1977).

The higher-boiling point phenols have been found to have the most pronounced inhibiting effect on peroxide formation (Maga, 1987).

2.2.2.3.2. Furans.

Maga (1987) reported that furans also contribute to the overall sensory properties of wood smoke and they tend to soften the heavy smokey aromas associated with phenolic compounds. He also reported that a furan fraction, in which six furans were identified, processes a sweet, fragrant, floral aroma, which was also felt to soften smokey aromas.

2.2.2.3.3. Carbonyl compounds.

Carbonyl compounds are also thought to contribute to the overall sensory properties of wood smoke, as this class represent the largest number of compounds identified from wood smoke. Fiddler et al (1970) isolated a polyfunctional carbonyl subfraction from wood smoke that possessed a caramel aroma which the authors felt modified the flavour of
phenols. However, Kim et al (1974) reported that carbonyls contribute very little to overall aroma.

It has also been established that the surface colouring of smoked food involves complicated carbonyl-amino reactions and is directly connected with the loss of the carbonyl groups of smoke, since more carbonyls present in smoke results in a higher colour intensity of the product.

However, colour development on the surface of the fish may arise from the physical characteristics of the smoke condensate itself rather than chemical reactions.

Clifford et al (1980) demonstrated that protein-bound lysine, via its terminal amino group, is considered to be a major source of the amino group in carbonyl-amino reactions. Tang (1978) also observed an additional loss of arginine and histidine. Ziemba (1969) reacted free amino compounds of proteins with smoke extracts and obtained a brown colouring on the surface of meat. Other workers reported similar findings with regard to reactions of phenolic compounds with amino groups of protein.

In addition, Gilbert & Knowles (1975) attributed some of the colouration to the hydrolysis of the surface proteins by the acidic compounds of the smoke. There are some smoke components which are known to be highly reactive towards amino groups but do not or only slightly contribute to colour development in smoked products.
Since the reaction between carbonyls and amino groups (browning reaction) is essentially a condensation followed by a rearrangement and dehydration, it is very important to have conditions that allow the above phenomena to occur. One factor, pH has a very definite effect with the darker colours being produced at high pH values and thus acidification of smoke constituents decreases the brown colouration. Furthermore, after the absorption of smoke, it is necessary for the temperature to be high to form a darker colour and the surface to be dry to accelerate the dehydration aspect of the reaction (Hollenbeck, 1977). The same author treated meats with liquid smoke and found that conditions of low temperature and high humidity developed very little, if any, brown colour. Thus, when the meat was held under relatively low humidity with high temperatures, a very dark brown colour associated with heavily smoked products developed. Thus, it is possible to conclude that smokehouse conditions are very critical with respect to the formation of the brown colouration. These browning reactions also have an undesirable influence in that, they destroy essential amino acids. It has been observed that the destruction of essential amino acids, such as lysine, in smoked fish products becomes less by smoking and by flavouring with liquid smokes (Chen & Issenberg, 1972).

2.2.2.3.4. Polycyclic Aromatic Hydrocarbons.

Polycyclic aromatic hydrocarbons (PAH) are well-known carcinogens and public interest in food has prompted numerous
studies on their occurrence in smoked foods. Not all PAHs are carcinogenic but show either a co-carcinogenic or an antagonistic effect (Hamm, 1977).

The amounts of these compounds in smoked foods have been extensively surveyed, Tilgner & Daun (1969); Dunn & Fee (1979); Potthast (1978 & 1979); Sikorski (1989) but the recent reports have concentrated on the presence of 3,4-benzopyrene in smoked foods and "smoke flavours". Although, 3,4-benzopyrene is the most commonly specifically determined polycyclic hydrocarbon in foodstuffs, its presence can only be regarded as an arbitrary indicator of carcinogens. About 65 other different polyaromatics are believed to be present in curing smoke, 25 of which have been identified (Tilgner & Daun, 1969).

The main factor which determines the amount of polycyclic aromatic hydrocarbons, produced in smoked fish, is the temperature. It has been reported that below a wood destruction temperature of 425 °C smoke can be produced without 3,4-benzopyrene (Tilgner & Miler, 1963) and that a smouldering sawdust temperature of 350-400 °C leads to minimum production of PAHs but also leads to the development of an unpleasant smoke flavour (Toth & Potthast, 1984). A linear increase in the production of amounts of polycyclic aromatic hydrocarbons is said to occur over the 400-1000 °C temperature range (Toth & Potthast, 1984).
Commercial kiln hot-smoked fish had levels of 3,4-benzopyrene similar to those smoked over a log fire, whereas cold smoking gave much lower levels (Toth & Potthast, 1984).

The polyaromatics also exist in "liquid smokes" and "smoke flavours". Gilbert & Knowles (1975) examined two commercial liquid smoke samples, which were shown to be free of 3,4-benzopyrene but five other PAHs were present in trace amounts. Additionally, the same authors analysed seven water soluble smoke flavours obtained from three different manufacturers, they found traces of anthracene, phenanthrene, pyrene, fluoranthrene and triphenylene in three samples, but no detectable PAHs in the remaining four. By contrast, Toth & Potthast (1984) detected 3,4-benzopyrene in all of fifteen commercially available liquid smoke preparations, but mostly at very low levels, furthermore in five of the samples pyrene and fluoranthrene were found in large amounts.

According to Gilbert & Knowles (1975) the variation in figures like the above for different liquid smoke preparations reflects the different production methods and it should be possible to produce products with nil or acceptably low levels of PAHs.

2.2.2.4. Effect of drying.

Drying is a part of the smoking process and occurs in parallel with smoking.
The preservative effect of smoking on fishery products is credited to a combination of surface drying, salting and deposition of antioxidant (phenolic) and antimicrobial constituents on the fish (Gilbert & Knowles, 1975). It is widely known that reducing the water activity (Aw) results in a reduction of microbial activity, at low water activities (0.1 – 0.2) however, other effects such as rancidity become important (Troller & Christian, 1978). According to Burgess et al (1965) drying has only a slight influence on the storage life of the smoked products and it mainly affects the texture of the products.

During drying, water is removed in two stages. During the first stage, while the surface of the fish is wet, the rate of drying depends only upon the state of the surrounding air, in particular its speed, temperature and how much moisture it already carries. This stage is called the constant rate period, because when conditions remain constant then the drying rate remains constant.

The second stage of drying occurs when most of the moisture on the surface of the fish has evaporated, then water can evaporate only as quickly as it reaches the surface from within. As the amount of water in the fish becomes less, the water molecules take progressively longer to reach the surface from the deeper lying parts and drying becomes progressively slower. This second stage is known as the falling rate period (Storey, 1982).
Proctor & Lyhiry (1956) tested the effect of dehydration on the amino acid content of shad & haddock and could not identify any significant difference between raw and dehydrated fish.

Olley et al (1989) reported that lipid oxidation is highest at low water activities, around 0.15, probably because of the activation of metal catalysts. Woolfe (1975) studied the change in peroxide values during storage of smoke dried herring. It was found that oxidation of the lipids had been initiated by the drying process, and the site of initiation appeared to be the bound lipids in contact with protein, while protein free lipid appeared to be stable and did not oxidize at all. Labuza et al (1970) investigated the rate of browning of ethyl linoleate-casein mixtures at various water activities and found the highest reaction rate at medium water activities (0.6-0.7).

2.2.2.5. Effect of heating.

2.2.2.5.1. Protein quality.

The heating of protein causes denaturation which is a disruption of the secondary and higher structures (Leward, 1979). The temperature at which denaturation occurs (denaturation temperature) varies for the different fish proteins and for the same protein between different fish species, being related to the environmental temperatures in which fish live (Aitken & Connell, 1979) i.e. those present
in organisms subjected to lower environmental temperatures are less stable than those present in organisms subjected to higher temperatures. The development of protein inextrac-tability according to Aitken & Connell (1979) commenced at 30-35 °C, and was 40% and 90% complete at 40 °C and 60-65 °C, respectively. When cod and hilsa were heated at a con-stant temperature of 30 °C, without allowing drying to oc-cur, a considerable fall in the extractable myofibrillar proteins occurred over about 40 hours (Howgate & Ahmed, 1972). The fall, which was greater with cod, was attributed to the fact that these species inhabits a colder water en-vironment than hilsa. In the same period of heating (40 hours) Aitken & Connell (1979) stated that only about one- quarter of the sarcoplastic proteins of cod and hilsa became inextractable and that this group as a whole is more stable than the myofibrillar group.

When fish muscle is heated, the fraction of nitrogen which is not precipitated by trichloroacetic acid increases. This so-called extractable fraction consists of ammonia, amines, TMAO, amino acids, lower peptides and proteins not coagu-lated by the acid. This phenomenon has been studied in cod and hilsa (Howgate & Ahmed, 1972) and presented by Aitken & Connell (1979) in carp, caspian sprats and herring. Much of the increase in extractable nitrogen in herring, is caused by the degradation of collagen to gelatin, of a molecular weight not precipitated by the acid and smaller peptides, but in addition a sizeable increase in ammonia derived from unknown sources occurs.
2.2.2.5.2. Lipid changes.

Enzymic hydrolysis of lipids ceases when fish tissue is heated at 100 °C for periods up to 90 minutes (Olley & Lovern, 1960). Heating cod for 30 minutes, at the same temperature, however, hardly affected phospholipase activity, which means that, in normally cooked fish, some enzymic hydrolysis of lipids in the stored material could occur.

Heating undoubtedly results in oxidation of lipids in fish but few systematic studies of the reaction have been recorded (Aitken & Connell, 1979). In one study a rapid increase of peroxide value was observed by Howgate & Ahmed (1972) in hilsa kept for about 40 hours at 30 °C. Fish heated at 220-240 °C for 40 minutes resulted in a significant diminution of polyunsaturated fatty acids, presumably through oxidative reactions (Quaglia et al, 1974). Thomas et al (1987) used microwave oven cooking to investigate the stability of polyunsaturated fatty acids (PUFA) in fresh fish of low, medium and high fat content. They found that the effect of cooking was minimal and the cooked fish retained their original PUFA composition and content. Bhuiyan et al (1986) studied the changes due to oxidative rancidity and the composition of major lipid classes and fatty acids in hot-smoked mackerel and reported that there was an increase in TBA and peroxide values but the values were still indicative of acceptable quality. The percentages of triglycerides and phospholipids did not change significantly and free fatty acids could barely be
detected. The overall fatty acid composition remained virtually unchanged after the hot-smoking process.

2.2.2.5.3. Browning of lipids & proteins.

During heating of mixtures of polyunsaturated methyl esters of cod oil acids with albumin, hydroperoxides present in the lipid phase were rapidly decomposed under conditions of roasting and frying with formation of both liposoluble and insoluble brown pigments (Pokorny et al, 1973). Pokorny (1981) also found that lipid hydroperoxides easily react with protein forming both light-brown and dark-brown products. It has been found that the intensity of browning and the percentage of non-extractable lipids increased with increasing degree of autoxidation and is proportional to the original content of oxidation products of lipids. Phospholipids are particularly reactive as they contain both polyunsaturated fatty acids and amines. The browning activity was shown to be highest in phosphatidyl ethanolamine, medium in phosphatidyl choline and lowest in sphingolipids.

Pokorny (1981) stated that the hydroperoxides and aldehydes are not the direct reacting substances. Free radicals derived from peroxidizing lipids are the principal reactants which polymerize proteins and destroy amino acids. Cysteine, methionine, tyrosine, alanine and lysine were the amino acids damaged by free radicals to the greatest degree.
Aldehydes belong to the main reaction products of lipid oxidation and are always present in oxidized lipids. A linear correlation was found by Pokorny (1981) between the amount of aldehydes reacted with protein and the degree of browning. Fujimoto (1970) found that unsaturated aldehydes gave more intense browning than saturated while Pokorny (1981) noted that hydroxyketones are more reactive than ketones.

El-Zeany & El-Tarras (1976) mixed highly rancid polyunsaturated fish oil methyl esters with various proteins, amino acids and amines. The browning products of the amine-aldehyde system were more soluble in lipid solvents than those of the amino acid-aldehyde systems. The authors attributed lipid soluble pigments as being formed from α-amino groups and lipid insoluble pigments from non α-amino groups. Pokorny (1981) noted that the chief amino acid attacked by autoxidising lipid (when the amino acid is contained within the protein) is lysine through its ε-amino group.

Factors affecting the rate of the browning reaction, between oxidized lipids and proteins and their control are demonstrated in Table 4.
Table 4: Browning reaction between oxidized lipids and proteins.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Stimulation</th>
<th>Inhibition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Higher than</td>
<td>Cold or frozen</td>
<td>Pokorny et al (1973, 1975)</td>
</tr>
<tr>
<td></td>
<td>40 °C</td>
<td>storage</td>
<td></td>
</tr>
<tr>
<td>Value of pH</td>
<td>Higher than 7</td>
<td>Lower than 5</td>
<td>El-Zeany &amp; El-Tarras (1976)</td>
</tr>
<tr>
<td>Catalysts</td>
<td>Transition</td>
<td>Antioxidants</td>
<td>El-Zeany et al (1975)</td>
</tr>
<tr>
<td></td>
<td>Transition</td>
<td>vitamin E</td>
<td></td>
</tr>
<tr>
<td>Composition of lipids</td>
<td>Polyunsaturation</td>
<td>Saturation or Monounsaturat.</td>
<td>Pokorny et al (1975)</td>
</tr>
<tr>
<td>Composition of nonlipids</td>
<td>Addition of ammonia or amines</td>
<td>Blocking of amino groups</td>
<td>Pokorny et al (1974)</td>
</tr>
<tr>
<td>Content of oxidation products</td>
<td>Use of oxidized oils, addition of aldehydes</td>
<td>Use of fresh oil</td>
<td>El-Zeany &amp; El-Tarras (1976)</td>
</tr>
</tbody>
</table>
2.2.2.6. Total effect of process parameters.

Smoking of fish, as mentioned before, is a preservation procedure which combines the processes of salting, heating, drying and the addition of smoke itself.

It may be supposed that fish is not a particularly rich source of vitamins (apart from vitamin D in fatty fish) and must therefore be primarily considered as providing protein and lipids. Thus, changes in the content and composition of protein and lipid are the most important criterion by which the effect of a process must be judged.

It has long been recognised by food technologists that fish proteins are especially labile. Thus, fish actomyosin is particularly easily "denatured" and rendered insoluble in dilute salt solutions. This denaturation, however, constitutes a technological rather than a nutritional problem because it is not associated with any loss of nutritive value (Cutting, 1962).

Therefore, the overall effect of the smoking process on fish protein could result in amino acids being affected by any or all the steps of the process. When the ε-amino group of lysine is blocked by either aldehydes or by a Maillard reaction, the reacted lysine is not susceptible to enzymic attack, and thus becomes unavailable (Bhuiyan et al, 1986). In the smoking process the loss of lysine and other essential amino acids is essentially proportional to the time and tem-
perature of processing and may exceed 55% destruction in traditional hot-smoke tropical operations (Caurie et al, 1974). Other workers have recorded much smaller losses, in a range of 6 to 33% (Hoffman et al, 1977). Clifford et al (1980) has confirmed that lysine is the most sensitive of the essential amino acids (a 25% destruction of lysine is accompanied by a 7% fall in other nutritional critical amino acids) and that losses are much greater in the outermost 5-10 mm of the fish and decline rapidly towards the centre. Accordingly one would expect lysine loss to be approximately inversely proportional to the thickness of the smoked fillet.

During the smoking process fish are subject to heating, smoking and atmospheric oxygen. All of these factors can accelerate the oxidative deterioration of the lipid of fish. Salt is another factor to be considered as salt has been reported to act as a prooxidant (Nambudiry, 1980). Hobbs (1982) stated that the antioxidative effect of smoke in smoked fish can be outweighed by the prooxidant effect of salt used during brining. However, there is other evidence to suggest that salt may have a protective action against the oxidation of lipid (Nambudiry, 1980). Toth & Potthast (1984) have shown that the phenolic fraction, deposited on the fish surface during smoking, is mainly responsible for the antioxidative activity. Heating finally undoubtedly results in oxidation of lipids in fish (Aitken & Connell, 1979).
2.2.3. NUTRITIONAL ASPECTS.

The flesh of fish contains small amounts of nearly all the vitamins with the exception of ascorbic acid, of which only insignificant amounts are present, and of vitamin D and A which is present in substantial quantities in the flesh of fatty fish.

Cutting (1962) in reviewing the literature then available made some comments and also reached a number of other conclusions which are still particularly relevant. In general, figures for the vitamin contents of cured fish products were few, and as might be expected from seasonal and other causes, e.g. variability in composition of raw material, rather variable. Differences in processing technique would also introduce variations. This means that analyses of products, no matter how accurately conducted, could be unrepresentative and unreliable.

Some general conclusions have been presented by some authors since then.

According to Castell et al (1965) & Tarr (1969) salting exhibits prooxidant activity in fish flesh and may increase the losses of some vitamins. Cutting (1962) reported that the salting process itself may induce a physical loss of water soluble constituents, including the B vitamins and according to Daum (1975) it would be logical to assume that such losses taking place will be proportional to the losses
of water occurring during the first stages of curing.

Losses of nutrients from smoking may result from interaction with smoke components. Furthermore, changes of nutritional value could also be expected because of the dehydration which occurs during smoking (Burt, 1989).

The vitamin and mineral contents of fresh and processed lean and fatty fish are given in Table 5.

Thiamine is the most sensitive to degradation by heat and the most important factors governing its loss in heated foodstuffs are temperature, time of heating, pH, the presence of metal ions (copper in particular), the form of the vitamin and the presence or absence of protective agents such as proteins. This last point probably accounts for the fact that thiamine in natural products is more stable to heat than it is in either aqueous or buffer solution (Priestley, 1979).

There is some loss of thiamine on smoking and complete destruction has been reported during salting and drying of certain tropical products (Cutting, 1962).

However, no loss of thiamine had been observed during processing by Paul & Southgate (1979) as shown in Table 5.
Table 5: Vitamin and mineral content of fresh & smoked cod (G. morrhua) and fresh & fried mackerel (S. scombrus) flesh.

<table>
<thead>
<tr>
<th>Vitamin/Mineral</th>
<th>Unit</th>
<th>Cod fresh/100g</th>
<th>Cod smoked/100g</th>
<th>Mackerel fresh</th>
<th>Mackerel fried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>ug</td>
<td>Tr</td>
<td>Tr</td>
<td>45</td>
<td>52</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>ug</td>
<td>Tr</td>
<td>Tr</td>
<td>17.5</td>
<td>21.1</td>
</tr>
<tr>
<td>Thiamin</td>
<td>mg</td>
<td>0.08</td>
<td>0.08</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>mg</td>
<td>0.07</td>
<td>0.07</td>
<td>0.35</td>
<td>0.38</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>mg</td>
<td>1.7</td>
<td>1.4</td>
<td>8.0</td>
<td>8.7</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>mg</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>mg</td>
<td>0.44</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>mg</td>
<td>0.33</td>
<td>0.32</td>
<td>0.70</td>
<td>0.84</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>ug</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Folic acid free</td>
<td>ug</td>
<td>8</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Folic total</td>
<td>ug</td>
<td>12</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>mg</td>
<td>0.2</td>
<td>0.2</td>
<td>1.0</td>
<td>0.96</td>
</tr>
<tr>
<td>Biotin</td>
<td>ug</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Sodium</td>
<td>mg</td>
<td>77</td>
<td>1170</td>
<td>130</td>
<td>150</td>
</tr>
<tr>
<td>Potassium</td>
<td>mg</td>
<td>320</td>
<td>390</td>
<td>360</td>
<td>420</td>
</tr>
<tr>
<td>Calcium</td>
<td>mg</td>
<td>16</td>
<td>14</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mg</td>
<td>23</td>
<td>25</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mg</td>
<td>170</td>
<td>190</td>
<td>240</td>
<td>280</td>
</tr>
<tr>
<td>Iron</td>
<td>mg</td>
<td>0.3</td>
<td>0.4</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Copper</td>
<td>mg</td>
<td>0.06</td>
<td>0.17</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>Zinc</td>
<td>mg</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Sulphur</td>
<td>mg</td>
<td>200</td>
<td>210</td>
<td>180</td>
<td>210</td>
</tr>
</tbody>
</table>

Tr = Trace amount
Source: Paul & Southgate (1979)
Smith (1988)
2.2.4. TOXICOLOGICAL ASPECTS.

A type of food poisoning can result from the ingestion of foods containing substantial amounts of histamine. This type of food poisoning is often called scombroid fish poisoning because of its frequent association with scombroid-type fish such as tuna and mackerel (Arnold & Brown, 1978; Omura et al, 1978; Taylor et al, 1978).

Histamine is formed in food from histidine by bacteria that possess the enzyme histidine decarboxylase, as shown in Fig. 4.

Fig. 4: The metabolism of histamine.

![Histidine Decarboxylase Reaction](image)


Many bacteria reportedly possess at least limited histidine decarboxylase activity (Taylor et al, 1978), but only Proteus morganii (Kawabata et al, 1956; Sakabe, 1973), Kleb-
siela pneumoniae (Taylor et al., 1979) have been implicated as causative organisms in the formation of toxicologically significant levels of histamine.

In the fishing industry, bacterial histamine production is controlled primarily by the use of low storage temperatures. The histamine-producing bacteria apparently reside in the gills and/or intestines of the fish (Taylor et al. 1977; Lerke et al., 1978) and any prolonged storage at elevated temperatures results in bacterial action on tissue histidine. Behling & Taylor (1982) studied the histamine formation by incubating samples of tuna at different temperatures. They found that the fastest production of histamine was at 37 °C.

The main fish products which contain high levels of histamine are smoked, canned, salted and dried fish (Table 6).

Limits on the allowable levels of histamine in fish do not exist in most countries. A hazard action level of 50mg / 100g, an amount thought to cause a health hazard, has been enacted by the USA for tuna and a defect action level (DAL) of 10mg / 100g has been established. Sweden has imposed a level of 20mg / 100g as the maximum amount of histamine permitted in fish offered for sale. Finally there is a recommended level of 5mg / 100g for mackerel, since with good manufacturing practice the histamine level does not go above that (Al-Weng, 1988).
Table 6: Histamine contents reported for various fish products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Histamine mg / 100g</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chunk light tuna</td>
<td>1.2 - 10.0a</td>
<td>Taylor et al (1978)</td>
</tr>
<tr>
<td>Albacore tuna</td>
<td>0.7 - 4.0b</td>
<td>Taylor et al (1978)</td>
</tr>
<tr>
<td>Mackerel</td>
<td>1.0 - 10.0c</td>
<td>Taylor et al (1978)</td>
</tr>
<tr>
<td>Dried</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sardine</td>
<td>12.4</td>
<td>Igarashi (1949)</td>
</tr>
<tr>
<td>Herring</td>
<td>30.1</td>
<td>Igarashi (1949)</td>
</tr>
<tr>
<td>Salted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herring</td>
<td>9.8</td>
<td>Igarashi (1949)</td>
</tr>
<tr>
<td>Salted / Dried</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mackerel pike</td>
<td>29.8</td>
<td>Igarashi (1949)</td>
</tr>
<tr>
<td>Scomberomorus sp.</td>
<td>15.0 - 65.0</td>
<td>Hanson et al (1985)</td>
</tr>
<tr>
<td>Rastrelliger sp.</td>
<td>50.0</td>
<td>Hanson et al (1985)</td>
</tr>
<tr>
<td>Euthynnus sp.</td>
<td>265 - 560</td>
<td>Hanson et al (1985)</td>
</tr>
<tr>
<td>Sardinell sp.</td>
<td>70 - 220</td>
<td>Hanson et al (1985)</td>
</tr>
<tr>
<td>Smoked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herring</td>
<td>34.5</td>
<td>Igarashi (1949)</td>
</tr>
<tr>
<td>Mackerel</td>
<td>148.0</td>
<td>Smith (1980)</td>
</tr>
<tr>
<td>Tuna</td>
<td>10.0</td>
<td>Hanson et al (1985)</td>
</tr>
</tbody>
</table>

\[a = 92\% \text{ of the samples had less than 5mg / 100g.}\]
\[b = 99\% \text{ of the samples had less than 4mg / 100g.}\]
\[c = 95\% \text{ of the samples had less than 5mg / 100g.}\]
2.3. ASSESSMENT OF QUALITY CHANGES DURING FROZEN STORAGE AND DURING SMOKING.

Numerous methods have been used for measuring the quality changes during frozen storage and smoking of fish, including chemical, physical, biological, instrumental, microbiological and sensory.

2.3.1. Protein changes.

The most common test used to study the denaturation that occurs in fish protein during frozen storage is related to the loss in solubility of proteins, including total proteins, salt-soluble proteins, or particular groups of proteins (i.e. myofibrillar proteins) or particular types, such as myosin and actin.

Such protein changes are better indicators when they are related to a lean fish, such as cod, rather than to a fatty one. Thus, the extractability of the proteins of frozen stored cod muscle in cold neutral 5% sodium chloride solution has been studied by many workers (Connell, 1964; Dyer & Dingle, 1961; Love, 1962a & b; Dyer, 1951).

Cowie & Mackie (1968) studying the extractability of cod protein, found that there is considerable variation in the details of the experimental methods. They showed how the value for protein extractable in 5% sodium chloride solution was influenced by the type of homogeniser and duration of
storage of homogenate. They concluded that the rate of change of protein extractability measured by any one extraction procedure will be meaningful as a guide of storage history, but when results are compared, it is essential to ensure that identical extraction procedures have been followed.

Various methods have been used by investigators to determine the free amino acid content in fish or other products. The copper salt method appeared to offer many advantages and after further modification by Pope & Stevens (1939) it was found to be possible to obtain correct results with tryptic digests of proteins.

For available lysine measurement, it is necessary to devise a method to measure the amount of unaltered lysine present in the protein. This is commonly done with a derivatizing agent that will react with the free ε-amino group of lysine in the protein and remain attached during a subsequent hydrolysis step. 1-Fluoro-2,4-dinitrobenzene (FDNB) has been extensively used for this purpose.

Carpenter & Ellinger (1955) were the first to use FDNB for the measurement of available lysine. The method, with some modifications, is still in wide use today. The method involves reaction of a protein with FDNB followed by acid hydrolysis, ε-DNP lysine is then measured spectrophotometrically at 435nm. This method has some limitations. Hydroxylysine and ornithine are measured as available lysine
causing an overestimation. The presence of carbohydrates in a sample has been also shown to affect the measurement of available lysine.

A chromatographic method for available lysine using the FDNB derivative has numerous advantages over the spectrophotometric measurement of the Carpenter (1960) method, as ε-DNP hydroxylysine and δ-DNP ornithine can be separated from ε-DNP lysine. Interfering compounds formed in the presence of carbohydrates during hydrolysis can also be separated from the compound of interest.

Chromatographic techniques used for available lysine include paper chromatography, ion-exchange column chromatography, liquid-liquid partition chromatography and thin layer chromatography. All these chromatographic techniques, however, are quite lengthy. Using HPLC, a rapid method has been developed for available lysine based on the FDNB procedure but without the problems associated with the Carpenter (1960) method (Peterson & Warthesen, 1979).

2.3.2. Lipid changes.

The investigation of lipid changes can involve simply measuring changes in the content of total lipid or phospholipid, and any decrease could be due to the occurrence of lipid hydrolysis or oxidation.
The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides. Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. However, this theory is limited due to the transitory nature of the peroxides, as shown in Fig. 3, Section 2.2.1.2., the peroxides are intermediate products in the formation of carbonyl and hydroxy compounds. Notwithstanding the transitory nature of the peroxides many analytical procedures have been described for peroxide value measurement.

The iodometric methods are widely used and these are based on the measurement of the iodine produced from potassium iodide by the peroxides present in the oil. Gray (1978) stated that the two principal sources of error in these methods are a) the absorption of iodine at unsaturated bonds of the fatty material and b) the liberation of iodine from potassium iodide by oxygen present in the solution to be titrated. The latter is often referred to as the oxygen error and leads to high results in the peroxide determination.

An alternative in the iodometric methods is a colorimetric assay of liberated iodine which was converted into a blue starch-iodine complex. This method has advantages for samples too small for convenient titration. Another colorimetric method, based on the oxidation of ferrous to ferric iron and the determination of the latter as ferric thiocyanate, has been suggested. This method has been found suitable for the estimation of fat peroxides in milk powder.
Infrared spectrometry is of particular value in the recognition of unusual functional groups. As the compounds form during the oxidation of fats, it is possible using infrared spectrometry to follow the course of oxidation e.g. the appearance of bands at about 2.93μm indicates formation of hydroperoxides (Gray, 1978).

The refractive index method has been studied by Gray (1978). In the induction period, when peroxide formation is low, the refractive index remained constant. During the secondary stage of relatively more peroxide formation, the refractive index sharply increased until the peroxide value reached to a maximum. This increase in refractive index was attributed by Gray (1978) to conjugation, known to precede hydroperoxide formation.

Polarographic methods have been also developed for quantitation of peroxides of fats. In the early stages of oxidation of fats, a linear relationship exists between wave height and peroxide value (Gray, 1978).

The AOACS (1973) official iodometric method for peroxide value determination is the most widely used and applied to all normal fats and oils. This method fails to adequately measure low peroxide values because of difficulties with the determination of the titration end point.
Lipid hydrolysis of both triglycerides and phospholipids is a common post mortem feature in fish and fish products. The major products are free fatty acids and glycerol. It is obvious, therefore, that the determination of free fatty acid content is a very good indicator for assessing lipid hydrolysis. The method described in (Section 4.4.5.) has been commonly used (Hardy, 1980).

Chromatographic methods have been used to determine the fatty acid profile changes. Gas liquid chromatography by capillary column has been widely employed mainly because of its sensitivity, however, the packed column also gives quite repeatable results.

2.3.3. Nutrient changes.

Of the vitamins, thiamine is the most sensitive to degradation by heat and its measurement has been studied as a good indicator of nutrient changes in marine products.

The measurement of thiamine has been based on different fluorometric methods which mainly depend upon the type of the product. The method used in this investigation (Section, 4.4.6.), is not applicable for products which have materials that absorb thiamine or which contain extraneous materials which affect thiochrome fluorescence (AOACS, 1984).
2.3.4. Texture changes.

The structure as well as some specific rheological properties of the muscle tissue of fish are governed by the myofibrillar proteins and the constituents of the connective tissue. However, after cooking the textural properties of muscle depend primarily upon the state of the myofibrillar proteins.

Instrumental measurements are quite difficult for fish due to its unique morphological and compositional features. All methods used are applicable to raw material, but very difficult to apply for cooked fish (Dunajski, 1979).

The instruments which have been used to study the texture of fish are: penetrometers which register the force required to penetrate the material or the depth of penetration (Main et al, 1972), cutting devices determining the energy input in cutting the fibres (Buttkus, 1963), shearing instruments which record the force needed to shear the test material (Dassow et al, 1962) and extrusion cells. All of them are only rarely used in experiments involving the texture of fish flesh, particularly cooked, whilst for other products they have been widely used.

After heating the forces acting between the myomeres and between the fibres become so weak that the muscles disintegrate easily and the muscle fibres remain as the sole elements of resistance in cooked fish flesh (Dunajski, 1979).
This disintegration which occurs to the cooked flesh of fish, under pressing, makes the use of penetometers for assessing the texture of the product more reasonable.

A combination between instrumental texture measurements and sensory evaluation by trained panellists could probably give a good estimation of the cooked fish texture.

2.3.5. Sensory assessment.

The sensory evaluation is the primary method in any system of quality assessment (Connell & Shewan, 1980), and the methods may be divided into two types: subjective and objective.

In the former, biases in judges are not minimized and personal opinion is allowed free reign. These methods are typified by the consumer trial in which a group of the ordinary public numbering, at least 20 to 50 are asked their views about or preferences for some sensory aspects of the product. Hatae et al. (1988) for example asked 387 housewife consumers, which were familiar with fish and good in judging textural properties.

In the latter, biases are deliberately minimized by the use of specially trained judges or assessors, who concentrate on a particular well-defined attribute of the product and who operate as a panel of one person but usually more (Connell & Shewan, 1980).
Subjective sensory tests of eating quality are undoubtedly those which ultimately count because they come closest to assessing those consumer responses which matter. However, because of the difficulty and expense normally involved in arranging fully representative consumer tests, this is rarely possible and instead, comparisons with the results of objective sensory tests are usually employed. In general, the results of subjective and objective sensory assessments correlate well, especially where the differences between samples tend to be large (Connell & Shewan, 1980).

A number of methods for the objective sensory analysis of foods have been described (Amerine et al, 1965; Drake & Johansson, 1974) but four have been used fairly frequently for fish:

1. Paired comparison.
2. Ranking.
3. Triangle.
4. Scoring of attributes.

The first method has been useful for a number of applications e.g. increases in the toughness of fish stored in the frozen state were determined by paired comparison with unfrozen controls (Love, 1966).

The determination of the threshold for detection of oil taints in fish and shellfish has been achieved using ranking (Connell & Shewan, 1980).
The ability of consumers to differentiate and recognise different species of fish or parts of fish by odour or taste has been measured using triangle and comparison tests on a number of occasions at Torry Research Station (Connell & Shewan, 1980).

Scoring is, however, by far the most commonly used method. The most common parameters have been used in scoring systems for fish products are texture, flavour, colour, salt and the overall appearance (Borderias et al., 1983; Connell & Howgate, 1968).
3. AIMS OF PRESENT WORK.

Most research on smoked fish has been concerned with the constituents of the wood smoke which are of great importance in such products. Little research has been carried out into the assessment of quality of smoked products, particularly hot-smoked fish.

Additionally, there is very little information concerning the quality and acceptability of hot-smoked products with prior frozen storage.

The aims of this study were therefore:

1. To investigate the effect of prior frozen storage on the quality of hot-smoked lean and fatty fish.

2. To study the relationship between taste panel (sensory) methods and instrumental methods, of texture assessment.

3. To relate the quality changes observed with changes at the molecular level of the component lipids and proteins.

4. To assess the effect of storage at -20 °C for up to 33 weeks on the quality of lean and fatty fish.

5. To assess the influence of packaging on the quality of lean and fatty fish during frozen storage.
6. To assess the level of histamine in smoked mackerel.

7. To establish the acceptability of previously frozen hot-smoked lean and fatty fish with particular reference to Greece.
4. EXPERIMENTAL.

4.1. MATERIALS & EQUIPMENT.

4.1.1. Fish.

Cod (Gadus Morrhua) Batch Ac were obtained from Grimsby market, on 18th of June 1990 and stored in ice before being further processed. The cod samples (1000 ± 100g in weight) were frozen within 24 hours of arrival at the laboratory. Further cod samples were obtained after 11 weeks and were also frozen within 24 hours and some further after 22 weeks.

Mackerel (Scomber Scombrus) Batch Am were obtained from Grimsby market, on 5th of July 1990 and also stored in ice before being further processed. The mackerel samples (200 ± 30g in weight) were also frozen within 24 hours. Further mackerel samples were also obtained as above.

4.1.2. Chemicals.

Standard PUFA 1 & PUFA 2 mixes were obtained from Supelco, inc. c/o Supeichsen U.K. (R.B. Radley & Co. Ltd) Sawbridgeworth, Herts.

Acid phosphatase from wheat germ was obtained from Sigma Chemical Company Ltd, Poole, Dorset.
All other reagents and solvents were analytical reagent (AR) grade when possible, otherwise general purpose reagent (GPR) grade.

4.1.3. Equipment.

A jackstone laboratory horizontal plate freezer was used to freeze the whole fish samples, down to a minimum central temperature of -20 °C, as monitored by a Comark microprocessor digital thermometer.

The frozen fish were stored in a large capacity cold store at -20 °C ±1.

An AFOS-Torry kiln was used for smoking the fish samples.

An MSE Europa 24M refrigerated centrifuge was used.

The homogenation of fish samples was performed using an Ystral 7801 top drive homogenizer.

The nitrogen determinations were carried out using a Tecator Kjeltec Auto 1030 Analyser.

Texture measurements were achieved by a Steven’s LFRA Texture Analyser.
Quantification of histamine was achieved by a Shimadzu Dual-Wavelength TLC Densitometer CS-930 which was connected to a Shimadzu data recorder DR-2.

Samples were applied to a TLC plate using the Camag Linomat III.

Fluorescence determinations were carried out with a Shimadzu Spectrophotometer RF-540.

Chromatographic separations of FAMEs was achieved using a Shimadzu GC-RIA instrument with a FI detector (for all packed column work) connected to a Shimadzu RPR-81 processor.

High performance liquid chromatography (HPLC) was performed using a Gilson system, consisting of a model 303 pump connected with a Gilson model 802 C manometric module, with a UV detector (PYE Unicam PU 4020) and a Shimadzu RPR-81 processor.

4.2. PROCESSES.

4.2.1. Freezing and Frozen storage.

48 whole cod samples were cleaned with cold water and placed in the horizontal plate freezer which was operated at -30.8 °C to -36.8 °C. The duration of freezing was about 3.5 hours, this being the time for the central temperature of
the whole cod to freeze down to \(-30\, ^\circ\text{C}\).

After being frozen the cod samples were equally divided into 4 batches and placed into the \(-20\, ^\circ\text{C}\) cold store as follows:

Batch Ac1: 12 whole cod samples equally placed (4 in each bag) in 3 plastic (polythene) bags and analysed after 11, 22 and 33 weeks.

Batch Ac2: 12 whole cod samples equally placed (4 in each bag) in 3 plastic (polythene) bags removed after 11, 22 and 33 weeks smoked and then analysed.

Batch Ac3: 12 whole cod samples equally placed (4 in each box) in 3 cardboard boxes and analysed after 11, 22 and 33 weeks.

Batch Ac4: 12 whole cod samples equally placed (4 in each box) in 3 cardboard boxes removed after 11, 22 and 33 weeks smoked and then analysed.

The same procedure was also followed with 120 whole mackerel samples.

The freezing times of cod and mackerel samples are demonstrated in Fig. 5.
Fig 5. Freezing time of cod and mackerel.

Temperature

Time (mins)

--- COD  --- MACKEREL
4.2.2. Smoking.

8 whole cod samples (4 from plastic bags and 4 from cardboard boxes) were removed from the freezer and immersed in water at room temperature for about 6 hours to allow thawing.

The thawed fish and an equal number of fresh cod were filleted, brined (in 20% salt solution) for 1.5 hours and allowed to dry at room temperature overnight. The fillets (24 in number) were subsequently placed in the Torry kiln and were smoked for 7 hours.

The exact smoking procedure was 1 hour at 30 °C, 2 hours at 40 °C and 4 hours at 70 °C. After smoking, the cod fillets were left in the kiln overnight and the next morning they were individually packed in plastic bags and stored at 4 °C.

The same smoking process was followed for the mackerel samples but instead of being filleted the fish were split before the brining process.
4.3. ANALYTICAL PROCEDURES.

4.3.1. Protein content.

This was determined using the Kjeldahl method. About 1g of fish was accurately weighed on an ash free filter paper, wrapped with this paper and placed in a Kjeldahl digestion tube. 20 ml of 98 % sulphuric acid and two Kjeltabs (3.5g K₂SO₄ and 0.4g CuSO₄ . 5 H₂O) were added and the tube was heated at 400°C on a digestion heating block until the contents became green and clear (about 2 hours).

After cooling, 75 ml of water was added and the tube was connected to the distillation head of the Autoanalyser. The titration cycle was started by closing the safety door. When the alkali pump dispensed alkali into the sample tube the ammonia produced was swept by steam into a receiver solution of boric acid and mixed indicator (methyl red and bromocresol green) in the titration vessel. Depending upon the colour of the indicator, standard hydrochloric acid 0.100 M was dispensed into the receiver solution in the titration vessel from the burette continuously during the distillation until the acid-base equivalent point was reached (colour change from green to red). The volume of standard acid required was the read from the digital display.

The percentage of crude protein was then calculated from:
\[ 0.14 \times (S-B) \]

\[
\% \text{ total nitrogen} = \frac{\text{-------------}}{W}
\]

Where: 
- \( S \) = ml of 0.100 M for sample.
- \( B \) = ml of 0.100 M for blank.
- \( W \) = weight of the sample (g).

Total nitrogen content was converted to crude protein by multiplying by the factor 6.25 (Commission of European Communities, 1979).

The Kjeltec Auto 1030 Analyser was calibrated using ammonium sulphate.

4.3.2. Lipid content.

The total lipid content was determined by the Bligh and Dyer (1959) method as modified by Hanson and Olley (1963).

The sample (about 20g for cod and 10g for mackerel) was accurately weighed into a 150 ml homogenising flask. About 8-10 ml of water (based on the known water content to give a total volume of 16 ml), 20 ml of chloroform (containing 0.01% BHT) and 40 ml of methanol were added. The mixture was homogenised for 2 minutes whilst being cooled in ice. A further 20 ml of chloroform was added and then the mixture was again homogenised for 30 seconds, when 20 ml of water was added and the mixture homogenised for a further 30 seconds.
The homogenate was transferred to centrifuge tubes and centrifuged for 20 minutes at 2500 rpm (after being balanced within 0.1g). 20 ml of the lower chloroform solution containing the lipid was pipetted into a "Quickfit" pear shaped flask of known weight.

The solvent was evaporated using a rotary film evaporator and with a rotary oil pump at 38 °C. The flask was wrapped in aluminium foil during evaporation to exclude light. The weight of the residue multiplied by 2 gave the weight of total lipid in the sample.

The lipid content was therefore calculated as follows:

\[
\text{weight of the extracted lipid} \times 2 \times \frac{\% \text{ of lipid}}{\text{weight of the sample}} \times 100
\]

4.3.3. Moisture content.

Moisture was determined by the EEC recommended method ISOR. 1442 (EEC, 1979).

About 5g of fish sample was added to a moisture dish, containing about 20g sand and a glass rob and accurately weighed. To this, about 5 ml of ethanol was added and the mixture stirred with the glass rob. The dish was then placed over a water bath at 80 °C and the ethanol allowed to evaporate (approximately 20 minutes). The sample was then
dried (to constant weight) in an air oven at 103 ± 1 °C for at least 24 hours. Samples were cooled in a desiccator and accurately reweighed.

The percentage moisture was calculated as follows:

\[
\text{initial weight} - \text{final weight} \\
\% \text{ moisture} = \frac{\text{initial weight} - \text{final weight}}{\text{weight of sample}} \times 100
\]

4.3.4. Salt content.

About 1.5g of fish was accurately weighed into a conical flask. The fish sample was warmed to about 80 °C in a water bath and 25 ml of standard 0.100 M silver nitrate, 10 ml of water and 10 ml of concentrated nitric acid were added.

The fish sample was digested by boiling for about 20 minutes. After being cooled 25 ml of water, 1 ml of ferric alum and 1 ml of nitrobenzene were added. The mixture was titrated with standard 0.100 M potassium thiocyanate until a permanent orange-red colour was seen.

A blank titration was also performed.

Exactly 25 ml of 0.100 M silver nitrate, about 25 ml of water, 10 ml concentrated nitric acid, 1 ml ferric alum and 1 ml of nitrobenzene were added into a conical flask. The mixture was titrated with 0.100 M potassium thiocyanate un-
til a permanent orange-red colour was appeared.

\[(T_1 - T_2) \times 0.100 \times 58.5 \times 100\]

\[% \text{ of sodium chloride} = \frac{\text{---------------------------}}{100 \times W}\]

Where:
\[T_1 = \text{ml of 0.100 M potassium thiocyanate for blank.}\]
\[T_2 = \text{ml of 0.100 M potassium thiocyanate for sample.}\]
\[W = \text{weight of fish sample (g).}\]
4.4. ASSESSMENT OF CHANGES DURING FROZEN STORAGE AND DUE TO SMOKING.

4.4.1. Salt-soluble protein.

The method used was that of Cowie and Little (1966). In the procedure adopted, soluble nitrogen, non-protein nitrogen and total nitrogen were determined.


To an ice-cold homogenation flask 1g of sample was accurately weighed, 15 ml of 5% sodium chloride, adjusted to pH 7.0-7.5, was added and the mixture was thoroughly homogenised for 2 minutes. The homogenate was transferred into a 100 ml volumetric flask and made up to the mark with 5% sodium chloride. The solution was then cooled at 4 °C for 1 hour and portions of this solution were centrifuged for 30 minutes at 6000 rpm whilst at 0 °C.

20 ml of the supernatant was pipetted into a Kjeldahl digestion tube and two Kjeltabs (3.5g K₂SO₄ and 0.4g CuSO₄ . 5 H₂O) and 20 ml of 98% sulphuric acid were also added. After digestion, the nitrogen content was determined as described in Section 4.3.1.
b). Non-protein nitrogen.

About 4g of fish was accurately weighed and homogenised in 50 ml of 15% trichloacetic acid (TCA) solution for 30 seconds. The extract was filtered using a Whatman No. 1 filter paper, 15 ml of the filtrate was digested and the nitrogen content determined as described in Section 4.3.1.

c). Total nitrogen.

This was determined as outlined in Section 4.3.1.

The percentage of salt-soluble protein is represented as below:

\[
\text{% Soluble} = \frac{\text{Salt soluble nitrogen - non-protein nitrogen}}{\text{Protein total nitrogen - non-protein nitrogen}} \times 100
\]

4.4.2. Free amino acids.

The copper method of Pope and Stevens (1939) was used to determine amino nitrogen.

Fish sample (10 to 15g) was accurately weighed and homogenised for 2 minutes in 120 ml of distilled water. 40 ml of 5% aqueous trichloroacetic acid was added and the mixture homogenised for a further 30 seconds. The protein
precipitate and other solid matter was removed by filtration using a Buchner funnel (with filter aid) and suction. About 50 ml of the filtrate was poured into a beaker and neutralised with 1 M sodium hydroxide using thymolphthalein as an indicator.

The neutralised filtrate was transferred to a separating funnel and extracted twice with 10 ml of light petroleum (60-80 °C) (to remove interfering peroxides).

To analyse the extracts, 10 ml of neutralised extract was transferred to a 50 ml volumetric flask and 30 ml of a copper phosphate suspension added (a mixture of 1 volume of 0.16 M copper II chloride solution; 2 volumes of 0.2 M trisodium phosphate decahydrate; and 2 volumes of borate buffer solution). The solution was diluted to volume with distilled water and the contents filtered through Whatman No. 5 filter paper. 10 ml of the filtrate was acidified with 0.5 ml glacial acetic acid, approximately 1g of potassium iodide added and the released iodine immediately titrated with 0.005 M sodium thiosulphate. 1% starch solution was added as an indicator towards the end of the titration.

1.00 ml of 0.005 M sodium thiosulphate = 0.14 mg free amino acid nitrogen.

\[ 0.14 \times \frac{X \text{ ml} (0.005\text{M Na}_2\text{S}_2\text{O}_3)}{50 \times 160 \times 100} \]
\[ \% \text{ FAA} = \frac{\text{Weight of the sample}}{\text{Weight of the sample}} \]
4.4.3. Peroxide value.

The same procedure was followed for extraction of lipid as described in Section 4.3.2.

The method used for peroxide value was adapted from that developed by Lea (1952). About (0.1 to 0.5g) of lipid was accurately weighed into a stoppered conical flask. 20 ml of chloroform was then added to dissolve the lipid. 50 ml of acetic : chloroform (60 : 40) was added followed by 1 ml of saturated potassium iodide.

The flask was rotated for 30 seconds and then placed in the dark. After 30 minutes, 100 ml of distilled water was added and the liberated iodine was titrated with 0.002 M sodium thiosulphate solution, adding 1% starch solution as an indicator towards the end of the titration.

A blank run was also performed omitting the lipid, and the peroxide value was calculated as milliequivalents (meq) peroxide oxygen per Kg of lipid using the following formula:
C X 1000 X (S - B)

\[ \text{Peroxide value} = \frac{\text{meq} \%}{\text{Kg lipid}} \]

Where

\( S = \) sample titre in ml.

\( B = \) blank titre in ml.

\( W = \) weight of sample in g.

\( C = \) molarity of thiosulphate solution.

4.4.4. Free fatty acid (FFA).

The same procedure was followed in this test for extraction of lipid as described in Section 4.3.2.

About (0.1 to 0.5g) of fish lipid was accurately weighed into a 100 ml conical flask, and then a mixture, of diethyl ether 25 ml and ethanol 25 ml, neutralised with 0.100 M sodium hydroxide, was added. After slight shaking to allow the lipid to dissolve, the lipid was titrated with 0.100 M sodium hydroxide using a microburette and phenolphthalein as indicator.

A blank determination was also carried out.

The percentage of free fatty acid as equivalent oleic acid was calculated as follows:
\[(S - B) \times 5.61\]
\[
\% FFA = \frac{W \times 2}{W}
\]

Where  
- \( S \) = sample titre in ml.  
- \( B \) = blank titre in ml.  
- \( W \) = weight of sample in g.

4.4.5. Fatty acid profile.

The lipid sample was obtained as described in Section 4.3.2.

The methylation was performed according to a simple and quick method of Humberside Polytechnic (1989).

Lipid sample, about 25-50g was accurately weighed into a screw cap tube and 1.5 ml of 0.5 M methanolic sodium hydroxide was added. The tube was tightly capped, mixed and heated at 100 °C (using a heating block) for 7-15 minutes. After being cooled, 2 ml of 14% boron trifluoride in methanol was added. The tube was again tightly capped, mixed and heated at 100 °C for 5 minutes.

The mixture was cooled to 30-40 °C and 1 ml of iso-octane was added. The tube was capped and shaken, using a "whirl mix" for 30 seconds. 5 ml of saturated sodium chloride was immediately added and the mixture was again shaken for a further 30 seconds.
The iso-octane was allowed to separate and then the iso-octane layer removed by Pasteur pipette and transferred to a small sample tube. A second extraction was achieved with an additional 1 ml of iso-octane. Two iso-octane extractions were combined and concentrated to approximately 1 ml with a stream of dry nitrogen. The concentrated extract was then ready for gas chromatography analysis.

The gas chromatography analysis was performed on a highly polar (SP 2330) 2 meter packed column, with nitrogen as a carrier gas at a flow rate of 22 ml/min. The initial temperature was 150 °C for 4 minutes rising to 230 °C at a rate of 5 °C/minute. The injection temperature was 230 °C and the peak area was recorded automatically as a percentage of total peak area by using a flame ionisation detector with a data processor (Section 4.1.3.).

4.4.6. Thiamine.

The thiamine was determined by the AOAC official methods of analysis (1984).

About 2.5g of fish sample was accurately weighed and 0.1 M hydrochloric acid equal in ml to more than 10 times the sample in weight (g) was added. The mixture was comminuted and when lumping occurred, agitated vigorously so that all particles came in contact with liquid. The mixture was digested for 30 seconds to 1 hour at 95-100 °C in a steam bath with frequent mixing and after cooling was diluted to
about 65 ml with 0.1 M hydrochloric acid and the pH adjusted to 4.0-4.5 with 5 ml 2 M sodium acetate buffer.

5 ml enzyme (10% acid phosphatase) was added and incubated for about 16 hours at 37 °C. After being cooled and adjusted to pH 3.5, using 1 M hydrochloric acid, the mixture was diluted to 100 ml with distilled water and filtered through Whatman No. 541 filter paper. This was designated as Assay Sample Solution.

0.01 ug/ml Assay Standard Thiamine Solution was also prepared.
1 ml from (1 ug/ml) standard thiamine solution was mixed with ca 50 ml 0.1 M hydrochloric acid, digested, cooled and diluted to 100 ml with 0.1 M hydrochloric acid. 5 ml from each Assay Solution was added into two 40 ml tubes (A & B) containing ca 1.5g sodium chloride.

To one of these tubes (A), while gently swirling, 3 ml of oxidizing reagent (4 ml of 1% potassium ferricyanide solution diluted to 100 ml with 15% sodium hydroxide) was immediately delivered (in a dark place because light destroys thiochrome) and the mixture was gently swirled again to ensure adequate mixing. Immediately afterwards 13 ml isobutanol was added and the tube was vigorously shaken for 15 seconds. The other tube (B) was similarly treated except that the oxidizing reagent was replaced with 15% sodium hydroxide solution (blank). After isobutanol had been added to all tubes, they were centrifuged at 2000 rpm for 15
minutes and 10 ml of the supernatant isobutanol was obtained from each.

An amount of the isobutanol extracted was decanted into a cell for thiochrome fluorescence measurement (Excitation wavelength 370, Emission wavelength 426).

The thiamine content of the oxidized Assay Sample Solution was determined by comparing the intensity of fluorescence of this solution with that from oxidized Assay Standard Solution.

Intensity of fluorescence is proportional to amount of thiamine present and measured with spectrofluorophotometer (Section 4.1.3.)

The thiamine content was calculated as follows:

\[
\text{ug thiamine.HCl in 5 ml Assay Solution} = \frac{(I - B)}{(S - D)}
\]

Where:  
\(I\) = Fluorescence of isobutanol extracted from oxidized Assay Sample Solution.  
\(B\) = Fluorescence of isobutanol extracted from Assay Sample Solution which had been treated with 3 ml 15% NaOH.  
\(S\) = Fluorescence of isobutanol extracted from oxidized Assay Standard Solution.
D = Fluorescence of isobutanol extracted from Assay Standard Solution which had been treated with 3 ml 15% NaOH

4.4.7. Available lysine.

The available lysine was determined by the Peterson & Warthesen (1979) method.

About 0.5g of fish sample was accurately weighed into a 250 ml boiling flask and boiling chips, 12.5 ml of 0.95 M sodium carbonate and 15 ml FDNB (1-fluoro-2,4-dinitrobenzene) solution (0.4 ml FDNB in 15 ml ethanol) were added. The mixture was shaken for 2 hours at room temperature. After shaking the boiling flask was transferred to a water bath to evaporate the ethanol. 30 ml of 8.1 M hydrochloric acid was added and the mixture refluxed for 16 hours. After refluxing and while hot, the mixture was quantitatively filtered through Whatman No. 1 filter paper into a 250 ml volumetric flask and the volume was adjusted to 250 ml with water. A small amount from the mixture was again filtered through a 0.2 um filter and the new filtrate was ready to be injected on HPLC.
The HPLC was operated using:
Microbondapac C18 column.
Mobile phase of 0.01 M acetate buffer pH 4.0 and acetonitrile (80:20 v/v).
2.0 ml/min flow rate of the mobile phase.
Detection at 436 nm.
Injection 10 ul.
Results were calculated by reference to the standards and the equation of the calibration curve (as shown in Fig. 6).

Fig. 6: Lysine calibration curve.

\[ Y = 160.1054X + 9.8395 \]
\[ r = 0.9984 \quad r = 42.2381 \quad P = 6E - 2\% \]
4.4.8. Histamine.

Histamine was determined by a thin layer chromatography method (TLC). This method is based on that developed at the Torry Research Station, Aberdeen (1984).

10g of fish sample was homogenised with 70 ml of methanol for 1 minute. The homogenate was then decanted into a 100 ml conical flask, covered with a watch glass, and placed in a water bath at 60 °C for 15 minutes. After cooling the volume was made up to 100 ml with methanol, mixed thoroughly and filtered using Whatman No. 1 filter paper. The filtrate was used to spot directly to the TLC plate.

From the stock standard solution, 1mg histamine/ml (165.6mg of histamine hydrochloride to 100 ml with 0.1 M hydrochloric acid) 0.5, 1.0, 1.5 and 2.0 ml were pipetted into four 100 ml volumetric flasks and made up to volume with 95% methanol. These standard solutions were equivalent to 5, 10, 15 and 20mg of histamine per 100g of fish flesh.

An appropriate amount of solvent (chloroform / methanol / ammonia) ratio 70/30/5 to cover the bottom of the chromatographic tank to a depth of 15 mm was added to the tank.

TLC plates were activated at 105 °C for 1 hour and allowed to cool in a desiccator before spotting with standards and sample. After cooling they were spotted (20 ul) using the
Camag Linomat III spotter and placed into the tank and left to run until the solvent front reached to within 2 mm of the top edge (about 2 hours). The plates then were removed and dried thoroughly in an oven at 100 °C for 10 minutes. After cooling the plates were sprayed with 0.5% ninhydrin in butanol. The spots were developed by placing the plates in a dark cupboard overnight and quantified using a scanning densitometer.

Results were calculated by reference to the standards and the equation of the calibration curve (as shown in Fig. 7).

Fig. 7: Calibration curve for Histamine determination.

\[ Y = 39.680X - 1.414 \]

- $r = 0.9980$  
- $t = 27.5157$  
- $P% \leq 1E - 2\%$
4.4.9. Texture (Steven's LFRA Texture Analyser).

The texture of the raw fish sample was measured using the Steven's LFRA Texture Analyser with a sharp conic probe (2.9 cm diameter, 4 cm height). Small cylinder-shaped containers (2 cm diameter, 1.1 cm height), which contained an exact equal volume of fish, obtained from under the dorsal fin of the fish sample, were used each time.

The smoked samples were measured using unskinned whole fillets.

The operation parameters for the texture analyser were: penetration distance 0.5 mm and speed 2.0 mm/min for both raw and smoked samples.

The texture of the fish samples were represented as the penetration force, which was indicated on the analyser as grams. The value of the penetration force indicates the degree of toughness of the fish flesh.

4.4.10. Taste panel assessment.

An objective sensory evaluation was performed using 10 expert panellists and a scoring method. Care was taken to use the same panellists each time.

The smoked samples were presented to the panellists in small, similar portions. The characteristics were assessed
as described in Table 7. The taste panellists were trained to concentrate on the attributes of smoked cod and mackerel and follow the correct evaluating procedures, i.e. assessing the surface colour of the sample and then flavour, texture and saltiness.
Table 7. TASTE PANEL SHEET

You are presented with FIVE (5) smoked fish samples, I would like you to classify them using the scales below, writing the code of each sample in the box of the number of your preference.

For instance: ANF if more than one sample has the same score please put the other codes above the box.

1. **COLOUR**
   - Yellow
   - Strong Yellow
   - Brown
   - Strong Brown

2. **TEXTURE**
   (a) **FIRMNESS**
   - Extreme Soft
   - Moderate Firmness
   - Extreme Firm

   (b) **JUICINESS**
   - Extreme Dry
   - Moderate Juiciness
   - Extreme Dry

3. **FLAVOUR**
   (a) **SMOKED FLAVOUR**
   - No smoked fish flavour
   - Weak smoked fish flavour
   - Moderate smoked fish flavour
   - Strong smoked fish flavour

   (b) **OTHER OFF-FLAVOUR**
   - None
   - Slight
   - Moderate
   - Strong

   (c) **SALTINESS**
   - Not Salty
   - Slight Salty
   - Moderate Salty
   - Strong Salty

4. **ACCEPTABILITY**
   - Extremely Unacceptable
   - Moderately Unacceptable
   - Moderately Acceptable
   - Extremely Acceptable
5. RESULTS AND DISCUSSION.

5.1. CHEMICAL COMPOSITION OF FRESH COD & MACKEREL.

5.1.1. Proximate analysis.

Since fish composition varies not only among species but also within the same species, proximate analysis was therefore carried out. Fish composition within the same species is mainly dependent upon sex, age, size, state of maturity, feeding conditions, locations of catch, water temperature etc., which has been studied extensively (Spinelli & Dassow, 1982).

As summarised in Table 8, the protein content of both cod and mackerel fell in a relative narrow range, particularly for mackerel and the mean mackerel value was slightly higher than the cod one. These values agree with previous work done on cod and mackerel (Murray & Burt, 1969). According to these authors the proximate composition of these types of fish are:

<table>
<thead>
<tr>
<th>Species</th>
<th>Scientific name</th>
<th>Moisture %</th>
<th>Protein %</th>
<th>Fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod</td>
<td>Gadus morrhua</td>
<td>78 - 83</td>
<td>15 - 19</td>
<td>0.1 - 0.9</td>
</tr>
<tr>
<td>Mackerel</td>
<td>Scomber scombrus</td>
<td>60 - 74</td>
<td>16 - 20</td>
<td>1.0 - 23</td>
</tr>
</tbody>
</table>
The lipid content of cod showed a narrow range as expected for a lean fish. On the contrary, the amount of lipid in mackerel was, as expected, for this period (July), which usually is about 10% (Ackman, 1980) and the wide range observed indicated the varying distribution of lipids in the body of fatty fish (e.g. under the skin 40%).

The lean fish, according to Jangaard et al (1967), generally have fatty livers (e.g. cod) which show seasonal effects, but in other species the muscle can show fluctuating levels of neutral fat (i.e. mostly triglyceride) e.g. sprats (Hardy & Mackie, 1969), menhaden (Dubrow et al, 1976), or various mackerel species (Ackman, 1980).

Ackman (1980) stated that in lean fish the distribution of the fat in muscle is best shown histologically instead of extracting the fat of the muscle. The same author also showed that the belly flap is a notoriously fatty section of many fish bodies (e.g. 29% of lipid in belly flap of mackerel, 18.3% lipid in dark muscle and 7.6% in light muscle).

The moisture content which was much higher in cod, showed a relative narrow range, particularly in mackerel despite the wider range of percentage lipid in this fish.

The salt content showed a very narrow range for both fish samples, being slightly higher in cod.
Table 8: Proximate analysis of fresh cod & mackerel.

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>COD</th>
<th>MACKEREL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range 17.53 - 18.76%</td>
<td>18.26 - 18.64%</td>
</tr>
<tr>
<td>PROTEIN %</td>
<td>Mean (SD) 18.12 (0.42)</td>
<td>18.41 (0.17)</td>
</tr>
<tr>
<td></td>
<td>Range 0.81 - 0.98%</td>
<td>8.42 - 12.40</td>
</tr>
<tr>
<td>LIPID %</td>
<td>Mean (SD) 0.88 (0.07)</td>
<td>11.09 (1.56)</td>
</tr>
<tr>
<td></td>
<td>Range 78.45 - 79.19%</td>
<td>69.82 - 69.87%</td>
</tr>
<tr>
<td>MOISTURE %</td>
<td>Mean (SD) 78.82 (0.30)</td>
<td>69.84 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Range 0.24 - 0.30</td>
<td>0.18 - 0.23</td>
</tr>
<tr>
<td>SALT %</td>
<td>Mean (SD) 0.27 (0.02)</td>
<td>0.20 (0.02)</td>
</tr>
</tbody>
</table>

Data are means of triplicate determinations, except (*) which are quadruplicate.

Standard deviations are shown in parentheses.
5.1.2. Lipid FAME composition.

The fatty acid profiles of cod and mackerel were determined as described in Section 4.3.5. and they were identified by comparing retention times with those of standard fatty acids.

Since the lipid content in different parts of fish varies, care should be taken to obtain representative samples. In the present study, the lipid was extracted as outlined in Section 4.3.2. and the fatty acid composition of fresh cod and mackerel as found in this study is shown in Table 9.

To carry out a comparison with previous work, the fatty acid compositions from Table 3 & Table 9 are combined in Table 10.

This comparison shows a general agreement in fatty acid composition between the samples and the literature data. This comparison also indicates that there is actually a tremendous variation in fatty acid pattern between different species (Section 2.2.1.2.). According to Stansby (1982) the fatty acid pattern should not be thought of as changing from species to species or from season to season or from one geographical location to another, but rather the patterns conform to a large extent to the type and amount of feed available and its fatty acid content. The same author stated that the fatty acid content of fish of a given species is not completely independent of the species. Most species have
Table 9: Fatty acid profiles of fresh cod & mackerel.

<table>
<thead>
<tr>
<th>Fatty acids %</th>
<th>Cod</th>
<th>Mackerel</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.6 (0.05)</td>
<td>3.6 (0.01)</td>
</tr>
<tr>
<td>16:0</td>
<td>17.9 (0.20)</td>
<td>18.9 (0.08)</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>3.4 (0.06)</td>
<td>4.4 (0.03)</td>
</tr>
<tr>
<td>18:0</td>
<td>3.9 (0.06)</td>
<td>5.8 (0.03)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:1n-7</td>
<td>11.4 (0.06)</td>
<td>22.7 (0.51)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0.9 (0.02)</td>
<td>1.2 (0.21)</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>0.5 (0.05)</td>
<td>0.8 (0.22)</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>2.7 (0.09)</td>
<td>9.2 (0.02)</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>3.7 (0.09)</td>
<td>6.9 (0.03)</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>16.2 (0.22)</td>
<td>8.7 (0.06)</td>
</tr>
<tr>
<td>22:1n-11</td>
<td>1.6 (0.28)</td>
<td>2.4 (0.45)</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.8 (0.14)</td>
<td>1.6 (0.01)</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>34.4 (0.17)</td>
<td>13.9 (0.23)</td>
</tr>
</tbody>
</table>

Saturated       | 23.4 (0.45)| 28.3 (0.21)|
Monounsaturated | 19.1 (0.57)| 38.5 (0.88)|
Polyunsaturated | 57.5 (0.28)| 33.1 (0.17)|

The values are means of triplicate determinations.
Standard deviations are shown in parantheses.
Table 10: Comparison of fatty acid profiles of cod & mackerel.

<table>
<thead>
<tr>
<th>Fatty acids %</th>
<th>Cs</th>
<th>C1</th>
<th>C2</th>
<th>Ms</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.6</td>
<td>1.4</td>
<td>0.5</td>
<td>3.6</td>
<td>8.6</td>
<td>2.8</td>
</tr>
<tr>
<td>16:0</td>
<td>17.9</td>
<td>19.6</td>
<td>17.5</td>
<td>18.9</td>
<td>17.6</td>
<td>17.5</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>3.4</td>
<td>3.5</td>
<td>2.1</td>
<td>4.4</td>
<td>9.6</td>
<td>6.0</td>
</tr>
<tr>
<td>18:0</td>
<td>3.9</td>
<td>3.8</td>
<td>4.8</td>
<td>5.8</td>
<td>2.2</td>
<td>5.8</td>
</tr>
<tr>
<td>18:1n-9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:1n-7</td>
<td>11.4</td>
<td>13.8</td>
<td>9.5</td>
<td>22.7</td>
<td>14.3</td>
<td>7.8</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0.9</td>
<td>0.7</td>
<td>1.2</td>
<td>1.2</td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.0</td>
<td>0.8</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>2.7</td>
<td>3.0</td>
<td>2.0</td>
<td>9.2</td>
<td>8.4</td>
<td>4.1</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>3.7</td>
<td>2.5</td>
<td>1.5</td>
<td>6.9</td>
<td>0.8</td>
<td>6.9</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>16.2</td>
<td>17.0</td>
<td>16.3</td>
<td>8.7</td>
<td>9.4</td>
<td>11.2</td>
</tr>
<tr>
<td>22:1n-11</td>
<td>1.6</td>
<td>1.0</td>
<td>2.1</td>
<td>2.4</td>
<td>9.4</td>
<td>0.7</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.8</td>
<td>1.3</td>
<td>2.0</td>
<td>1.6</td>
<td>1.2</td>
<td>4.1</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>34.4</td>
<td>29.8</td>
<td>36.1</td>
<td>13.9</td>
<td>8.7</td>
<td>22.8</td>
</tr>
</tbody>
</table>

Saturated 23.4 24.8 22.8 28.3 29.6 26.1
Monounsaturated 19.1 21.3 15.7 38.5 43.1 18.6
Polyunsaturated 57.5 51.8 57.1 33.1 25.3 49.3

Cs = Cod samples.                        Ms = Mackerel samples.
metabolic pathways that tend to produce fat of a pattern at least somewhat typical of that species. These metabolic processes cannot, however, prevail against the constantly changing feed availability pattern. The resulting fatty acids make-up of the oil in a fish at a given point is modified from that most typical of the species to the actual pattern, which is highly dependent upon the fatty acid composition of the feed ingested in recent periods of time by that fish.

It is also believed that fish with low lipid contents and which are small in size contain a higher percentage of polyunsaturated fatty acids and lower percentage of monounsaturated in their lipid than fatty fish. This is because in lean fish the lipid is present mostly in cell membranes as phospholipids and that the concentration of polyunsaturated fatty acids in membranes is relatively higher than that in the depot fat (Wang, 1990).

5.1.3. Available lysine & thiamine.


The measurement of available lysine in fish and fish products, particularly smoked, is very important because the loss of lysine is related to a decrease in protein quality, as lysine is one of the essential amino acids and the most susceptible to the smoking process.
The available lysine and thiamine content in fresh cod and mackerel is shown in Table 11.

According to Munro & Morrison (1965) the available lysine in fresh cod flesh, with 78.1% protein (on dried salt free basis), was 12.3g / 16g N (on a dried salt free basis) whilst the lysine in the cod samples of Table 11, with protein content 85% (on dried salt free basis), was 12.9g / 16g N.

The values for the mackerel samples were also similar to those obtained by Bhyiyan et al (1985), who found that in fresh mackerel, with 10.3% protein, the available lysine was 8.4% in protein, whilst in the mackerel samples of Table 11, with 18.4% protein, the available lysine was 11.35% in protein.

Table 11: Available lysine & thiamine, on wet basis, in the fresh cod & mackerel samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Available lysine %</th>
<th>Available lysine in protein %</th>
<th>Thiamine ug / 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod</td>
<td>2.37 (0.16)</td>
<td>13.0 (0.82)</td>
<td>9.0 (0.02)</td>
</tr>
<tr>
<td>Mackerel</td>
<td>2.09 (0.03)</td>
<td>11.3 (0.20)</td>
<td>32.0 (0.08)</td>
</tr>
</tbody>
</table>

The values are means of triplicate determinations, with standard deviations in parentheses.
b. Thiamine.

The amount of thiamine in both samples (cod & mackerel) was very low.

According to Murray & Burt (1969) and Paul & Southgate (1979) the thiamine content in cod flesh is 0.7–0.8 ug / g and in mackerel is 0.9–1.0 ug / g (Section 2.2.3.).

However, Gordon et al (1979) also found the level of thiamine in cod to be very low at 0.22 ug / g, quite close to the value for the fresh cod samples found in the present work.

As fish is a good source of protein the loss of available lysine has a greater nutritional importance than thiamine or any other vitamin, particularly for smoked products. However, because of its sensitivity to heat the thiamine level is a good indicator of general nutritional change during thermal treatment and this is of greater relevance to the later hot smoking.

5.1.4. Histamine in mackerel.

Food poisoning outbreaks associated with the consumption of scombroid fish products containing high levels (above 50mg / 100g) of histamine have been reported with irregular frequency for a number of years (Kimata, 1965; Smith, 1980; Hanson et al, 1985).
Scombroid fish belong to the Scomberesocidae and Scombridae families and include such important commercial varieties as tuna, abiacore, bonito and mackerel. Scombroid fish characteristically contain high tissue levels of free histidine. Histamine is formed in the tissues of these fish during storage by bacterial decarboxylation of the histidine. Such bacterial species according to Taylor et al (1978) are Proteus morganii, Hafnia alvei, Enterobacter aerogenes and Klebsiella pneumoniae (Section 2.2.4.).

In the fresh mackerel samples histamine was not detected (Section 5.2.5.) using the TLC method as described in Section 4.4.8. Salguere & Mackie (1979) reported the histamine levels of mackerel stored at 0°C and on the first day of storage the histamine level was 0.007mg / 100g. That indicates that histamine in fresh fish requires a more accurate method to be detected than that used in this study.
5.2. THE EFFECT OF FROZEN STORAGE ON COD & MACKEREL.

The cod and mackerel samples were frozen and then stored in plastic bags or cardboard boxes (Section 4.2.1.), until being analysed every 11 weeks.

Protein denaturation, changes in free amino acid content, lipid oxidation and hydrolysis and losses of available lysine and vitamins, as a consequence of long frozen storage periods, are all involved in the loss of fish quality and nutritive value. The protein changes (in relation to texture) are usually the main problem in lean fish whilst lipid oxidation is often the major problem in fatty fish and can lead to development of rancid flavour.

5.2.1. Protein & amino acids.

From reference to Table 12, it can be observed that no overall significant differences occurred in the true protein content during frozen storage of either cod or mackerel.

The moisture content also showed no significant variation and this was probably due to the effective process (freezing) and protection of packaging during frozen storage. In spite of the type of packaging having a strong effect on dehydration of frozen stored products, both bags and boxes in this work gave very similar results.
Table 12: True protein of cod & mackerel, on wet basis, during frozen storage.

<table>
<thead>
<tr>
<th></th>
<th>True Protein %</th>
<th>Moisture %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bags</td>
<td>Boxes</td>
</tr>
<tr>
<td><strong>COD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>17.7 (0.82)</td>
<td>17.7 (0.82)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>17.0 (0.84)</td>
<td>16.8 (0.10)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>14.2* (0.10)</td>
<td>13.8* (0.46)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>16.4 (0.28)</td>
<td>17.0 (0.84)</td>
</tr>
<tr>
<td><strong>MACKEREL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>15.7 (0.16)</td>
<td>15.7 (0.16)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>16.6 (2.00)</td>
<td>16.7 (1.80)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>16.9 (0.69)</td>
<td>16.5 (0.16)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>17.4 (0.35)</td>
<td>17.7 (0.03)</td>
</tr>
</tbody>
</table>

Where F = Frozen  W = weeks

The values are means of triplicate determinations.

Standard deviations are shown in parentheses.

* Significant difference at 95% level.
However, in the 22 weeks frozen stored cod samples a significant decrease, at the 95% level (using t-test), in true protein content occurred, compared to the values at other times. This was either due to fish to fish variation of protein in the cod samples (lower protein content) or due to an unknown experimental error and this difference was also reflected by the higher value of non-protein nitrogen (NPN), which was 0.59% compared to 0.33% for the others. This difference in NPN was probably an effect of different qualities of the raw material (cod samples).

When fresh fish muscle is macerated in 5% sodium chloride solution at chill temperatures, about 90% of the protein dissolves. If the muscle is frozen and stored at sub-zero temperatures and then thawed and macerated, the amount which dissolves in the salt solution is diminished to an extent dependent upon the freezing process, the storage temperature, duration of storage and the type of fish.

According to Ironside & Love (1958) frozen fish gradually deteriorate in quality, becoming tougher to eat. An accurate objective means of assessing this deterioration would have obvious advantages in research on methods of freezing and cold storage, and the diminution of salt soluble protein is an obvious choice of indicator.

The protein solubility of cod and mackerel samples during frozen storage is demonstrated in Fig. 8.
Fig. 8: % SSP of Cod & Mackerel during frozen storage.

The values are means of triplicate determinations, with coefficients of variation: 0.4 & 0.9% for cod obtained from bags and boxes respectively; 3.8 & 2.9% for mackerel obtained from bags and boxes respectively.
There was a more rapid diminution of protein solubility in cod samples compared to mackerel.

Thus, 72.1% salt soluble protein was found in the fresh cod samples, a value which indicates that the cod samples were chilled for some time before purchasing, which became after 11 weeks frozen storage 49.6% to 45% for those being packaged in plastic bags and cardboard boxes, respectively. This was a decrease in protein solubility of 31% and 37.6% respectively. This diminution was continued and after a further 11 weeks (22 weeks total) the values were 29.8% and 23.5% for those being packaged in plastic bags and cardboard boxes, respectively. This was a further decrease of 39.9% and 47.8%. The decrease in solubility of protein ceased after 22 weeks frozen storage of the cod samples.

A slight increase which was observed after 33 weeks frozen storage was probably due to the different parts of the cod samples used.

According to Ironside & Love (1958) the cod muscle has different proportions of soluble and insoluble material in different parts of the fish, so that if, for instance, some of the caudal end, which contained the largest proportion of insoluble material, were inadvertently trimmed off, the figure for the whole fish or fillet would be slightly altered.
Thus the overall estimation for cod protein solubility was as follows:

Fresh cod 72.1%

Frozen stored 33 weeks in plastic bags 29.8 to 32% (30.9%)

Frozen stored 33 weeks in cardboard boxes 23.5 to 29.6% (26.6%). The diminution in solubility after 33 weeks frozen storage was 57 & 63% for the cod samples packaged in plastic bags and cardboard boxes, respectively.

The difference obtained from the different packaging was not significant (at 95% level, using t-test) and was probably due to the different parts from the cod samples used.

Similar results were found by other investigators. Ironside & Love (1958) who studied the denaturation of cod protein during frozen storage, found that over a period of about 20 weeks at -14 °C the soluble protein declined steadily from about 85 to almost 28%. They also noticed that from 20 to 38 weeks there was no obvious decline in soluble protein, which remained for the most part between 25 & 32%. Cowie & Mackie (1968) studied the protein solubility in cod fillets stored at -7, -14 and -29 °C. The purpose of their investigation was to compare the influence of different types of homogenation used in the protein solubility test. Their results, obtained using the same type of homogenation that was used in this work, were also similar. The soluble protein in cod fillets at -7 °C declined from 80 to 25% after 20 weeks cold storage and no further diminution was observed. At -14 °C the protein solubility reduced from about 80 to 40% after
20 weeks and continued to decline after this period. Finally those cod fillets being stored at -29 °C showed a diminution in soluble protein from 75 to 50% after 82 months of storage.

Castell et al (1973) studied the effect of formaldehyde on salt soluble proteins and they found that addition of formaldehyde to fresh cod muscle, to give a concentration of 10 to 200 ppm (i.e. a similar level of formaldehyde to that produced during frozen storage of gadoid fillets), brought about marked decreases in the soluble protein content during holding periods of 24 hours or less at 0 °C. They concluded that in these protein changes more than one mechanism is involved. They also concluded that the more rapid and more extensive denaturation, of most gadoid fillets in frozen storage than of fillets of non-gadoid species, appears to be directly related to the presence of a muscle enzyme of the former group, that is capable of producing formaldehyde from trimethylamine oxide, which is absent in the muscle of the non-gadoid species so far tested.

In line with this, the protein solubility changes in the mackerel samples was found to be entirely different.

The 84.04% soluble protein which was found in the fresh mackerel samples became 74 & 79% after 11 weeks frozen storage of mackerel samples packaged in plastic bags and cardboard boxes, respectively. After 22 weeks frozen storage the soluble protein was found to be 71.1 & 73.3% and after
33 weeks frozen storage the percentage soluble protein became 63.4 & 65%, respectively.

The slight difference observed for the different type of packaging was not significant (at 95% level, using t-test) and was probably due to the part of the fish obtained.

Thus, the decrease in protein solubility in mackerel samples was:
Fresh mackerel 84.04%
Frozen stored 33 weeks in plastic bags 63.4%, extent of diminution 24.5%
Frozen stored 33 weeks in cardboard boxes 65%, extent of diminution 22.7%.

Jiang & Lee (1985) found quite different results in protein solubility in mackerel samples. They found that after 12 weeks frozen storage of mackerel at -20 °C. the soluble protein was 66.5%, while in this investigation it was 74 to 79%.

Another factor which affects the protein denaturation during frozen storage is the quantity and composition of free amino acids (Section 2.2.1.1.).

The free amino acid content was as shown in Table 13.

A constant significant (at 95% level, using t-test) increase of free amino acids was observed in mackerel, while in cod
Table 13: Changes in free amino acid content, on wet basis, in
cod & mackerel during frozen storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Free amino acids mg / g.</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bags</td>
<td>Boxes</td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>2.19 (0.35)</td>
<td>2.19 (0.35)</td>
<td></td>
</tr>
<tr>
<td>Frozen 11 weeks</td>
<td>2.67 (0.17)</td>
<td>2.56 (0.08)</td>
<td></td>
</tr>
<tr>
<td>Frozen 22 weeks</td>
<td>2.77* (0.10)</td>
<td>3.48* (0.60)</td>
<td></td>
</tr>
<tr>
<td>Frozen 33 weeks</td>
<td>4.08* (0.38)</td>
<td>5.88* (0.46)</td>
<td></td>
</tr>
<tr>
<td>MACKEREL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>6.95* (0.27)</td>
<td>6.95* (0.27)</td>
<td></td>
</tr>
<tr>
<td>Frozen 11 weeks</td>
<td>8.20* (0.38)</td>
<td>7.80* (0.22)</td>
<td></td>
</tr>
<tr>
<td>Frozen 22 weeks</td>
<td>10.80* (0.05)</td>
<td>10.50* (0.01)</td>
<td></td>
</tr>
<tr>
<td>Frozen 33 weeks</td>
<td>10.70 (0.42)</td>
<td>11.20 (0.69)</td>
<td></td>
</tr>
</tbody>
</table>

The values are means of triplicate determinations.
Standard deviations are shown in parentheses.
* Significant difference at 95% level.
the increase appeared to be significant only after 33 weeks frozen storage. Comparing these results with those presented by Jiang & Lee (1985), they were found to be quite similar. They found, for a fish without much dark muscle, such as carp, 2.02mg/g in fresh material and after 12 weeks frozen storage it was 5.48mg/g, whilst in this work the results of cod were 2.19mg/g in fresh condition and after 33 weeks frozen storage it was 4.08 to 5.88mg/g.

According to Ikeda (1982) there is a specific difference in the free amino acid patterns of muscle between dark- and white- fleshed fish, e.g. sometimes more than 1,000mg/100g of histidine occurs in the former group.

Thus, the results for mackerel were much higher and generally agreed with those reported by Jiang & Lee (1985). They found 5.31mg/g free amino acids in fresh mackerel which became 10.12mg/g after 12 weeks frozen storage. In this investigation the free amino acid content was 6.95mg/g in fresh mackerel and became 10.7-11.2mg/g after 33 weeks frozen storage, which is quite a slower increase.

Correlating the free amino acid content in fish, particularly mackerel, with the protein solubility the results agreed with those presented by Jiang & Lee (1985). According to them the higher the free amino acid content in the fish the less stable the muscle protein during frozen storage. Thus, they found 10.12mg/g free amino acids and the protein solubility was 66.5% after 12 weeks
of frozen storage. In this investigation when the free amino acid content was 10.7 to 11.2 mg/g, a slightly higher value than that reported by Jiang & Lee (1985), after 33 weeks frozen storage, the protein solubility was 63.4 to 65%, slightly lower than that reported by the authors. This indicates that for mackerel the free amino acid content is likely to be a factor which influences the protein denaturation during frozen storage. In Fig. 9, the correlation between soluble protein and free amino acids in mackerel samples is presented.
A good negative correlation ($r = -0.9272$) is observed which is significantly different from zero, and indicates that the variables are related. Thus, this supports the proposal that the higher the concentration of free amino acids the higher the denaturation of protein in mackerel.
5.2.2. Lipid.

The total lipid content, as demonstrated in Table 14, was virtually constant during this period (33 weeks) of frozen storage of both fish. This reflects the good freezing process and frozen storage conditions, and also the positive effect of packaging.

Table 14: Lipid content in cod & mackerel, on wet basis, during frozen storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Lipid %</th>
<th>Moisture %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bags</td>
<td>Boxes</td>
</tr>
<tr>
<td>COD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>0.88 (0.16)</td>
<td>0.88 (0.16)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>1.09 (0.20)</td>
<td>1.10 (0.20)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>0.75 (0.03)</td>
<td>0.79 (0.02)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>0.92 (0.07)</td>
<td>0.90 (0.10)</td>
</tr>
<tr>
<td>MACKEREL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>11.09 (1.56)</td>
<td>11.09 (1.56)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>12.80 (0.32)</td>
<td>12.80 (0.63)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>10.60 (0.75)</td>
<td>10.90 (0.94)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>13.10 (2.75)</td>
<td>10.80 (1.64)</td>
</tr>
</tbody>
</table>

The values are means of triplicate determinations. Standard deviations are shown in parentheses.
Lipid in fish may undergo deterioration by both oxidative and hydrolytic processes. Of these two processes, oxidation is the most important in the deterioration of frozen fish products, causing flavour and colour change (Hardy, 1980).

In this work oxidation was not detected in the cod samples. This agrees with the results of many investigations and the reasons why lean fish oxidize very slowly are not clear. According to Hardy (1980) in such fish the lipid is bound to protein often within the cell membrane matrix and it may be supposed that radical reactivity will be severely restricted so that lipid peroxy radical attack on nearby lipid molecules will not occur. Studies carried out by the same author on the frozen storage of cod and haddock showed that only phospholipids oxidized, neutral lipids and free fatty acids did not participate in any radical reaction.

McGill et al (1977) stated that one certain effect of the oxidation in lean fish like cod is the introduction of odours, often called cold-storage flavours. The compounds most responsible for this are unsaturated carbonyls and in particular hept-cis-4-enal, a by product of the oxidation of the n-3 polyenoic acids that are present.

Sikorski et al (1976) also stated that oxidation in such lipids possibly results in protein denaturation, as found using a model system containing phospholipids and proteins.
The oxidation in mackerel, as shown in Fig. 10 by the peroxide value determination, was as expected, for a fish which possesses lipid reserves in the flesh and is more susceptible to oxidation.

Ke et al (1977) studied the changes in oxidation of lipid in various parts of frozen mackerel and found that the peroxide value after two months frozen storage at -15 °C was about 5meqO₂/ Kg lipid and the lipid content of their samples was 11.8%, including the skin which contains the highest concentration in lipids.

In this investigation, at the same approximate time of storage with lipid content 11.09% in the flesh, the peroxide value was 10.4meqO₂/ Kg. After the first 11 weeks of frozen storage the oxidation appeared to increase rapidly, particularly in the mackerel samples being packaged in the cardboard boxes. This indicates the important effect of packaging on lipid oxidation and the requirement that the access to oxygen must be taken in account, particularly in fatty fish products.
**Fig. 10**: PV of Mackerel during frozen storage.

PV meq$\text{O}_2$/g lipid

Frozen storage (weeks)

A = mackerel frozen stored in plastic bags.
B = mackerel frozen stored in cardboard boxes.

The values are means of triplicate determinations, with mean coefficients of variation: 8.6 & 11% for mackerel obtained from bags and boxes respectively.
The hydrolysis, as shown in Fig. 11 as percentage of free fatty acids, appeared very rapid in cod samples whilst in mackerel it was slow.

A major factor which affects lipid hydrolysis is the type of lipid in the fish. Ke et al (1977) demonstrated that the formation of free fatty acids by lipid hydrolysis in mackerel frozen stored at -15°C, takes place at a moderate rate. The same authors also studied the rates of the formation of free fatty acids in different parts of frozen mackerel and found that the subcutaneous lipids were very slowly hydrolysed, perhaps because they are nearly pure triglycerides, whilst the white and dark muscle were moderately hydrolysed in line with their higher phospholipid content. They also found that at -30°C hydrolysis for all parts of the fish had been effectively retarded.

The hydrolysis which occurred in cod samples was very similar to that reported by Olley and Lovern (1960). They found a rapid increase of free fatty acids in cod samples stored at -15 and -22°C. In their investigation the free fatty acid content after 25 weeks frozen storage at -15°C was about 48%, whereas in this work it was 32-36% after 22 weeks storage at -20°C, while in those being stored at -22°C after 25 weeks, according to the same authors, it was 20%.

The above order in values indicates the important influence of the temperature on lipid hydrolysis. The lower the temperature the less hydrolysis occurs.
Fig. 11: % FFA of Cod & Mackerel during frozen storage.

F.F.A.% in lipid.

Frozen storage (weeks)

A = Cod frozen stored in plastic bags.  C = Mackerel frozen stored in plastic bags
B = Cod frozen stored in cardboard boxes.  D = Mackerel frozen stored in cardboard boxes

The values are means of triplicate determinations, with mean coefficients of variation: 13.4 & 9.8% for cod samples obtained from bags & boxes respectively; 5 & 8.5% for mackerel samples obtained from bags & boxes respectively.
Phospholipolysis is the major type of lipid hydrolysis, thus, the increase in free fatty acid content during frozen storage was quite normal as cod contain 85-90% phospholipids.

However, the consequences of hydrolysis on acceptability are not clear. The major effect of lipid hydrolysis is the influence of free fatty acids on protein denaturation and thus on the texture qualities of the fish. The high correlation observed in this investigation between soluble protein and free fatty acids is shown in Fig. 12.

Fig. 12: Correlation between % SSP & % FFA during frozen storage of Cod.

\[
Y = -1.2668X + 74.3272 \\
r = -0.9833 \quad t = 5.4051 \quad P% = 11.65%
\]
The $r$ value is significantly different from zero and it gives a very good negative correlation that shows that the variables are related. Thus, this high correlation strengthens the aspect that the free fatty acid content are responsible for the denaturation of cod protein.

The fatty acid profiles for cod and mackerel during frozen storage are shown in Table 15 & 16 respectively.

The fatty acid profiles of cod remained quite similar and some slight changes, in the % of total fatty acids, were mainly attributed to the variation of fatty acids in the body of the fish and to the different individual fish analysed.

Generally, it can be observed, that a very slight increase in monounsaturated fatty acids (from 19.1 to 20.9%) occurred after 33 weeks frozen storage of the cod samples, at the expense of polyunsaturated fatty acids which very slightly decreased (from 57.5 to 56.2%). A further interchange between fatty acids can be also observed. Thus, a decrease of the n-3 polyunsaturated fatty acids was accompanied by an increase of n-6 as well as by an increase of 20:1n-9 after 33 weeks frozen storage of the cod samples.

Hardy et al (1979) found that, in cod samples stored at -10 °C for 200 days, the phospholipids showed an increase in the C18 fatty acids with a decrease in the C16 & C20 components, and in the free fatty acids the proportion of the C22 fatty
Table 15: Fatty acid profiles in cod during frozen storage at -20 °C (in plastic bags).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>% Fresh</th>
<th>F. 11 W.</th>
<th>F. 22 W.</th>
<th>F. 33 W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.6 (0.05)</td>
<td>2.1 (0.11)</td>
<td>2.1 (0.19)</td>
<td>2.4 (0.05)</td>
</tr>
<tr>
<td>16:0</td>
<td>17.9 (0.20)</td>
<td>19.9 (2.02)</td>
<td>18.7 (0.07)</td>
<td>16.6 (0.20)</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>3.4 (0.06)</td>
<td>3.7 (0.19)</td>
<td>3.5 (0.12)</td>
<td>4.1 (0.03)</td>
</tr>
<tr>
<td>18:0</td>
<td>3.9 (0.06)</td>
<td>3.1 (0.10)</td>
<td>4.0 (0.01)</td>
<td>3.8 (0.17)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>11.4 (0.06)</td>
<td>8.3 (0.42)</td>
<td>9.8 (0.13)</td>
<td>10.3 (0.13)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0.9 (0.02)</td>
<td>0.8 (0.06)</td>
<td>0.7 (0.01)</td>
<td>1.6 (0.11)</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>0.5 (0.05)</td>
<td>0.6 (0.09)</td>
<td>0.2 (0.05)</td>
<td>0.6 (0.04)</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>2.7 (0.09)</td>
<td>3.2 (0.17)</td>
<td>2.9 (0.03)</td>
<td>4.2 (0.03)</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>3.7 (0.09)</td>
<td>4.5 (0.14)</td>
<td>4.9 (0.02)</td>
<td>7.3 (0.05)</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>16.2 (0.22)</td>
<td>17.0 (0.09)</td>
<td>18.2 (0.09)</td>
<td>14.2 (0.23)</td>
</tr>
<tr>
<td>22:1n-11</td>
<td>1.6 (0.28)</td>
<td>1.7 (0.14)</td>
<td>1.4 (0.11)</td>
<td>2.3 (0.43)</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.8 (0.14)</td>
<td>1.5 (0.17)</td>
<td>2.1 (0.03)</td>
<td>1.3 (0.08)</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>34.4 (0.17)</td>
<td>33.2 (2.38)</td>
<td>30.9 (0.06)</td>
<td>31.2 (0.46)</td>
</tr>
</tbody>
</table>

Saturated: 23.4 (0.45) 25.1 (0.13) 24.8 (0.21) 22.8 (0.53)
Monounsat.: 19.1 (0.37) 16.9 (0.66) 17.6 (0.16) 20.9 (0.47)
Polyunsat.: 57.5 (0.28) 57.6 (0.93) 57.0 (0.20) 56.2 (0.29)

F = Frozen  W = Weeks

The values are means of triplicate determinations.
Standard deviations are shown in parentheses.
Table 16: Fatty acid profiles in mackerel during frozen storage at -20 °C (in plastic bags).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>% Fresh</th>
<th>F. 11 W.</th>
<th>F. 22 W.</th>
<th>F. 33 W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>3.6 (0.01)</td>
<td>5.9 (0.06)</td>
<td>6.7 (0.06)</td>
<td>6.8 (0.38)</td>
</tr>
<tr>
<td>16:0</td>
<td>18.9 (0.08)</td>
<td>16.7 (0.10)</td>
<td>18.0 (0.19)</td>
<td>18.5 (0.63)</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>4.4 (0.03)</td>
<td>5.3 (0.06)</td>
<td>5.7 (0.04)</td>
<td>9.0 (0.97)</td>
</tr>
<tr>
<td>18:0</td>
<td>5.8 (0.03)</td>
<td>4.1 (0.03)</td>
<td>4.9 (0.03)</td>
<td>4.0 (0.20)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>22.7 (0.51)</td>
<td>22.3 (0.04)</td>
<td>19.9 (0.09)</td>
<td>20.5 (0.65)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>1.2 (0.21)</td>
<td>1.6 (0.01)</td>
<td>2.0 (0.04)</td>
<td>3.0 (0.33)</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>0.8 (0.22)</td>
<td>1.5 (0.03)</td>
<td>1.7 (0.05)</td>
<td>1.4 (0.05)</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>9.2 (0.02)</td>
<td>8.6 (0.02)</td>
<td>9.0 (0.03)</td>
<td>9.3 (0.05)</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>6.9 (0.03)</td>
<td>10.2 (0.01)</td>
<td>9.5 (0.02)</td>
<td>5.4 (0.76)</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>8.7 (0.06)</td>
<td>7.3 (0.03)</td>
<td>7.8 (0.02)</td>
<td>11.5 (0.59)</td>
</tr>
<tr>
<td>22:1n-11</td>
<td>2.4 (0.45)</td>
<td>2.1 (0.01)</td>
<td>2.4 (0.01)</td>
<td>1.7 (0.22)</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.6 (0.01)</td>
<td>1.4 (0.01)</td>
<td>1.2 (0.02)</td>
<td>0.0</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>13.9 (0.23)</td>
<td>12.1 (0.19)</td>
<td>11.5 (0.11)</td>
<td>8.9 (1.21)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Saturated</th>
<th>Monounsat.</th>
<th>Polyunsat.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28.3 (0.21)</td>
<td>38.5 (0.88)</td>
<td>33.1 (0.17)</td>
</tr>
<tr>
<td></td>
<td>26.7 (0.06)</td>
<td>38.3 (0.06)</td>
<td>34.1 (0.04)</td>
</tr>
<tr>
<td></td>
<td>29.6 (0.05)</td>
<td>37.0 (0.06)</td>
<td>33.7 (0.02)</td>
</tr>
<tr>
<td></td>
<td>29.3 (0.80)</td>
<td>40.5 (1.31)</td>
<td>30.2 (0.74)</td>
</tr>
</tbody>
</table>

F = Frozen  W = weeks

The values are means of triplicate determinations.
Standard deviations are shown in parentheses.
acids increased primarily at the expense of the C18 component. They concluded that the relatively small change that occurred in the phospholipids may possibly indicate that there was a slight preferential hydrolysis of the C20 over the C18 fatty acids, whereas the small change of the C22 acids was indicative of little oxidation.

A similar conclusion could be also extracted from this investigation.

The changes of fatty acid profiles, in the % total fatty acids, appeared much greater in the mackerel samples. Thus, notwithstanding the variation of fatty acids in the body of mackerel, it was observed that an overall decrease of polyunsaturated fatty acids was accompanied by an increase of monounsaturates and with a smaller increase of saturated fatty acids (Table 16). Some other small changes, which have been observed, were probably a result of the distribution of fatty acids in the body of mackerel. Some other significant observations were the decrease in 22:6n-3, the overall disappearance of 22:5n-3, which both indicated lipid oxidation, and the increase in 16:1n-7, 18:2n-6 and 14:0 during frozen storage of the mackerel samples for 33 weeks.

The results in this investigation agree with those reported by Ke et al (1977). They studied the lipid oxidation in various parts of mackerel frozen, at -15 °C for 2 months. They found no changes in fatty acid profiles, after this period of storage, in white and dark muscle while the
peroxide value in these parts of the mackerel were 0.0 & 1.2meq\\_kg lipid respectively. However, at the same time in the skin lipids the 18% decrease of 22\_6n-3 observed was accompanied by a peroxide value of 11.4mg / Kg lipid (the lipid content in the skin was 26.3%).

In this work the 22\_6n-3 decreased after 11 weeks of frozen storage by 13% and this was accompanied by a peroxide value of 8.7meq\\_kg lipid (with lipid content 11%). However, after 33 weeks frozen storage when the peroxide value was 56.9meq\\_kg lipid (in the plastic bags samples) the decrease of 22\_6n-3 was 36%, indicating that the increase in peroxide value is not exactly proportional to the decrease of 22\_6n-3. Thus, while in the first frozen storage period, in this investigation as well as in that by Ke et al (1977), it was observed that an increase in peroxide value of 10meq\\_kg lipid was accompanied by about a 15% decrease of 22\_6n-3, this proportion became lower during a further period of frozen storage. Thus, the decrease of 8% in 22\_6n-3 was for a 10meq\\_kg lipid increase in peroxide value after 22 weeks and became 6.3% for a further 10meq\\_kg lipid increase in peroxide value after 33 weeks frozen storage.

This is evidence that the initial oxidation is mainly related to the destruction of 22\_6n-3, whilst later some other fatty acids, e.g. 22\_5n-3, are also oxidized.
Similar results for fatty acid profiles in jack mackerel stored at 5 °C, were reported by Shono & Toyomizu (1973). They found that polyunsaturated fatty acids decreased with oxidation. The most predominant unsaturated fatty acid (22:6n-3) decreased the most, while monounsaturated and saturated fatty acids showed comparatively little decrease.

5.2.3. Texture.

The change of texture during frozen storage of both fish samples is presented in Fig. 13, which shows a significant difference between them.

The thawed samples of cod showed an increase from a penetration force of 32g for the fresh sample to 72 & 77g after being stored for 11 weeks in plastic bags and cardboard boxes, respectively. These results coincide with a decrease in protein solubility (Section 5.2.1., Fig. 8) and an increase in free fatty acids content (Section 5.2.2., Fig. 10) in the cod samples after 11 weeks frozen storage. This indicates the strong influence of these two factors on the cod texture, as shown in Table 17.

However, after this initial period of time the texture of the cod samples appeared relatively constant despite the further changes of the other two factors. This limited texture change during frozen storage may be related to a limit in the level of formaldehyde being produced in the frozen flesh.
Fig. 13: Texture measurements by Steven's Texture Analyser of Cod & Mackerel during frozen storage.

Texture (g).

Frozen storage (weeks)

A = Cod frozen stored in plastic bags.  
B = Cod frozen stored in cardboard boxes.  
C = Mackerel frozen stored in plastic bags.

The values are means of 15-20 determinations with mean coefficients of variation: 6 & 5% for cod samples obtained from bags and boxes respectively; 8.5% for both mackerel samples.
Table 17a: Relation between Texture and % SSP of the cod samples during frozen storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Texture (g)</th>
<th>% SSP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bags</td>
<td>Boxes</td>
</tr>
<tr>
<td>Fresh</td>
<td>32 (1.25)</td>
<td>32 (1.25)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>72 (7.97)</td>
<td>77 (3.25)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>67 (3.52)</td>
<td>66 (4.44)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>68 (2.49)</td>
<td>67 (3.94)</td>
</tr>
</tbody>
</table>

Table 17b: Relation between Texture and % FFA of the cod samples during frozen storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Texture (g)</th>
<th>% FFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bags</td>
<td>Boxes</td>
</tr>
<tr>
<td>Fresh</td>
<td>32 (1.25)</td>
<td>32 (1.25)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>72 (7.97)</td>
<td>77 (3.25)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>67 (3.52)</td>
<td>66 (4.44)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>68 (2.49)</td>
<td>67 (3.94)</td>
</tr>
</tbody>
</table>

F = Frozen    W = Weeks

The values of texture are means of 15 - 20 determinations, while the other values are means of triplicate determinations. Standard deviations are shown in parentheses.
The samples of mackerel showed a relatively constant texture during frozen storage. A slight decrease was observed in the first 11 weeks storage, despite the slight decrease in soluble protein and the small increase in free amino acids (Section 5.2.1.). An increase in the penetration force, however, was observed in the further 11 weeks of frozen storage and afterwards remained constant, as shown in Table 18.

In mackerel the total lipid content is a factor which influences the texture of the fish during frozen storage, possibly overwhelming the effect of the denaturated protein.

5.2.4. Available lysine & thiamine.


The available lysine, as shown in Fig. 14, showed a slight steady decrease for both fish samples during frozen storage. The loss of available lysine appeared to be higher in the cod samples, with a total loss of 35.8% over the 33 week period, whilst in mackerel, at the same time, the loss was 22.9%.

The loss of lysine in the cod samples was greater during the initial 11 week period and this was probably due to fish to fish variation, while for mackerel the available lysine content was virtually constant during the first 11 weeks.
Table 18a: Relation between Texture and % SSP of the mackerel samples during frozen storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Texture (g)</th>
<th>% SSP Bags</th>
<th>% SSP Boxes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>27 (1.58)</td>
<td>84.0 (1.81)</td>
<td>84.0 (1.81)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>21 (1.50)</td>
<td>74.0 (5.68)</td>
<td>79.0 (3.99)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>33 (3.77)</td>
<td>71.1 (2.06)</td>
<td>73.3 (1.64)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>33 (3.19)</td>
<td>63.4 (1.44)</td>
<td>65.0 (1.36)</td>
</tr>
</tbody>
</table>

Table 18b: Relation between Texture and mg / g FAA of the mackerel samples during frozen storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Texture (g)</th>
<th>mg / g FAA Bags</th>
<th>mg / g FAA Boxes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>27 (1.58)</td>
<td>6.95 (0.27)</td>
<td>6.95 (0.27)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>21 (1.50)</td>
<td>8.20 (0.38)</td>
<td>7.80 (0.22)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>33 (3.77)</td>
<td>10.80 (0.05)</td>
<td>10.50 (0.01)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>33 (3.19)</td>
<td>10.70 (0.42)</td>
<td>11.20 (0.69)</td>
</tr>
</tbody>
</table>

F = Frozen    W = Weeks

The values of texture are means of 15-20 determinations, while the other values are means of triplicate determinations. Standard deviations are shown in parentheses.
Fig. 14: Available lysine, on wet weight basis, in Cod & Mackerel during frozen storage.

Available lysine %

Frozen storage (weeks)

The values are triplicate determinations, with mean coefficients of variation: 15% & 2.4% for cod and mackerel, respectively.
Poulter & Lawrie (1977) studied the nutritional consequences of the changes occurring during frozen storage of cod and found that there was a statistically significant decrease in the FDNB-available lysine (8.47g / 16g N to 8.13g / 16g N) whilst in this investigation the decrease of available lysine (Fig. 14) was not significant at the 95% level.

b. Thiamine.

The influence of frozen storage on the thiamine content is demonstrated in Fig. 15.

Thiamine is the most sensitive vitamin to heating, however, this water soluble vitamin appeared to be quite sensitive to thawing loss, particularly in the cod samples.

Thus, the thiamine content in cod appeared to be constant in the first 11 week period of storage but in the next 11 weeks a significant (at 95% level, using t-test) loss was observed which was 50% of the total thiamine content. During the last 11 weeks, however, the thiamine content remained virtually constant (4 to 3ug / 100g)

Conversely the thiamine content in mackerel remained virtually constant, with some statistically insignificant (at 95% level, using t-test) slight changes during the period of frozen storage.
Fig. 15: Thiamine content of Cod & Mackerel during frozen storage.

Thiamine ug/100g

Frozen storage (weeks)

The values are means of triplicate determinations, with mean coefficients of variation: 15.8% & 11.4% for cod and mackerel, respectively.
5.2.5. Histamine in mackerel.

The histamine levels during 33 weeks storage of mackerel are shown in Fig. 16.

Although there was a steady increase in the histamine content of mackerel during the period of frozen storage, these levels of histamine are very small in comparison to the detected amounts of free-histidine contained in the muscle of such a fish (1000mg / 100g).

According to Eitenmiller et al (1982) there are three quantitative groups of histamine intoxications:

1. Mild poisoning due to consumption of 8-40mg / day.
2. Moderate intensity disorders 70-1000mg / day.
3. Severe incidents 1500-4000mg / day.

The levels in this investigation were much lower than the levels described above.

Salguero & Mackie (1979) studied the histamine formation in mackerel during storage at 23, 10, 2, 0, and -30 °C under sterile and non-sterile conditions. They found that little histamine was produced under both (sterile and non-sterile) conditions at 0 °C after 25 days (0.24mg / 100g). According to the same authors it was not a surprise that the histamine level in the mackerel samples stored at -30 °C for 8 months became only 0.52mg / 100g from a level 0.08mg / 100g in the fresh fish.
Fig.16: Histamine content of Mackerel during frozen storage.

The values are means of triplicate determinations, with 10.5% coefficient of variation.
Morii et al (1988) also found an increase in the histamine level to about 1mg / 100g during storage of mackerel samples in ice for 12 days.

These results indicate that the level of histamine in mackerel after 33 weeks frozen storage was as expected.
5.3. THE EFFECT OF SMOKING ON PREVIOUSLY FROZEN COD.

For all molecular changes observed during frozen storage of the cod samples, the effect of packaging was insignificant (at 95% level), thus, the values from the previous section and quoted in this work will be the mean of 3 determinations from the cod samples packaged in plastic bags and another 3 determinations from those packaged in cardboard boxes i.e. means of 6 determinations. Some apparent significant differences observed due to packaging, particularly in soluble protein were probably due to the variation of fish being analysed (Section 5.2.1.).

Additionally, because the smoked products were not influenced by the type of packaging, during frozen storage, the overall means (from those packaged in plastic bags and those in cardboard boxes prior to smoking) will also be used for results of determinations on smoked fish.

The unprocessed and smoked fresh cod samples were analysed for % moisture, % protein, % lipid and % salt. The results, as shown in Table 19, indicate that, as expected, the moisture content decreases due to the drying part of the smoking process (Section, 2.2.2.4.). The loss of water was accompanied by an increase in salt, which penetrated into the flesh during the salting process (Section, 2.2.2.2.), and increases in percentage protein and lipid.
Table 19: Proximate analysis, on wet weight & dried salt free basis, of fresh unprocessed & fresh smoked Cod.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unprocessed</th>
<th>Smoked</th>
<th>Unprocessed</th>
<th>Smoked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>78.4</td>
<td>59.5</td>
<td>78.8</td>
<td>58.6</td>
</tr>
<tr>
<td></td>
<td>78.8</td>
<td>58.6</td>
<td>79.2</td>
<td>56.8</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>78.8 (0.30)</td>
<td>58.3 (1.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17.5</td>
<td>28.1</td>
<td>83.8</td>
<td>81.9</td>
</tr>
<tr>
<td></td>
<td>18.0</td>
<td>31.4</td>
<td>86.2</td>
<td>91.4</td>
</tr>
<tr>
<td>Protein</td>
<td>18.3</td>
<td>30.8</td>
<td>87.5</td>
<td>95.6</td>
</tr>
<tr>
<td></td>
<td>18.8</td>
<td></td>
<td>89.7</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>18.1 (0.42)</td>
<td>30.1 (1.42)</td>
<td>86.8 (2.14)</td>
<td>87.6 (4.14)</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.9</td>
<td>1.9</td>
<td>4.2</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>1.3</td>
<td>4.1</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>1.1</td>
<td>3.9</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.2</td>
<td>4.7</td>
<td>6.4</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.9 (0.07)</td>
<td>1.6 (0.44)</td>
<td>4.2 (0.29)</td>
<td>4.7 (1.27)</td>
</tr>
<tr>
<td>Salt</td>
<td>0.24</td>
<td></td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.26</td>
<td></td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td></td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.27 (0.03)</td>
<td>7.4 (0.44)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.3.1. Protein and amino acids.

The true protein content was virtually constant during the 33 week frozen storage period of the cod samples (Section 5.2.1.) as also shown in Table 20, however, a steady loss of true protein content, which increased with extent of prior frozen storage, appeared in the smoked products, as a result of the smoking process (which includes salting, smoking, drying and heating) (Section 2.2.2.1.). Thus, a loss of 6% protein was observed during smoking of the fresh fillets of cod, which became 10% for the fish that had undergone 11 weeks frozen storage at -20 °C prior to smoking and was followed by a protein loss of 21% for the cod fillets which had been stored at -20 °C for 33 weeks and then smoked.

A probably erroneous result, was obtained for the cod fillets which had been stored frozen for 22 weeks prior to smoking. The low value of true protein, which was obtained for the unprocessed cod was probably due to fish to fish variation or unknown experimental error and the high non-protein nitrogen content of these cod samples (Section 5.2.1.).

According to Cutting (1962) salting allows dehydration as a result of osmotic effects, which also allows the loss of water soluble constituents and some soluble protein along with the fluid. This loss of protein depends upon the freshness of the raw material.
This may also be concluded from this work, i.e. the smoking and salting processes allow the loss of some soluble protein, the extent of which depends upon the quality of the raw material which has been smoked.

Table 20: Effect of smoking on true protein of previously frozen cod.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% True protein</th>
<th>% Loss of protein</th>
<th>% Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frozen</td>
<td>Smoked</td>
<td>Frozen</td>
</tr>
<tr>
<td>Fresh</td>
<td>85.0 (0.36)</td>
<td>79.9 (1.96)</td>
<td>6.0</td>
</tr>
<tr>
<td>F.11 W.</td>
<td>85.5 (0.29)</td>
<td>76.5 (1.03)</td>
<td>10.5</td>
</tr>
<tr>
<td>F.22 W.</td>
<td>71.8 (0.33)</td>
<td>80.7 (0.61)</td>
<td>80.2 (0.19)</td>
</tr>
<tr>
<td>F.33 W.</td>
<td>84.2 (0.61)</td>
<td>66.0 (0.55)</td>
<td>21.6</td>
</tr>
</tbody>
</table>

F = Frozen     W = Weeks

The values presented in the Table are means of sixfold determinations (Section 5.3.). Standard deviations are shown in parentheses. Expressed on a dry, salt free basis.

According to Opstvedt (1989) about 90% of the fish protein is denaturated at about 60 to 65 °C (Section 2.2.2.5.).

The denaturation of protein in the cod fillets due to smoking process (heating at 70 °C for 4 hours) was, as expected, quite rapid, as shown in Fig. 17. From the same figure the
Fig. 17: Effect of smoking on protein solubility of previously frozen cod.

The values are means of sixfold determinations, with mean coefficients of variation: 0.5 & 3.9% for frozen and smoked cod samples respectively.
rapid decrease of protein solubility during frozen storage of the cod samples can also be observed (Section 5.2.1.).

From this figure it can also be concluded that the % soluble protein of the raw material does not affect the final protein solubility of the smoked cod fillets. Thus, the protein solubilities of all smoked cod fillets, fresh and frozen for 11, 22 and 33 weeks, was from 12 to 15% while those of the unsmoked samples varied from 72.1 to 26.6% (Section 5.2.1.). Therefore, the percentage denaturation observed for each cod sample due to smoking was quite different and it was 84.2, 72.7, 49.0 and 50.0 for fresh cod and cod frozen for 11, 22 and 33 weeks respectively.

The protein denaturation, however, does not appear to affect protein utilisation. Zagret (1969) analysed the amino acid content and tested the protein quality of five samples of traditionally smoked fish, in feeding tests with rats. For comparison a sample of commercial herring meal was included. All samples of smoked fish had an excellent protein quality which was equal to or better than that of the commercial herring meal. Munro & Morrison (1965) also tested the protein quality of medium (48% and 20% NaCl) salted and cold smoked commercial samples of cod in comparison with fresh cod. All samples were cooked and freeze-dried before feeding trials with rats. No differences were found between salted and smoked cod and fresh cod with regard to protein quality (protein efficiency ratio and gross protein value). According to Opstvedt (1989), however, there is a reduction of 40%
in the content of α-amino-nitrogen of the final product compared with fresh fish, most of which occurs during the heating step. Ziemba (1967) also concluded from his work that smoke carbonyls react with amino acids and that oxidative browning may take place if the smoke is produced with limited amounts of air.

The effect of smoking on the free amino acid content of cod samples is demonstrated in Fig. 18. The increase which had been observed during frozen storage (Section 5.2.1.), as also shown in the figure, was accompanied by a 22.4% reduction in the free amino acid content due to smoking of the fresh cod and this reduction became 17% for the smoked cod samples with prior frozen storage of 22 weeks and 13.8% for those smoked cod samples which had been stored at -20 °C for 33 weeks. This reduction was probably due to the reactions between free amino acids and carbonyl compounds from the smoke, with these reactions also being responsible, to a lesser or greater extent, for the surface colour formation of the cod fillets.

However, a different result was obtained for the smoked cod samples which had been frozen stored for 11 weeks. In those samples an increase in free amino acid content, at 7.5 percent, was observed due to smoking. One possible explanation for this result may be that it was an effect of the particular process, as this gave the darkest cod fillets, indicating that the surface of the fillets was probably covered by the smoke particles which may have inhibited the
Fig. 18: Effect of smoking on free amino acids of previously frozen cod (on dried salt free basis).

The values are means of sixfold determinations, with mean coefficients of variation: 5 & 6.9% for frozen and smoked cod samples respectively.
reactions between amino acids and carbonyl compounds from the smoke. According to the taste panel results the colour of these cod fillets was the brownest and the panellists gave an average score of 6.6 with a SD of 0.80 whilst all other samples, even those which had been smoked after 33 weeks frozen storage, had an average of less than 6.2 with a SD above of 1.00. As it can be observed from the taste panel sheet used in this work (Table 7) the greater the score, the greater the degree of browning of the product.

5.3.2. Lipid.

Smoking did not affect the total lipid content of the cod samples, as this remained virtually constant during all processes. This is, as expected, because fish such as cod which are deficient in muscle tissue depots, contain structural lipid associated with membranes. With this initial low level of lipid, it is, therefore, quite difficult to have any appreciable loss.

No changes in peroxide value were found for the cod samples at any stage of processing, indicating that very little lipid oxidation had occurred. A peroxide value could only be detected in the fresh smoked cod fillets and in those frozen stored for 11 weeks prior to smoking, but the values were very low (4μeqO₂/ Kg lipid). Some lipid oxidation may be expected during the smoking process, because the cod fillets are subject to heating, smoking and atmospheric oxygen. All these factors together with the high salt concentration of
the cod fillets (Section 5.3.7.) can accelerate the oxidative deterioration of the fish.

According to Hardy et al (1979) cellular phospholipids do not oxidize rapidly, although they contain the most unsaturated lipids in all fish, and this effect is believed to be caused by the physical disposition of the lipids making it difficult for them to participate in the oxidation chain reaction.

Thus, the oxidation in cod with 85-90% phospholipids was insignificant.

In comparison hydrolysis of the lipid during frozen storage (Section 5.2.2.) and during smoking was quite rapid, as determined by its major product which is the free fatty acid content, as shown in Fig. 19.

Thus, apart from the increase of free fatty acid content during frozen storage, an additional increase was observed due to curing in all cod samples being hot smoked. This increase seemed to be limited, thus in the cod samples being frozen stored for 33 weeks, which contained a very high amount of free fatty acids (49.35% in lipid) the increase due to smoking was very slight (2%).

Hardy (1980) stated that smoking enhances hydrolysis. Salting, according to the same author, does not seem to have a marked effect on the rate although, in salted cod,
Fig. 19: Effect of smoking on free fatty acids of previously frozen cod (on dried, salt free basis).

The values are means of sixfold determinations, with mean coefficients of variation: 12 & 7.8% for frozen and smoked cod samples respectively.
hydrolysis ceased when the lipid contained 50% free fatty acids.

The same phenomenon could be also observed in this work, the hydrolysis possibly ceased when the lipid contained about 50% free fatty acids.

The fatty acid profiles of smoked cod fillets are presented in Table 21. Table 22 demonstrates the total saturated, monounsaturated and polyunsaturated fatty acids, present in the lipid of both frozen and smoked fish, for ease of comparison.

The fatty acid profiles of cod, during frozen storage, remained quite similar and some slight changes were mainly attributed to the variation of fatty acids in the fish body (Section 5.2.2.). The slight changes being observed during frozen storage, as also shown in Table 22, where the decrease of polyunsaturates which was accompanied by the slight increase of monounsaturated fatty acids. The same result could be also concluded for the changes in fatty acid profiles due to the smoking process. Thus, an increase in monounsaturates was observed for all fish upon smoking, with a final increase of 3.7% (19.1 to 22.8%) at the expense of polyunsaturated fatty acids of 3% (57.5 to 54.5%), using the fresh raw material as a reference point. The saturated fatty acids virtually remained unaltered.
Table 21: The fatty acid profiles of smoked cod fillets.

<table>
<thead>
<tr>
<th>Fatty acids %</th>
<th>Fresh smoked</th>
<th>Frozen 11 W. then smoked</th>
<th>Frozen 22 W. then smoked</th>
<th>Frozen 33 W. then smoked</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.3 (0.01)</td>
<td>1.7 (0.12)</td>
<td>1.7 (0.03)</td>
<td>2.0 (0.07)</td>
</tr>
<tr>
<td>16:0</td>
<td>16.4 (0.19)</td>
<td>16.5 (0.07)</td>
<td>17.3 (0.04)</td>
<td>16.5 (0.43)</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>2.4 (0.03)</td>
<td>3.0 (0.04)</td>
<td>3.4 (0.03)</td>
<td>4.5 (0.11)</td>
</tr>
<tr>
<td>18:0</td>
<td>4.0 (0.08)</td>
<td>3.9 (0.09)</td>
<td>4.6 (0.04)</td>
<td>4.3 (0.13)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>12.8 (0.12)</td>
<td>16.3 (0.08)</td>
<td>10.0 (0.24)</td>
<td>11.5 (0.13)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>2.9 (0.02)</td>
<td>3.6 (0.14)</td>
<td>0.7 (0.01)</td>
<td>2.4 (0.20)</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>1.6 (0.01)</td>
<td>2.0 (0.01)</td>
<td>0.2 (0.01)</td>
<td>1.0 (0.08)</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>2.7 (0.01)</td>
<td>3.0 (0.06)</td>
<td>3.4 (0.22)</td>
<td>4.0 (0.07)</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>3.2 (0.06)</td>
<td>5.2 (0.09)</td>
<td>7.1 (0.05)</td>
<td>6.8 (0.14)</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>11.5 (0.02)</td>
<td>13.1 (0.06)</td>
<td>17.9 (0.05)</td>
<td>15.2 (0.01)</td>
</tr>
<tr>
<td>22:1n-11</td>
<td>1.5 (0.08)</td>
<td>1.6 (0.10)</td>
<td>1.8 (0.24)</td>
<td>2.8 (0.26)</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.0 (0.04)</td>
<td>1.3 (0.04)</td>
<td>2.2 (0.42)</td>
<td>1.2 (0.05)</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>38.5 (0.32)</td>
<td>28.2 (0.27)</td>
<td>29.4 (0.11)</td>
<td>27.9 (0.47)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Saturated 21.7 (2.20)</th>
<th>Monoenic 19.4 (0.66)</th>
<th>Polyenic 58.7 (0.13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22.1 (0.18)</td>
<td>23.9 (0.13)</td>
<td>53.4 (0.17)</td>
</tr>
<tr>
<td></td>
<td>23.6 (0.08)</td>
<td>18.6 (0.27)</td>
<td>57.5 (0.20)</td>
</tr>
<tr>
<td></td>
<td>22.8 (0.55)</td>
<td>22.8 (0.31)</td>
<td>54.5 (0.30)</td>
</tr>
</tbody>
</table>

The values presented in this Table are means of triplicate determinations of the cod samples being stored at -20 °C in plastic bags prior to smoking. Standard deviations are shown in parentheses.
Table 22: Fatty acid composition as obtained in both conditions (frozen & smoked).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Saturated</th>
<th>Monoenic</th>
<th>Polyenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>23.4 (0.45)</td>
<td>19.1 (0.57)</td>
<td>57.5 (0.28)</td>
</tr>
<tr>
<td>Fresh smoked</td>
<td>21.7 (2.20)</td>
<td>19.4 (0.66)</td>
<td>58.7 (0.13)</td>
</tr>
<tr>
<td>Frozen 11 weeks</td>
<td>25.1 (0.13)</td>
<td>16.9 (0.66)</td>
<td>57.6 (0.93)</td>
</tr>
<tr>
<td>Frozen 11 weeks then smoked</td>
<td>22.1 (0.18)</td>
<td>23.9 (0.13)</td>
<td>53.4 (0.17)</td>
</tr>
<tr>
<td>Frozen 22 weeks</td>
<td>24.8 (0.21)</td>
<td>17.6 (0.16)</td>
<td>57.0 (0.20)</td>
</tr>
<tr>
<td>Frozen 22 weeks then smoked</td>
<td>23.6 (0.08)</td>
<td>18.6 (0.27)</td>
<td>57.5 (0.20)</td>
</tr>
<tr>
<td>Frozen 33 weeks</td>
<td>22.8 (0.53)</td>
<td>20.9 (0.47)</td>
<td>56.2 (0.29)</td>
</tr>
<tr>
<td>Frozen 33 weeks then smoked</td>
<td>22.8 (0.55)</td>
<td>22.8 (0.31)</td>
<td>54.5 (0.30)</td>
</tr>
</tbody>
</table>

All values presented are means of triplicate determinations of the cod samples being stored at -20 °C in plastic bags. Standard deviations are shown in parentheses.

Comparing also the Tables 15 (Section 5.2.2.) and 21 some marked changes can be observed. The most important is the small decrease due to smoking, in almost all cases, of 22:6n-3 and of 20:5n-3 which indicates that little oxidation occurred due to the smoking process. This small decrease of the above n-3 fatty acids was accompanied by a small increase of all C18.
All other slight changes were probably from the distribution of the fatty acids in the body of the fish and due to the different individual fish analysed.

5.3.3. Texture.

Instrumental measurements on cooked fish have been found to be unreliable as a result of the unique morphological and compositional features of fish (Section 2.3.4.). Devices used for rheological testing of food, even those found to be applicable to red meat, are in general, unsuitable for fish and particularly when being cooked, because the forces acting between the myomeres and fibres become so weak that the muscles disintegrate easily (Section 2.3.4.).

Borderias et al (1983) studied the texture of 5 raw and cooked fish species (sardine, rainbow trout, conger eel, horse mackerel and blue whiting) using an Instron Model 1140 Texturometer. They tested the shearing of the fish samples using a Kramer shear cell and a Warner-Bratzler cell. They observed that the values were significantly higher in the shear tests performed using the Warner-Bratzler cell than in the Kramer shear cell. They also found that none of the species investigated showed variations attributable to species differences. They finally found that there was no significant correlation between any of the indices obtained from the instrumental analyses and the taste panel results, for fish fillets. All these indicate the difficulty and unreliability of texture instrumental measurements on cooked
Because of the above difficulties and because the penetration force has been suggested as a measure suitable to avoid disintegration of the muscles during the instrumental measurements, the Steven's LFRA Texture Analyser was used in this investigation (Wang 1990).

The results of the penetration force required for all fresh, frozen and smoked cod fillets are demonstrated in Fig. 20.

It can be observed from the figure that the texture of all smoked samples were very similar, despite their different histories of frozen storage and notwithstanding the observed increase of the texture due to frozen storage, particularly in the initial 11 weeks period (Section 5.2.3.) as also shown in Fig. 20. Similar results for all smoked samples were also obtained for soluble protein (Section 5.3.1.) which indicated that the denaturation of protein due to heating is independent of the protein solubility before heating (Section 5.3.1.) and influences the texture of the heated products to the same extent.

The main factors which probably contributed to the high texture values of the smoked products were the lower amount of water content in these products, compared to the unprocessed ones, and probably the high salt concentration in the smoked cod fillets.
Fig. 20: Effect of smoking on texture of previously frozen cod using the Steven's Texture Analyser.

The values are means of 15-20 measurements, with mean coefficients of variation: 6% & 13% for frozen and smoked cod samples respectively.
5.3.4. Available lysine and thiamine.


The FDNB available lysine is one of the most critical nutritional factors in smoked fish products. It has been established that the surface colouring of smoked fish involves complicated carbonyl-amino reactions (Section 2.2.2.3.3.).

Clifford et al (1980) confirmed that lysine is the most sensitive of the essential amino acids in such reactions and that losses, due to smoking, are much greater in the outermost 5-10mm of the fish and decline rapidly toward the centre. The same authors also confirmed that destruction of lysine at 25% level is accompanied by a 7% fall in other nutritionally critical amino acids, including histidine, arginine etc.

The percentage available lysine in the frozen and smoked fish is presented in Fig. 21.

From the figure it can be concluded that the overall destruction of available lysine due to smoking was actually dramatic. The FDNB available lysine was 11.3% in fresh unprocessed cod samples, and had a small but insignificant loss due to the long period of frozen storage. Thus, after 33 weeks frozen storage the available lysine was 7.65% and became due to smoking 0.79% (all these values on dried salt free basis). This is an overall destruction due to smoking
Fig. 21: Effect of smoking on available lysine of previously frozen cod (on dried salt free basis).

The values are means of triplicate determinations, with mean coefficients of variation: 8.6 & 18.6% for frozen and smoked cod samples respectively.
of 93%, compared to a loss of 34.5% for the fresh smoked cod fillets and losses of 31.2, 64.0 and 90.0% for the 11, 22 and 33 weeks frozen stored cod samples respectively. This destruction of available lysine indicates that the frozen cod fillets are more susceptible than fresh to the reactions that cause lysine to become unavailable e.g. carbonyl-amino acids reactions.

It is of interest that a darker yellow/brown colour was obtained for the smoked cod fillets which had been previously frozen stored (Section 5.3.7.) compared to smoking fresh cod fillets.

Opstvedt (1989) reported that the loss in available lysine in minced smoked tilapia was about 18% when the total smoking operation was only 4 hours, 2 hours at 30 °C and another 2 hours at 70 °C. According to the same author, there is a reduction of 40% in the content of a-amino-nitrogen, due to smoking of the final product compared with fresh fish.

b. Thiamine.

The thiamine content of the fresh, frozen and smoked cod fillets are presented in Fig. 22.

Fish, in general, are not a good source of vitamins (except the vitamin D in fatty fish) and the amount of all vitamins in fish flesh is very low (Section 2.2.3.). Moreover, various factors can introduce variations, in those very
Fig. 22: Effect of smoking on thiamine of previously frozen cod (on dried salt free basis).

Thiamine ug/100g

Frozen storage (weeks)

The values are means of triplicate determinations, with mean coefficients of variation: 15.5 & 3.0% for frozen and smoked cod samples respectively.
small amounts of vitamins, thus, any analysis of these in the products could be unrepresentative and unreliable (Section 2.2.3.).

In this work the thiamine was chosen to be analysed as a general nutritional figure of the smoked products, because it is the vitamin most sensitive to heating.

However, despite of its reported sensitivity to heating, the thiamine in the cod samples, in this work, appeared to be very stable during the smoking process despite temperatures of 70 °C for 4 hours. Stability of thiamine due to the smoking process was also found by Paul & Southgate (1979) (Table, 5). Thus, thiamine seemed to be affected more by the frozen storage and thawing procedures, as shown in Fig. 22 and (Section 5.2.4.) rather than by the smoking process.

Thus, the thiamine remained at the same approximate level in fresh and frozen 11 weeks unprocessed and smoked samples. Heating, as a part of the smoking process, did not influence the thiamine content of these cod samples. However, a loss of thiamine content at about 50% was observed after 22 weeks frozen storage and thawing giving a value of 22ug/100g which remained virtually unaltered due to smoking of these samples. Little change occurred after this time.
5.3.5. Relation of lipid quality and taste panel results for off-flavours.

Evidence of lipid oxidation, which is associated with the production of off-flavours, was not found during the 33 week period of frozen storage of the cod samples by the peroxide value determination and only very slightly indicated by the decrease of 22:6n-3 (Section 5.2.2.)

The oxidation of lipid also occurred to a very small extent in the cod fillets due to the smoking process as identified by the low peroxide value detected after smoking of the fresh and 11 weeks frozen cod samples. A slight but steady decrease of 22:6n-3 also occurred due to the smoking processes (Section 5.3.2.).

However, this slight oxidation was not identified by the taste panellists. The majority of them (5-7) gave all samples a score of 0.0, which is equal to no off-flavour, in the taste panel sheet (Table, 7). Others (2-4) scored the samples 2 to 4, which is equal to slight off-flavour, in the taste panel sheet and only 1 panellist expressed a higher score equal to moderate off-flavour for some of the smoked cod fillets. No statistically significant (at 95% level) development of off-flavour was therefore detected in these samples after smoking (Table 23).
Table 23: Taste panel results for off-flavours with the lipid changes due to smoking of cod samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Score</th>
<th>Mean</th>
<th>% 22:6 n-3</th>
<th>PV meq/kg</th>
<th>% FFA in Kg lipid</th>
<th>lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.S.</td>
<td>0.89(1.20)</td>
<td>0.70(1.01)</td>
<td>1.0(1.34)</td>
<td>38.5(0.32)</td>
<td>4.3(0.56)</td>
<td>11.5(0.95)</td>
</tr>
<tr>
<td></td>
<td>1.40(1.80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.05(2.79)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 F.S.</td>
<td>0.78(1.03)</td>
<td>1.8(2.09)</td>
<td>28.2(0.27)</td>
<td>4.9(0.86)</td>
<td>31.7(5.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.80(2.44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.89(1.29)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 F.S.</td>
<td>2.80(3.12)</td>
<td>1.9(2.24)</td>
<td>29.4(0.11)</td>
<td>0.0</td>
<td>47.3(1.45)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.00(2.32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33 F.S.</td>
<td>1.20(1.32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.70(1.75)</td>
<td>1.5(1.53)</td>
<td>27.9(0.47)</td>
<td>0.0</td>
<td>50.4(1.94)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F.S. = Fresh smoked.  
11 F.S. = 11 weeks frozen stored then smoked.  
22 F.S. = 22 weeks frozen stored then smoked.  
33 F.S. = 33 weeks frozen stored then smoked.  

The fresh, 11 weeks frozen and 22 weeks frozen smoked cod fillets were all presented to 10 panellists on three different occasions, while the 33 weeks frozen stored prior to smoking samples were presented once with the values given for those which had been frozen stored in bags and boxes respectively. Standard deviations are shown in parentheses.
Additionally, no significant difference (at 95% level of significance) was found between the cod samples stored at -20 °C in plastic bags and cardboard boxes prior to smoking. All means of the values in the Table 23 were between 1.0 and 2.0 which according to the taste panel sheet (Table, 7) are in the middle of none-slight off-flavour scale.

The lipid hydrolysis which was the major deterioration which occurred in the lipid of cod samples during frozen storage and subsequent smoking (Section 5.3.2.) had no consequences on the flavour acceptability of cod fillets. According to Sikorski et al (1976) lipid hydrolysis by itself has no nutritional significance and although in taste panel sheets the terms rancid and soapy are often used as descriptors, these authors stated that no correlation appears to have been carried out between the development of these flavours and fatty acid production. Lovern (1962) stated that accumulation of free fatty acids in fish oils is undesirable due to the secondary reactions catalysed, such as increased susceptibility to oxidation and the consequent development of off-flavours.

5.3.6. Relation of texture and taste panel results for firmness and juiciness.

Connell (1969) studied the changes in eating quality of frozen stored cod, using the same scale (0-9) for firmness and found that changes in firmness with storage time were observed at high storage temperatures e.g. 0 °C, whilst at
-14 °C a slight increase was observed within the first six weeks (from 2.3 to 4.0) and then the increase was much slower, reaching a value of 4.5 at 50 weeks. However, at storage temperatures of -22 and -29 °C the firmness remained virtually constant. The same author also stated that the texture of cod is dependent upon the ultimate pH of the flesh, cod which has a low ultimate pH being tougher or firmer than cod of high ultimate pH.

Similar results, to those described by the above author, were found in this investigation for the firmness and juiciness of the smoked cod fillets, as shown in Fig. 23.

No significant difference (at 95% level of significance) was found by the 10 panellists in firmness and juiciness of the smoked fish with different previous frozen storage histories.

The firmness and juiciness as well as the instrumental texture measurements depend primarily upon the state of the myofibrillar proteins and the moisture content of the cod samples. Thus, a summary table of the soluble protein, moisture content, texture and firmness of the smoked cod samples is presented in table 24.
Fig. 23: Firmness & Juiciness of smoked, previously frozen Cod (taste panel results).

The values are means of three different presentations of the cod samples to 10 panellists, with mean coefficients of variation: 33 & 36% for firmness and juiciness respectively.
Table 24: % SSP, % moisture content, firmness and (g) texture in smoked cod fillets.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% SSP</th>
<th>% moisture</th>
<th>(g) texture</th>
<th>Firmness</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. S.</td>
<td>11.4 (0.62)</td>
<td>58.3 (1.13)</td>
<td>170 (28.28)</td>
<td>5.2 (1.66)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>12.9 (0.58)</td>
<td>59.6 (0.82)</td>
<td>168 (6.38)</td>
<td>5.1 (1.57)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>13.7 (0.26)</td>
<td>66.5 (0.78)</td>
<td>164 (4.03)</td>
<td>5.3 (1.98)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>15.3 (0.63)</td>
<td>62.1 (1.33)</td>
<td>174 (10.46)</td>
<td>6.1 (1.49)</td>
</tr>
</tbody>
</table>

F. S. = Fresh smoked.
F. 11 W. = Frozen stored 11 weeks then smoked.
F. 22 W. = Frozen stored 22 weeks then smoked.
F. 33 W. = Frozen stored 33 weeks then smoked.

The values for SSP and moisture are means of sixfold determinations. Standard deviations are shown in parentheses.

It can be seen, from the table 24, that no significant overall change in any of these parameters occurred after smoking of fish with various previous frozen storage histories.
It is possible to conclude that the texture of the smoked cod fillets was not influenced by the freezing and frozen storage history of the cod samples.

5.3.7. General discussion.

The colour and the smoked flavour of the smoked cod fillets are presented in Fig. 24 and Table 25.

With reference to Table 25, a significant increase (at 95% level of significance) was observed by the panellists for the surface colour development between the fresh smoked cod and cod frozen 11 weeks prior to smoking, with the first process, and between fresh smoked cod and cod frozen for 11 & 22 weeks prior to smoking, with the second process. However, after the third smoking process the colour development on the surface of all cod fillets (fresh smoked and frozen 11, 22 and 33 weeks prior to smoking) was quite similar and no significant difference (at 95% level) was observed. Moreover, the overall colour development, as illustrated in Fig. 24, was also statistically insignificant.
Fig. 24: Colour & Smoked flavour of smoked, previously frozen Cod (taste panel results).

Scores.

Frozen storage (weeks)

0 11 22 33

0 11 22 33

The values are means of three different presentations of the cod samples to 10 panellists, with mean coefficients of variation: 33 & 35% for colour and smoked flavour respectively.
These different results obtained probably indicate that the major factor affecting colour formation on the surface of the cod fillets was the smoke itself. Thus, it was observed that the final surface colour of the smoked fish was influenced by the position of the fillets in the kiln. For example, the cod fillets layered on the top shelf of the kiln (the fresh samples in the first two processes) developed less brown surface colour than those layered on the middle or on the bottom shelves of the kiln (all other samples in the first two processes and the fresh cod fillets in the third process).

Table 25: Taste panel results for surface colour of the cod fillets.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st process</th>
<th>2nd process</th>
<th>3rd process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh smoked 11 weeks frozen</td>
<td>3.0 (1.67)</td>
<td>3.9 (1.97)</td>
<td>6.4 (1.74)</td>
</tr>
<tr>
<td>then smoked</td>
<td>5.9 (1.01)</td>
<td>6.4 (2.01)</td>
<td>5.1 (1.90)</td>
</tr>
<tr>
<td>22 weeks frozen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>then smoked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33 weeks frozen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>then smoked</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values are means from 10 panellists. Standard deviations are shown in parentheses. The colour which represents each number is shown in Table 7 (taste panel sheet).
The above situation indicates that particles of the smoke, which contain the greater proportion of high molecular weight components, were deposited on the fish under the influence of gravity forming the surface colour of the cod fillets layered on the middle and the bottom shelves (Section 2.1.2.1.).

Furthermore, the overall colour results also show a good relationship with the destruction of reactive lysine (Section 5.3.4.) in the products due to smoking. The higher the destruction of lysine the greater the carbonyl-amino acids reactions and possibly the browner the colour developed on the surface of the product.

The smoked flavour was identified as being between weak and moderate by the 10 panellists, with no significant differences (at 95% level), for the various smoked cod fillets.

Freezing and frozen storage influence the salt penetration rate in the fish muscle (Section 2.2.2.2.). This effect was also observed in this investigation and the results are demonstrated in Fig. 25. From this figure it can also be observed that freezing rather than frozen storage history had the strongest influence on the salt penetration in the cod fillets. Thus, the greatest difference in salt content was obtained between the fresh smoked cod fillets and those frozen stored 11 weeks prior to smoking.
From the same figure it can be additionally observed that a very good relationship exists, between the salt content of the cod fillets and the scores for saltiness given by the panellists.

Using the salt content in the fresh smoked cod fillets as a reference point, it can be observed that the increase was 38, 47 and 67% for the cod samples being frozen stored for 11, 22 and 33 weeks prior to smoking respectively.

All cod samples were assessed as moderately acceptable products as characterized by the 10 panellists, as shown in Table 26. Also in Table 26 are figures of some parameters which may influence the acceptability of the products.
Fig. 25: Salt concentration & Saltiness (taste panel results) of smoked, previously frozen Cod.

The values for salt concentration are means of triplicate determinations, while the values for saltiness are means of three different presentations of the cod samples to 10 panellists, with mean coefficients of variation: 18 & 19% for salt content and saltiness respectively.
### Table 26: Acceptability of the smoked cod fillets in relationship with almost all other parameters analysed.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>F. S.</th>
<th>F. 11 W.</th>
<th>F. 22 W.</th>
<th>F. 33 W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% SSP (g)</td>
<td>11.4 (0.62)</td>
<td>12.9 (0.58)</td>
<td>13.6 (0.26)</td>
<td>15.3 (0.63)</td>
</tr>
<tr>
<td>Texture</td>
<td>170.0 (28.28)</td>
<td>168.0 (6.38)</td>
<td>164.0 (4.03)</td>
<td>174.0 (10.46)</td>
</tr>
<tr>
<td>Firmness</td>
<td>5.2 (1.66)</td>
<td>5.1 (1.57)</td>
<td>5.3 (1.98)</td>
<td>6.1 (1.49)</td>
</tr>
<tr>
<td>Juiciness</td>
<td>4.7 (1.77)</td>
<td>4.7 (1.67)</td>
<td>4.8 (1.72)</td>
<td>5.0 (1.70)</td>
</tr>
<tr>
<td>PV meq0.5/Kg lipid</td>
<td>4.3 (0.56)</td>
<td>4.9 (0.56)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Off - flavours</td>
<td>1.0 (1.34)</td>
<td>1.8 (2.09)</td>
<td>1.9 (2.24)</td>
<td>1.5 (1.53)</td>
</tr>
<tr>
<td>% Salt</td>
<td>6.4 (0.30)</td>
<td>8.8 (0.15)</td>
<td>9.4 (0.37)</td>
<td>10.7 (0.27)</td>
</tr>
<tr>
<td>Saltiness</td>
<td>5.4 (1.46)</td>
<td>6.7 (1.64)</td>
<td>7.6 (0.95)</td>
<td>7.4 (0.94)</td>
</tr>
<tr>
<td>Colour</td>
<td>4.4 (1.79)</td>
<td>5.8 (1.64)</td>
<td>5.9 (1.82)</td>
<td>6.1 (1.83)</td>
</tr>
<tr>
<td>Smoked flavour</td>
<td>4.5 (1.69)</td>
<td>5.8 (1.56)</td>
<td>4.9 (1.80)</td>
<td>5.1 (2.13)</td>
</tr>
<tr>
<td>Acceptability</td>
<td>5.8 (1.78)</td>
<td>5.2 (2.29)</td>
<td>4.3 (2.20)</td>
<td>5.0 (2.12)</td>
</tr>
<tr>
<td>Available lysine (ug / 100g)</td>
<td>7.4 (0.16)</td>
<td>6.6 (0.25)</td>
<td>3.3 (1.40)</td>
<td>0.8 (0.43)</td>
</tr>
<tr>
<td>Thiamine</td>
<td>41.0 (0.01)</td>
<td>42.0 (0.01)</td>
<td>22.0 (.01)</td>
<td>19.0 (0.02)</td>
</tr>
</tbody>
</table>

F. S. = Fresh smoked.
F. 11 W. = Frozen stored 11 weeks then smoked.
F. 22 W. = Frozen stored 22 weeks then smoked.
F. 33 W. = Frozen stored 33 weeks then smoked.
From this summary table it can be concluded that the acceptability of the smoked cod fillets, as expressed by the 10 panellists, was possibly only slightly influenced by the salt content and by the surface colour formed. All other parameters, which could influence the acceptance of the products, appeared unaltered despite the various frozen storage histories prior to smoking. Thus, the acceptability was classified as moderate, for the fresh smoked cod fillets, becoming moderately unacceptable, for the 22 week frozen stored cod samples prior to smoking. However, these slight differences observed in acceptability were statistically insignificant (at 95% level, using t-test).

Therefore, it can be concluded that these cod fillets are moderately acceptable products and their acceptance is independent upon their frozen storage history (at least 33 weeks at -20 °C) prior to smoking.

However, the nutritional effect due to smoking on the cod fillets deserves to be mentioned. A high level of destruction of available lysine was observed which indicates a very low protein quality in these products. A loss of available lysine was identified due to smoking even in the fresh smoked cod fillets and it can be proposed that this is a result of attack by the smoke carbonyl compounds on the cod fillets protein (smoke carbonyl-amino reactions). Lysine, as mentioned before, is the most sensitive (reactive) amino acid in such a carbonyl attack (Section 5.3.4.).
A second nutritional effect observed was the loss of thiamine. This was mainly a result by the frozen storage history of the cod samples rather than of the smoking process (Section 5.3.4.).

However, these products are primarily produced to confer traditional flavours and to be relished as condiments and not for nutritional purposes.
5.4. THE EFFECT OF SMOKING ON PREVIOUSLY FROZEN MACKEREL.

For almost all molecular changes observed during frozen storage of the mackerel samples, the effect of packaging was insignificant (at 95% level), thus, the values from section 5.2. and quoted in this work will be the mean of 3 determinations from the mackerel samples packaged in plastic bags and another 3 determinations from those packaged in cardboard boxes. The only exception to this was the peroxide value of mackerel, which was significantly influenced by the type of packaging but only after 33 weeks frozen storage. Similarly one mean value will be used for the smoked mackerel products.

The results (for % moisture, % protein, % lipid and % salt), shown in Table 27, indicate that, as expected, the moisture content decreases due to the drying part of the smoking process (Section 2.2.2.4.). This decrease is less than that found for the cod fillets, because of the lower moisture content of the raw mackerel samples, and because of the skin presence, which functions as a barrier to any loss (mackerel samples processed gutted, Section 4.2.2.).
Table 27: Proximate analysis, on wet and dried salt free basis, of fresh unprocessed and fresh smoked mackerel.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unprocessed</th>
<th>Smoked</th>
<th>Unprocessed</th>
<th>Smoked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% on wet basis</td>
<td>% on dried salt free basis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>69.8 (0.02)</td>
<td>63.0 (0.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18.6</td>
<td>62.2</td>
<td>22.7</td>
<td>64.2</td>
</tr>
<tr>
<td>Protein</td>
<td>18.3</td>
<td>61.1</td>
<td>22.0</td>
<td>62.2</td>
</tr>
<tr>
<td>Lipid</td>
<td>11.9</td>
<td>39.6</td>
<td>14.2</td>
<td>40.2</td>
</tr>
<tr>
<td>Salt</td>
<td>0.16</td>
<td>1.65</td>
<td>0.22</td>
<td>1.71</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>69.9</td>
<td>63.1</td>
<td>62.2</td>
<td>64.2</td>
</tr>
</tbody>
</table>
5.4.1. Protein and amino acids.

The true protein content was constant during the 33 week frozen storage period of the mackerel samples (Section 5.2.1.) and remained at this value after smoking (Table 28). This is in contrast to the cod fillets as these exhibited a protein loss which was dependent upon the frozen storage history prior to smoking. This stability in true protein content of mackerel samples was probably a result of the skin, which functions as a barrier, and from the different water-lipid-protein proportions in the two fish. Thus, the skin would prevent migration of water soluble proteins into the brine during the salting process.

Table 28: Effect of smoking on true protein of previously frozen mackerel.

<table>
<thead>
<tr>
<th>Sample</th>
<th>True protein Frozen</th>
<th>True protein Smoked</th>
<th>Moisture Frozen</th>
<th>Moisture Smoked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>52.4 (0.55)</td>
<td>54.8 (0.64)</td>
<td>69.8 (0.02)</td>
<td>63.0 (0.70)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>52.2 (1.94)</td>
<td>53.7 (0.61)</td>
<td>67.9 (1.10)</td>
<td>64.2 (0.44)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>54.6 (0.92)</td>
<td>52.7 (0.99)</td>
<td>71.7 (2.84)</td>
<td>60.0 (3.18)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>53.1 (1.12)</td>
<td>51.9 (1.87)</td>
<td>66.6 (1.63)</td>
<td>63.7 (0.84)</td>
</tr>
</tbody>
</table>


The values presented in the Table are means of sixfold determinations (Section 5.4.). Standard deviations are shown in parentheses. Expressed on a dry, salt free basis.
The denaturation of protein in the mackerel samples during frozen storage was much slower than in cod (Section 5.2.1.). However, despite this difference obtained during frozen storage, a similar extensive denaturation occurred due to smoking, as shown in Fig. 20 for cod and 26 for mackerel. Thus in both fish, this denaturation after smoking was independent of the frozen storage history and the extent of protein denaturation prior to smoking. This result may be expected because according to Opstvedt (1989) 90% of the fish protein is denaturated at about 60 to 65 °C, and in this smoking process both samples were exposed at 70 °C for 4 hours (Section 4.2.2.)

Thus, the protein solubilities of all smoked mackerel samples, fresh and frozen 11, 22 and 33 weeks, were from 10 to 14.5% while those of the unprocessed samples were from 84 to 64.2% (Section 5.2.1.). Therefore, the percentage drop in % SSP which occurred due to smoking was 86, 81, 87 and 82 for fresh mackerel and mackerel frozen for 11, 22 and 33 weeks respectively.
Fig. 26: Effect of smoking on protein solubility of previously frozen Mackerel.

The values are means of sixfold determinations, with mean coefficients of variation: 3.3 & 7% for frozen and smoked mackerel samples respectively.
The situation with the free amino acid content, as shown in Fig. 27, appeared quite interesting.

A steady increase in free amino acid content had been noticed during the frozen storage history of the mackerel samples (Section 5.2.1.) as also shown in Fig. 27.

The increase in free amino acid content was continued during the smoking processes of the fresh mackerel samples and those frozen stored for 11 weeks prior to smoking (Fig. 27), indicating that while a further protein hydrolysis occurred due to smoking there was possibly very little, if any, loss of free amino acids in carbonyl-amino acid reactions. Moreover, this indicated that the lipids in these two mackerel samples (fresh and frozen 11 weeks) was in a very good condition, thus, interactions between carbonyls from lipids and free amino acids had not occurred.

However, the situation appeared entirely different in the two other samples which contained higher levels of free amino acids after freezing. Thus in these fish, which had been frozen stored for 22 and 33 weeks prior to smoking, the free amino acid content decreased by 23 \(\text{~}~37\%\) respectively due to smoking. This indicates that carbonyl-amino reactions may have occurred in these samples and were quite extensive, because the reactions may have involved the free amino acids from the frozen mackerel samples and the free amino acids being produced by the further protein hydrolysis due to smoking.
Fig. 27: Effect of smoking on free amino acids of previously frozen mackerel (on dried salt free basis).

The values are means of sixfold determinations, with mean coefficients of variation: 31 & 33% for frozen and smoked mackerel samples respectively.
It may be assumed that the required carbonyls, for reaction with the free amino acids were mainly produced by the oxidized lipids, because the skin functioned as a barrier and did not allow the attack of the smoke carbonyls.

The decrease in free amino acid content due to smoking observed in the fresh cod fillets is more likely to be due to the attack of the smoke carbonyl compounds (Section 5.3.1.) as those samples were skinned.

5.4.2. Lipid

The distribution of lipid in a fish such as mackerel is very important and the analysis of lipid in such a fish requires much attention.

The total lipid content in these mackerel samples is presented in Table 29 on both a wet weight and a dry salt free basis, to enable a clear interpretation of any changes. The high values of the standard deviations indicate the considerable variation in distribution of the lipids in the body of a fatty fish, e.g. mackerel contains 40% of the total lipid as subdermal fat, therefore, large sample differences are quite possible.

In general, the lipid content of the mackerel samples remained constant during frozen storage (Section 5.2.2.), however, the lipid content of the smoked mackerel samples seemed to be influenced by their frozen storage history.
Table 29: Lipid content in the mackerel samples.

<table>
<thead>
<tr>
<th>% Lipid</th>
<th>Fresh</th>
<th>F. 11 W.</th>
<th>F. 22 W.</th>
<th>F. 33 W.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On wet weight basis.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unprocessed</td>
<td>11.1 (1.56)</td>
<td>12.8 (0.63)</td>
<td>10.7 (0.85)</td>
</tr>
<tr>
<td></td>
<td>Smoked</td>
<td>14.5 (1.29)</td>
<td>11.8 (2.99)</td>
<td>11.8 (2.82)</td>
</tr>
<tr>
<td></td>
<td>On dried salt free basis.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unprocessed</td>
<td>37.0 (5.22)</td>
<td>40.1 (4.65)</td>
<td>38.0 (2.63)</td>
</tr>
<tr>
<td></td>
<td>Smoked</td>
<td>41.0 (3.66)</td>
<td>34.2 (8.21)</td>
<td>30.9 (6.77)</td>
</tr>
<tr>
<td>% Moisture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unprocessed</td>
<td>69.8 (0.02)</td>
<td>67.9 (1.10)</td>
<td>71.7 (2.84)</td>
</tr>
<tr>
<td></td>
<td>Smoked</td>
<td>63.0 (0.70)</td>
<td>64.2 (0.44)</td>
<td>60.0 (3.18)</td>
</tr>
</tbody>
</table>

F. 11 W. = Frozen 11 weeks.
F. 22 W. = Frozen 22 weeks.
F. 33 W. = Frozen 33 weeks.

The values presented in the Table are means of sixfold determinations. Standard deviations are shown in parentheses. Expressed on a wet weight and on a dry salt free basis.
Thus, a small significant fat loss (at 95% level) upon smoking, was observed in the mackerel samples which had been frozen stored for 22 and 33 weeks prior to smoking, while no loss was noticed in the fresh smoked and a slight insignificant loss occurred in mackerel samples frozen stored for 11 weeks prior to smoking. This type of loss may be expected in fatty fish, because of the depot fat present, in comparison to cod which contains mainly structural lipids.

Although the peroxide values of Fig. 28 are means of the fish packed in bags and boxes, the peroxide values were actually 56.9 and 108 meqO₂/ Kg lipid, in the mackerel samples being frozen stored for 33 weeks in plastic bags and cardboard boxes respectively (Section 5.2.2.). However, the peroxide value was 9.5 meqO₂/ Kg lipid for both samples after being smoked. The same phenomenon, but at a lesser extent, was also observed for the mackerel samples frozen stored for 22 weeks. This breakdown of hydroperoxides possibly gave the reactant carbonyls which attacked the free amino acids in these two smoked mackerel samples (the 22 and 33 week frozen stored prior to smoking) (Section 5.4.1.). This breakdown of hydroperoxides may also induce off-flavours in these products.

In the fresh and 11 weeks frozen stored samples the situation was quite different, a slight increase in peroxide value was observed during smoking while the peroxide value in both unprocessed mackerel samples was quite low.
Fig. 28: Effect of smoking on peroxide value of previously frozen mackerel.

The values are means of sixfold determinations, with mean coefficients of variation: 9.8 & 14% for frozen and smoked mackerel samples respectively.
Woolfe (1975) found that when whole fresh fish was smoked the skin acted as a barrier to penetration into the flesh by smoke constituents and as a result the oxidation increased due to smoking.

Bhuiyan et al (1986) while studying the stability of lipid during smoking of fresh Atlantic mackerel fillets, found an increase in peroxide value from 1.30 to 8.96 meqO$_2$/Kg lipid during the process, which was attributed to increased conversion of some unsaturated fatty acids on the surface of the mackerel fillets into peroxides.

A similar but lower rate of increase in hydroperoxides, from 1.72 to 3.50 meqO$_2$/Kg lipid, was observed in fresh split smoked mackerel samples in this investigation. This probably indicates that the skin is not only a barrier of the flesh to smoke constituents but it also functions as a barrier to penetration of the flesh by some factors (oxygen, light e.t.c.) which accelerate the lipid oxidation.

Thus, as expected, the oxidation in mackerel samples appeared quite extensive because of the depot fat and the high percentage in their lipid of triglycerides, while in cod samples oxidation was minor (Section 5.3.2.).

In contrast to oxidation, hydrolysis of the lipid in the mackerel samples, as shown in Fig. 29, occurred to a much lesser extent than in cod samples (Section 5.3.2.) both during frozen storage and due to the smoking process.
Fig. 29: Effect of smoking on free fatty acids of previously frozen Mackerel.

The values are means of sixfold determinations, with mean coefficients of variation: 10 & 14.5 for frozen and smoked mackerel samples respectively.
This again may be a result of the different types of lipid of these two fish. According to Hardy (1979) triglyceride containing tissues of many species, are subject to hydrolysis of these lipids but this appears to be less common than phospholipids. Thus, the hydrolysis which occurred in mackerel, which contains mainly triglycerides in its lipid, was low and the only significant increase in % FFA (at 95% level) which was observed was due to the first 11 weeks frozen storage. The small increase in free fatty acid content noticed during the smoking process was insignificant.

Similar results were obtained by Bhuiyan et al (1986) who studied the stability of lipid during hot-smoking of fresh Atlantic mackerel. They found that the free fatty acids could be barely detected in the smoked products.

The fatty acid profiles of smoked mackerel samples are presented in Table 30. Table 31 demonstrates the total saturated, monounsaturated and polyunsaturated fatty acids, present in the lipid of all samples in both conditions (frozen and smoked), for ease of comparison.
Table 30: Fatty acid profiles of smoked mackerel samples.

<table>
<thead>
<tr>
<th>Fatty acids %</th>
<th>F. S.</th>
<th>F. 11 W.</th>
<th>F. 22 W.</th>
<th>F. 33 W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>6.3 (0.02)</td>
<td>6.5 (0.06)</td>
<td>6.1 (0.04)</td>
<td>7.0 (0.34)</td>
</tr>
<tr>
<td>16:0</td>
<td>17.8 (0.03)</td>
<td>16.7 (0.10)</td>
<td>18.1 (0.32)</td>
<td>18.3 (0.30)</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>5.9 (0.02)</td>
<td>5.6 (0.08)</td>
<td>6.8 (0.04)</td>
<td>8.5 (1.14)</td>
</tr>
<tr>
<td>18:0</td>
<td>5.9 (0.02)</td>
<td>4.8 (0.04)</td>
<td>5.9 (0.03)</td>
<td>3.5 (0.13)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>19.2 (0.01)</td>
<td>22.1 (0.09)</td>
<td>19.9 (0.26)</td>
<td>18.1 (0.25)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>1.6 (0.01)</td>
<td>2.1 (0.02)</td>
<td>1.8 (0.06)</td>
<td>3.8 (0.69)</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>1.5 (0.01)</td>
<td>1.8 (0.02)</td>
<td>1.5 (0.03)</td>
<td>1.9 (0.13)</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>6.9 (0.03)</td>
<td>8.2 (0.03)</td>
<td>9.1 (0.07)</td>
<td>9.7 (0.13)</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>7.7 (0.01)</td>
<td>9.1 (0.04)</td>
<td>7.5 (0.02)</td>
<td>4.7 (0.90)</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>10.2 (0.06)</td>
<td>6.9 (0.03)</td>
<td>6.8 (0.07)</td>
<td>11.5 (0.05)</td>
</tr>
<tr>
<td>22:1n-11</td>
<td>2.0 (0.09)</td>
<td>2.0 (0.03)</td>
<td>2.4 (0.02)</td>
<td>2.5 (0.32)</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.5 (0.01)</td>
<td>1.4 (0.05)</td>
<td>1.2 (0.01)</td>
<td>0.0</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>12.8 (0.06)</td>
<td>12.1 (0.21)</td>
<td>11.3 (0.21)</td>
<td>10.4 (1.69)</td>
</tr>
</tbody>
</table>

The values are means of triplicate determinations of the mackerel samples stored at -20 °C in plastic bags prior to smoking. Standard deviations are shown in parentheses.
Table 31: Fatty acid composition as obtained in both conditions (frozen & smoked) from mackerel samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Saturated</th>
<th>Monoenic</th>
<th>Polyenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>28.3 (0.21)</td>
<td>38.5 (0.88)</td>
<td>33.1 (0.17)</td>
</tr>
<tr>
<td>Fresh smoked</td>
<td>30.0 (0.08)</td>
<td>34.0 (0.12)</td>
<td>35.3 (0.07)</td>
</tr>
<tr>
<td>Frozen 11 weeks</td>
<td>26.7 (0.06)</td>
<td>38.3 (0.06)</td>
<td>34.1 (0.04)</td>
</tr>
<tr>
<td>Frozen 11 weeks then smoked</td>
<td>28.0 (0.16)</td>
<td>37.9 (0.11)</td>
<td>33.4 (0.10)</td>
</tr>
<tr>
<td>Frozen 22 weeks</td>
<td>29.6 (0.05)</td>
<td>37.0 (0.06)</td>
<td>33.7 (0.02)</td>
</tr>
<tr>
<td>Frozen 22 weeks then smoked</td>
<td>30.1 (0.22)</td>
<td>38.2 (0.25)</td>
<td>30.1 (0.06)</td>
</tr>
<tr>
<td>Frozen 33 weeks</td>
<td>29.3 (0.80)</td>
<td>40.5 (1.31)</td>
<td>30.2 (0.74)</td>
</tr>
<tr>
<td>Frozen 33 weeks then smoked</td>
<td>28.8 (0.46)</td>
<td>38.8 (1.64)</td>
<td>32.3 (0.81)</td>
</tr>
</tbody>
</table>

The values presented in the Table are means of triplicate determinations of the mackerel samples being stored at -20°C in plastic bags. Standard deviations are shown in parentheses.

In general, from Table 31 it can be observed that while there were some slight changes in the fatty acid profiles during frozen storage of the mackerel samples, i.e. the decrease of polyunsaturated, accompanied by an increase of monounsaturated and a slight increase of saturated fatty acids, during the smoking process the fatty acid profiles...
appeared virtually constant. A comparison between the fresh unprocessed mackerel samples with almost all smoked mackerel samples, even those which had a 33 week frozen history prior to smoking, showed that the fatty acid profiles remained unaltered due to smoking. Comparing also Tables 16 (Section 5.2.2.) and 30 the same conclusion can be extracted, i.e. that no changes occurred in the fatty acid profiles due to smoking, despite the frozen storage history and the slight changes which occurred in the frozen mackerel samples.

Bhuiyan et al (1986) investigated the stability of polyunsaturated fatty acids during hot smoking of Atlantic mackerel, using fresh fish in fillet form, and found that the overall fatty acid composition remained virtually unchanged after the smoking process.

5.4.3. Texture.

The texture measurements, the associated difficulties of which have been discussed in Section 2.3.4. and 5.3.3., were performed using the Steven's LFRA Texture Analyser, which was suggested as being the most suitable for such difficult products such as cooked fish (Section 5.3.3.).

The results of the penetration force required each time for all fresh, frozen stored and smoked mackerel samples are demonstrated in Fig. 30.
Fig. 30: Effect of smoking on texture of mackerel using the Steven's Texture Analyser.

The values are means of 15-20 measurements, with mean coefficients of variation: 9% & 15% for frozen and smoked mackerel samples respectively.
It can be observed from the figure that the texture of the frozen mackerel samples as well as of the smoked ones remained virtually constant, and a slight increase which occurred in the smoked mackerel samples with increasing prior frozen storage was statistically insignificant (at 95% level). These results may be as expected, because the major factor which influences the fish texture is the protein and this is extensively denaturated in all mackerel samples by the heating process (Section 5.4.1.).

The changes in texture of the mackerel samples upon smoking were quite similar to those of the cod samples, despite the increase in firmness of the cod samples during frozen storage (Section 5.3.3.). The only difference between the two types of smoked samples was that the mackerel smoked samples were softer than those of cod. This was probably because the tenderness of muscles should be greater in fish containing more lipid, while in lean species the tenderness increases with the water content. Thus, the mackerel samples contained relatively more lipid after smoking while the cod smoked fillets had lost water due to the smoking process.

5.4.4. Available lysine and thiamine.


The results for FDNB available lysine for frozen and smoked mackerel are presented in Fig. 31.
Fig. 31: Effect of smoking on available lysine of previously frozen Mackerel (on dried salt free basis).

The values are means of triplicate determinations, with mean coefficients of variation: 2.2 & 13% for frozen and smoked mackerel samples respectively.
It may be deduced from Fig. 31 and Fig. 27 (free amino acid changes), that the carbonyl-amino reactions were not extensive in the mackerel flesh, particularly for the fresh smoked and 11 weeks frozen stored prior to smoking mackerel samples. Thus, no significant (at 95% level) loss of lysine occurred in these two smoked mackerel samples, while the free amino acid content increased due to smoking (Section 5.4.1.). This indicates that proteins and free amino acids had not been attacked by the smoke carbonyls during smoking. This was probably a result of the skin which acted as a barrier to the penetration of smoke constituents into the flesh of the mackerel samples during smoking.

This is in contrast to the cod fillets where the loss of lysine in these two samples (fresh smoked and 11 weeks frozen stored prior to smoking) was quite extensive presumably caused by the extensive smoke carbonyl attack on the surface of the fillets and in the outermost 5-10 mm of the fish.

However, the loss of available lysine and the destruction of free amino acids upon smoking was quite extensive, at 50 and 63% for available lysine, in the 22 and 33 week frozen stored prior to smoking mackerel samples respectively. It may be concluded that this was mainly a result of interactions between lipid carbonyls and proteins (loss of available lysine) and free amino acids (Section 5.4.1.). This conclusion is supported by the high peroxide value obtained in these two thawed mackerel samples and the destruction of
these peroxides which occurred during smoking (Section 5.4.2).

Therefore, it could be concluded that the loss of available lysine in the mackerel samples was mainly a result of interactions between lipid oxidation products and proteins, while the destruction of available lysine in cod fillets was a result of the extensive attack by smoke carbonyl compounds on proteins.

b. Thiamine.

In contrast to cod, in the mackerel samples no loss of thiamine during frozen storage or due to thawing (Section 5.2.4.) was observed, as shown in Fig. 32.

Thus, the thiamine content in mackerel appeared to be influenced by the heating process, as expected for both samples (cod & mackerel), because the vitamin B is reported to be sensitive.

However, it can be observed from the Fig. 32 that thiamine was quite stable during smoking (heating) in the fresh and 11 weeks frozen stored mackerel samples, thus, the slight decrease noticed was insignificant (at 95% level). Therefore, significant losses of thiamine content, at 29 and 32%, due to heating appeared only in the 22 and 33 week frozen stored mackerel samples respectively.
Fig. 32: Effect of smoking on thiamine of previously frozen Mackerel (on dried salt free basis).

The values are means of triplicate determinations, with mean coefficients of variation: 10.7 & 6% for frozen and smoked mackerel samples respectively.
Stability of thiamine due to heating (frying) in fresh mackerel samples was also found by Paul & Southgate (1979) (Section 2.2.3., Table 5).

It could be concluded that the overall thiamine loss in mackerel samples was 44% mainly due to the smoking (heating) process, while the overall loss of thiamine content in cod fillets was 50% mainly due to the frozen storage history and subsequent thawing.

5.4.5. Relation of lipid quality and taste panel results for off-flavours.

Lipid oxidation, which is associated most with the production of off-flavours, was quite extensive in the mackerel samples, particularly in those which had been frozen stored for 22 and 33 weeks (Section 5.2.2.), to the contrary of cod samples where very little lipid oxidation occurred (Section 5.3.2.).

Thus, the peroxide values in the thawed mackerel samples with 22 and 33 weeks frozen storage history were very high and the peroxides of these two samples were destroyed due to the smoking process. This destruction of peroxides indicated that some secondary products (non-volatile and volatile carbonyl compounds) had been produced, which could be responsible for off-flavours in processed products. However, as shown in Table 32, no off-flavour was identified by the 10 panellists, with all values presented in the table 32 being
Table 32: Taste panel off-flavour results for smoked mackerel with PV changes due to smoking.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Off-flavour</th>
<th>Overall off-flavour</th>
<th>PV meqO₂/kg lipid in thawed</th>
<th>PV meqO₂/kg lipid in smoked</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. S.</td>
<td>1.60 (1.35)</td>
<td>1.23 (1.15)</td>
<td>1.72 (0.02)</td>
<td>3.50 (0.23)</td>
</tr>
<tr>
<td></td>
<td>1.80 (2.13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>1.30 (1.42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00 (1.09)</td>
<td></td>
<td>10.40 (0.98)</td>
<td>10.80 (0.98)</td>
</tr>
<tr>
<td></td>
<td>2.40 (2.42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>1.00 (1.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.70 (1.18)</td>
<td></td>
<td>24.60 (6.90)</td>
<td>4.62 (1.49)</td>
</tr>
<tr>
<td></td>
<td>2.50 (3.11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. 33 W.*</td>
<td>3.20 (2.52)</td>
<td></td>
<td>57.00 (0.94)</td>
<td>9.43 (0.93)</td>
</tr>
<tr>
<td>#</td>
<td>2.20 (2.36)</td>
<td>2.70 (2.44)</td>
<td>108.00 (1.64)</td>
<td>9.66 (0.88)</td>
</tr>
</tbody>
</table>

F. S. = Fresh smoked.
F. 11 W. = Frozen 11 weeks then smoked.
F. 22 W. = Frozen 22 weeks then smoked.
F. 33 W. = Frozen 33 weeks then smoked.

The fresh, 11 weeks frozen and 22 weeks frozen smoked mackerel samples were all presented to 10 panellists on three different occasions. Standard deviations are shown in parentheses. The values of off-flavour and PV, as obtained by the plastic bags and cardboard boxes stored samples, are presented in the Table only for the 33 weeks frozen stored samples because of their highly significant difference in PV.

* = Frozen stored for 33 weeks in plastic bags.
# = Frozen stored for 33 weeks in cardboard boxes.
between none and slight off-flavour, from the off-flavour scale in the taste panel sheet (Table, 7). Some differences, which may be noticed, were insignificant (at 95% level) and they were given by a minority of the panellists (2-4 panellists).

It can be concluded that the panellists were not able to detect any rancid flavours in these smoked mackerel samples.

5.4.6. Relation of texture and taste panel results for firmness and juiciness.

The results for firmness and juiciness obtained by the 10 panellists for the smoked mackerel samples, as shown in Fig. 33, were quite similar to those obtained for cod. These results indicate quite acceptable smoked products with respect to their texture. The only difference observed between the smoked mackerel samples and smoked cod samples was that the mackerel samples was softer than the cod ones and this was identified by the 10 panellists as well as by the Steven's LFRA Texture Analyser (Section 5.4.3.).

Thus, while the firmness for the smoked cod fillets was between 5.5 and 6.1 (a little higher than moderate), the firmness for the smoked mackerel samples was between 3.4 and 4.9 (a little lower than moderate). Additionally while the juiciness for smoked cod samples was from 4.7 to 5.0 (moderate), the juiciness for the smoked mackerel samples was from 5.2 to 5.5 (a little higher than moderate).
Fig. 33: Firmness & Juiciness of smoked, previously frozen Mackerel (taste panel results).

The values are means of three different presentations of the smoked mackerel samples to 10 panellists, with mean coefficients of variation: 50 & 31% for firmness and juiciness respectively.
It can be concluded that both smoked samples (mackerel & cod) had a moderate firmness and juiciness very acceptable by the panellists and some of them expressed a liking for the texture of both products during the trials.

No significant difference (at 95% level) was observed for the 10 panellist results for the smoked mackerel samples, either for firmness or for juiciness, with the different previous frozen storage histories. This was also apparent from the Steven's LFRA Texture Analyser results (Section 5.4.3.), thus, indicating a very good relationship between instrumental and sensory results. This, also indicates that the texture of the smoked mackerel products were independent of the freezing and frozen storage history of the samples prior to smoking.

The firmness and juiciness as well as the instrumental texture measurements depend primarily upon the state of the myofibrillar proteins and the lipid content of the mackerel samples. A summary table of the soluble protein, lipid content and texture of the smoked mackerel samples is quite helpful.
Table 33: % SSP, % lipid content and (g) texture in smoked mackerel.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% SSP</th>
<th>% lipid</th>
<th>(g) texture</th>
<th>firmness</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. S.</td>
<td>12.0  (0.82)</td>
<td>41.0 (3.66)</td>
<td>101 (23.68)</td>
<td>3.8 (2.14)</td>
</tr>
<tr>
<td>F. 11 W.</td>
<td>14.4 (1.04)</td>
<td>34.2 (8.21)</td>
<td>112 (18.62)</td>
<td>4.1 (1.72)</td>
</tr>
<tr>
<td>F. 22 W.</td>
<td>9.4 (0.64)</td>
<td>30.9 (6.77)</td>
<td>120 (11.84)</td>
<td>4.9 (1.74)</td>
</tr>
<tr>
<td>F. 33 W.</td>
<td>13.0 (0.94)</td>
<td>31.3 (3.85)</td>
<td>113 (12.65)</td>
<td>3.4 (2.42)</td>
</tr>
</tbody>
</table>

F. S. = Fresh smoked
F. 11 W. = Frozen stored 11 weeks then smoked
F. 22 W. = Frozen stored 22 weeks then smoked
F. 33 W. = Frozen stored 33 weeks then smoked

The values for SSP and lipid are means of sixfold determinations and the texture are means of 15-20 determinations. Standard deviations are shown in parentheses.

5.4.7. Histamine.

The results for histamine content in thawed and smoked mackerel samples are presented in Fig. 34 indicating that histamine can be formed in both conditions to a less or a greater extent, and its formation depends upon the existence of the factors which catabolise histidine.
Fig. 34: Effect of smoking of previously frozen mackerel on histamine formation (on dried salt free basis).

The values are means of triplicate determinations, with mean coefficients of variation: 2.3 & 3.2% for frozen and smoked mackerel samples respectively.
From Fig. 34 it can be observed that there was a significant increase (at 95% level) in histamine formation in fresh, frozen 11 and frozen 33 weeks mackerel samples due to the smoking process. These increases were very similar and no significant differences found between them, indicating that histamine formation was independent of the frozen storage histories of the mackerel samples prior to smoking.

The mackerel samples which had been frozen stored for 22 weeks prior to smoking showed a different result, that histamine formation was not influenced by the smoking process.

However, despite the large increase of histamine due to smoking in the mackerel samples which had been frozen stored for 33 weeks prior to smoking, the level 9.4mg / 100g found would not expected to cause symptoms of scombrotoxin poisoning.

Levels above 50mg / 100g and 100mg / 100g have been reported and Table 6 (Section 2.2.4.) also shows some high histamine levels found in cured fish from south East Asia. In Britain during the period 1979 to 1980, 79 incidents of scombrotoxin poisoning were recorded. Of these, 52 were associated with smoked mackerel. Histamine levels were determined on samples from 63 of the 79 incidents. Levels of < 50mg / 100g of fish were found in 25 incidents, between 50 and 100mg / 100g in 8 and > 100mg / 100g in 30 (Al-Weng, 1988).
5.4.8. General discussion.

Table 34: Acceptability of the smoked mackerel samples in relationship to almost all other parameters analysed.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fresh Smoked</th>
<th>Frozen 11W. then smoked</th>
<th>Frozen 22W. then smoked</th>
<th>Frozen 33W. then smoked</th>
</tr>
</thead>
<tbody>
<tr>
<td>% SSP</td>
<td>12.0 (0.82)</td>
<td>14.4 (1.04)</td>
<td>9.4 (0.64)</td>
<td>13.0 (0.94)</td>
</tr>
<tr>
<td>(g) texture</td>
<td>101.0 (23.68)</td>
<td>112.0 (18.62)</td>
<td>120.0 (11.84)</td>
<td>113.0 (12.66)</td>
</tr>
<tr>
<td>Firmness</td>
<td>3.8 (2.14)</td>
<td>4.1 (1.72)</td>
<td>4.9 (1.74)</td>
<td>3.4 (2.42)</td>
</tr>
<tr>
<td>Juiciness</td>
<td>5.5 (1.77)</td>
<td>5.2 (1.59)</td>
<td>5.3 (1.47)</td>
<td>5.5 (1.92)</td>
</tr>
<tr>
<td>PV meq O₂/kg</td>
<td>3.5 (0.23)</td>
<td>10.8 (0.98)</td>
<td>4.6 (1.49)</td>
<td>9.4 (0.93)</td>
</tr>
<tr>
<td>lipid</td>
<td>1.2 (1.15)</td>
<td>1.6 (1.64)</td>
<td>1.4 (1.80)</td>
<td>2.7 (2.44)</td>
</tr>
<tr>
<td>Off-flavours</td>
<td>1.6 (0.21)</td>
<td>1.7 (0.18)</td>
<td>1.9 (0.22)</td>
<td>1.8 (0.49)</td>
</tr>
<tr>
<td>% Salt</td>
<td>3.9 (1.67)</td>
<td>3.6 (1.87)</td>
<td>3.9 (1.97)</td>
<td>3.8 (1.89)</td>
</tr>
<tr>
<td>Saltiness</td>
<td>5.6 (1.35)</td>
<td>5.4 (1.33)</td>
<td>5.7 (1.58)</td>
<td>5.1 (1.72)</td>
</tr>
<tr>
<td>Colour</td>
<td>5.9 (1.67)</td>
<td>5.5 (1.47)</td>
<td>5.9 (1.87)</td>
<td>5.2 (2.23)</td>
</tr>
<tr>
<td>Available</td>
<td>lysine</td>
<td>5.7 (0.25)</td>
<td>5.7 (0.18)</td>
<td>2.3 (0.26)</td>
</tr>
<tr>
<td></td>
<td>Thiamine</td>
<td>99.0 (0.03)</td>
<td>95.0 (0.02)</td>
<td>84.0 (0.04)</td>
</tr>
</tbody>
</table>

From the summary Table 34 it can be observed that the acceptability of the smoked mackerel samples, as expressed by the 10 panellists, was virtually constant (moderately
acceptable) for all samples despite their different previous frozen storage history. It can be also noticed that all parameters which may be expected to influence the acceptability of the samples were also very similar, i.e. they had not been influenced by freezing and the frozen storage history of the samples. Some very slight differences found were insignificant (at 95% level).

Therefore, it can be concluded that split smoked mackerel samples are moderately acceptable products, and for a minority of the panellists (2-4) extremely acceptable, and their acceptance is independent upon their frozen storage history (at least 8 months) prior to smoking. A similar conclusion had been also stated for the smoked cod fillets (Section 5.3.7.).

However, some quality effects should be noticed for the smoked mackerel samples. The acceptance observed suggests that such a smoking process is more suitable for a fish such as mackerel, which allow the presence of skin during smoking. Thus, the first two smoked mackerel products (fresh and 11 weeks frozen), in addition to their general acceptance, were high quality products and no significant quality losses were observed due to smoking in those two samples. Moreover, their lipid quality was quite high and no extensive oxidation was detected (Section 5.4.2.).

However, the other two smoked mackerel samples, which had been frozen stored for 22 and 33 weeks prior to smoking,
showed a similar loss in quality to the smoked cod fillets. A considered loss in available lysine, which was mainly a result from oxidized lipid and protein interactions, as well as in thiamine due to the smoking process was observed. Moreover, the oxidized lipids in the smoked mackerel samples possibly induced rancid flavours which, however, were not identified by the panellists because of the characteristic smoked flavour.

Therefore, the 22 and 33 weeks frozen stored mackerel samples were lower quality products and the frozen storage history prior to smoking influenced the extent of quality changes, as also observed for all cod fillets. These quality changes, however, were not detected by the panellists and as mentioned before (Section 5.3.7.) such products are not usually consumed for their high nutritional value but for their traditional flavours.

The overall score, expressed by the 10 panellists for the colour and the smoked flavour, was similar for all smoked mackerel samples and they are both presented in Fig. 35.

The formation of the colour upon smoking is believed to originate from an uptake of coloured smoke constituents, oxidation and polymerization of smoke compounds (e.g. phenol, aldehydes) and reaction of smoke compound (carbonyls) with proteins and amino acids. The smoked products are expected to possess an attractive surface colouring (Section 2.2.2.3.).
Fig. 35: Colour & Smoked flavour of smoked, previously frozen mackerel.

The values are means of three different presentations to 10 panellists, with mean coefficients of variation: 27 & 44% for colour and smoked flavour respectively.
In the case of the smoked mackerel samples the attractive surface colouring was obtained on the skin of the products and on its splitted belly. The skin functioned as a barrier to the penetration of coloured smoke constituents and other smoke compounds in the flesh of the fish.

Therefore, the colour of the smoked mackerel flesh was very similar for all products, as also identified by the panelists.

The characteristic flavour of the smoked mackerel samples, which possibly covered any other flavour which may develop, was identified by the 10 panellists as virtually the same in all samples. The phenolic smoke components acted without being inhibited by the skin protection.

Fig. 36 demonstrates that the salt penetration in the mackerel samples was much slower than in cod fillets and this resulted from the effective skin protection. Moreover, it was not influenced by freezing and the frozen storage history of the mackerel samples, and the slight increase observed was insignificant (at 95% level). A very good relationship was shown between the salt penetration of the smoked mackerel samples and the saltiness.
Fig. 36: Salt concentration & Saltiness (taste panel results) of smoked, previously frozen Mackerel.

The values for salt concentration are means of triplicate determinations, while the values for saltiness are means of three different presentations of the smoked mackerel samples to 10 panellists, with mean coefficients of variation: 9 & 50% for salt content and saltiness respectively.
5.5. OVERALL DISCUSSION.

Both smoked products, cod and mackerel, appeared quite attractive with a characteristic traditional smoked flavour and all of them were expressed, by the 10 panellists as moderately acceptable products (Section 5.3.7. and 5.4.8.). A slight preference which was expressed by some panellists for the smoked mackerel samples was mainly due to the strong saltiness of the smoked cod fillets.

This acceptance of the smoked products indicates that the process chosen in this investigation (Section 4.2.2.), with the exception of the brining time of the cod fillets, was probably suitable for both fish.

It can be also concluded that acceptability of both smoked products was not influenced by their frozen storage history prior to smoking even for those samples (cod and mackerel) with 33 weeks frozen storage history prior to smoking. Thus, the extensive decrease in protein solubility (from 72 to 29%) and the increase in firmness (from 32 to 74g) during frozen storage of the cod samples, and the slight decrease in soluble protein (from 84 to 64%) which was accompanied by a virtually constant firmness during frozen storage of the mackerel samples gave similar changes after the smoking process.

The rapid increase in % FFA (from 0.5 to 49% in lipid) in the cod samples and of PV (from 1.7 to 82meq / Kg lipid) in
the mackerel samples, both during frozen storage, had no effect on the eating quality of the smoked products.

The degree of browning of the smoked cod fillets was found to depend upon the position of the fillet in the kiln, but all fish were acceptable to the panellists.

The final level of histamine found in all smoked mackerel samples would not be expected to cause symptoms of scombrotxin poisoning (Section 5.4.7.).

Therefore, it is possible to produce consumer acceptable smoked cod and mackerel products from fish which have been previously frozen stored for at least 8 months.

Notwithstanding that such products are primarily consumed for their traditional and desirable tastes and flavours, some nutritional effects found in this investigation deserve to be mentioned. Thus, the two fish samples, cod and mackerel, used in this investigation, appeared to be affected nutritionally in different ways and to different extents.

The loss of available lysine, and therefore the loss of protein quality, in the cod fillets during smoking occurred at a considerable rate even for the fresh samples, indicating the extensive smoke carbonyl attack on the amino groups of protein. This loss of available lysine increased with increased time of frozen storage prior to smoking, and became almost complete for the smoked cod fillets which had been
frozen stored for 33 weeks prior to smoking (Section 5.3.4.). In comparison, for the mackerel the available lysine remained unaltered in the fresh smoked and in the frozen stored for 11 weeks and then smoked samples. This indicates that no carbonyl-amino reactions of any type occurred in those two mackerel samples and their protein quality was quite high. This was facilitated by the preventative action of the skin to the penetration of smoke constituents in the flesh of the mackerel samples during smoking. A loss in available lysine, however, appeared in the 22 and 33 weeks frozen stored mackerel samples which were then smoked and this was primarily a result of interactions of oxidized lipid carbonyls and amino groups of protein (Section 5.4.4.).

Very little lipid oxidation occurred in the cod samples, but the progress of lipid oxidation in the mackerel samples was quite different. Thus, in the fresh and frozen stored 11 weeks mackerel samples the peroxides were at low levels, before and after smoking, indicating high quality lipid in these two smoked samples. However, in the 22 and 33 weeks frozen stored mackerel samples the oxidation was quite extensive. The secondary carbonyl products in these two smoked samples may have interacted with amino protein groups and accounting for the consequent loss in available lysine which was observed. These lipid carbonyls produced also indicated the possibility of rancid flavours in those two smoked mackerel products (Section 5.4.2.), however, these were not detected by the panellists.
On the contrary, hydrolysis was quite extensive in the cod fillets (unprocessed and smoked) (Section 5.3.2.) while very little production of FFA occurred in the mackerel samples (Section 5.4.2.).

The thiamine appeared quite stable to heating for both species of fish in the fresh and 11 weeks frozen stored and then smoked products. The same stability for fresh processed cod and mackerel products was found by Paul & Southgate (1979) (Section 2.2.3., Table, 5). However, despite a great loss of thiamine observed in the 22 weeks thawed cod samples due to the long frozen storage history and thawing process (leaching), vitamin B1 in cod did not appear to be influenced by the smoking process (Section 5.3.4.). Conversely the loss of thiamine in mackerel samples, which was observed in the 22 and 33 weeks frozen stored samples upon smoking, was a result of the heating effect, and no significant loss was identified due to the long frozen storage history and the thawing processes of the smoked mackerel samples (Section 5.4.4.).

However, despite these molecular changes, which had an effect on the quality of the products, as mentioned before, they were of similar acceptability and suitable to be consumed as traditional smoked fish products.

Traditional processed smoked fish, notwithstanding recent developments (e.g. liquid smoke) is still desired by many people as an attractive condiment. The new developments may
make possible a more hygienic product but without the desirable characteristics of a traditional smoked product such as flavour and colour, or at least to a lesser extent.
6. CONCLUSIONS.

1. The general acceptance of both hot-smoked fish (cod and mackerel) was not influenced by their frozen storage histories prior to smoking for at least 8 months at -20 °C, as expressed by the 10 panellists.

2. The prior frozen history of either cod or mackerel did not influence the development of texture, flavour and colour in the smoked products.

3. A very good relationship in the results of texture analysis of cod fillets and mackerel samples was observed between instrumental (Steven's Texture Analyser) and taste panel results by the 10 panellists.

4. The extensive denaturation which occurred during frozen storage of the cod samples, from 72% salt soluble protein in fresh to 29% in the 33 weeks frozen stored, did not influence the extent of denaturation of smoked products which had % salt soluble protein of 11.5 to 15%.

5. The protein denatured to a much lesser extent during frozen storage of the mackerel samples, from 84% in fresh to 64% in the 33 weeks frozen stored. However, the levels of salt soluble protein obtained for the smoked mackerel samples were similar to those of cod, i.e. from 9.5 to 14.5%.
6. Despite the increase in free amino acids, from 10.5mg / g in fresh cod to 25mg / g in the 33 weeks frozen stored and from 23mg / g in fresh mackerel to 35mg / g in the 33 weeks frozen stored, and the observed loss on smoking due to the carbonyl-amino reactions, this did not significantly influence the colour formed on the surface of both fish.

7. The lipid hydrolysis in the cod samples during frozen storage and due to smoking was quite extensive, from 0.69% FFA in lipid of the fresh to 49% FFA in lipid after 33 weeks frozen storage, and from 3.5% FFA in lipid of fresh smoked to 50% FFA in lipid after 33 weeks frozen storage followed by smoking. However, very little lipid oxidation was observed. These processes had no consequence for the flavour of the smoked cod.

8. In contrast to cod, oxidation in mackerel was quite extensive and appeared to be influenced by the type of packaging during frozen storage. Thus, the peroxide value increased from 1.7meqO₂/kg lipid in the fresh samples to 57meq O₂/kg lipid in those frozen stored for 33 weeks in plastic bags, and to 108meqO₂/kg lipid in those frozen stored for the same time in cardboard boxes. The hydroperoxides, which developed during frozen storage were broken down during the smoking process. However, off-flavours were not identified by the 10 panellists, indicating that the characteristic smoked flavour does not allow the detection of any other flavour.
9. The surface reactions involving the smoke carbonyls appeared quite extensive, even for the fresh samples, with a loss of available lysine from 37% in fresh smoked to 90% in the 33 weeks frozen stored and then smoked.

10. In contrast to the cod fillets, the skin on the mackerel appeared to prevent surface reactions involving smoke carbonyls. Thus, the available lysine remained unaltered in the fresh smoked and 11 weeks frozen stored and smoked mackerel samples. However, an extensive loss of available lysine in mackerel samples of 50 and 63% was noticed in the 22 and 33 weeks frozen stored samples after smoking, and this was probably a result from the interactions between oxidized lipid carbonyls and amino protein groups.

11. Thiamine appeared quite stable in the cod samples. A loss of 50% due to thawing (leaching) was found only after 22 weeks frozen storage, while the heating process during smoking had no influence on thiamine in cod.

12. In contrast to cod samples a loss of 44% thiamine in mackerel due to smoking (heat at 70 °C) appeared in the fish frozen stored for 22 weeks and then smoked.
13. Histamine in smoked mackerel remained at levels which would not be expected to cause symptoms of scombroid poisoning, being 4mg / 100g in fresh smoked and 9.4mg / 100g in the 33 weeks frozen stored and then smoked mackerel samples. Its formation appeared to be independent of the previous frozen storage histories of the mackerel samples.

14. This investigation shows that an acceptable smoked product could be attained from both fish (cod and mackerel) even after being frozen stored for up to 8 months.
7. SUGGESTIONS FOR FUTURE WORK.

1. To study the storage life and storage changes of this type of hot-smoked product, with regard to the effect of modern packaging, e.g., modified and controlled atmosphere, and under different storage conditions.

2. To carry out further investigation of the interactions between smoke constituents and fish, particularly those responsible for colour formation.

3. In order to obtain further knowledge regarding the effect of the smoking process on nutritional value, the effect of smoke constituents on essential amino acids, vitamins and fatty acids should be studied.

4. To study the effect of smoking on phospholipids, fatty acids and triglycerides and to investigate the relevance of whether they are free or membrane-bound.

5. To compare the effect of hot smoking and smoke preparations on nutritional value and quality of cod and mackerel.

6. The consumer preference for traditional smoked products or for those produced by smoke preparations (e.g., liquid smoke) should be carried out.
7. Study of the effect of prior frozen storage on employing different processes e.g. cold smoking, milder hot smoking etc.
REFERENCES.


