

This item was submitted to [Loughborough's Research Repository](#) by the author.
Items in Figshare are protected by copyright, with all rights reserved, unless otherwise indicated.

Modified atmosphere packaging of freshwater fish

PLEASE CITE THE PUBLISHED VERSION

PUBLISHER

© Kimani, Eunice Wangari

PUBLISHER STATEMENT

This work is made available according to the conditions of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) licence. Full details of this licence are available at:
<https://creativecommons.org/licenses/by-nc-nd/4.0/>

LICENCE

CC BY-NC-ND 4.0

REPOSITORY RECORD

Kimani, Eunice W.. 2019. "Modified Atmosphere Packaging of Freshwater Fish". figshare.
<https://hdl.handle.net/2134/38318>.

LOUGHBOROUGH
UNIVERSITY OF TECHNOLOGY
LIBRARY

AUTHOR/FILING TITLE

KIMANI, E W

ACCESSION/COPY NO.

016757/02

VOL. NO.

CLASS MARK

Loan copy

1 JUL 1994

001 6757 02



MODIFIED ATMOSPHERE PACKAGING OF FRESHWATER FISH

BY

EUNICE WANGARI KIMANI

A Master's Thesis

Submitted in partial fulfilment of the requirements for the award of:

Masters of Philosophy of the Loughborough University of Technology.

March 1987

**© KIMANI, EUNICE WANGARI 1987
Humberside College of Higher Education**

Loughborough University of Technology Library	
Date	Mar 88
Class	
Acc. No.	016757/02

ACKNOWLEDGEMENT

I wish to express my gratitude to my Supervisors, Messrs John W. Garbutt and John A. Hanna for their guidance, critical evaluations, suggestions, assistance and expert advice throughout the study.

My thanks to Dr. S.W. Hanson and Dr. W.R.B. Arthur for their administration and general assistance.

My very special thanks to Dave Taylor and Leslie Stewart, and to all the technicians (Food and Analytical Laboratories), Humberside College of Higher Education.

My special thanks is extended to the Research Students and Research Assistants, Humberside College of Higher Education who voluntarily and tirelessly participated in the tedious organoleptic tests, making this study a success.

My sincere thanks to the British Council for their financial support throughout my study, their representative in Grimsby Mr. J. Moy and the programme officer, Miss R. Fergusson. Lastly, I would wish to express my appreciation to my Employers, The Permanent Secretary, Ministry of Tourism and Wildlife, and the Director of Fisheries Department, Kenya Government for nominating me to undertake this study.

DEDICATION

To my loved ones, Kimani Senior, Kimani Junior, and Irungu for their ceaseless love and understanding. My sister Wanjiku, who sacrificed her time for the welfare of my family.

ABSTRACT

A preservation method that could extend the shelflife of fresh, freshwater fish would be of nutritional and economic importance to a nation like Kenya, where 90% of all the fish landed comes from the freshwater environment, and where fresh fish is highly valued.

In this study, modified atmosphere packaging of freshwater fish (Rainbow trout, *Salmo gairdneri*) was investigated for its potential in extending the shelflife, in comparison to the traditional method, (ice storage) currently being used in Kenya.

Fish stored in several gas compositions were investigated, (100% CO₂, 60/40 % CO₂/N₂, 50/50 % CO₂ /N₂ , and 50/50 % CO₂/O₂) for the inhibitory effect on microorganisms, the effect on the quality as judged by a panel using the TFRU system, and by use of the GR Torrymeter.

Each of the gas compositions used demonstrated an inhibitory effect on microorganisms with the microbial load of the skin samples remaining below 10⁴ CfU/cm² for 21 days. Maximum inhibition of the microbial flora was achieved by 100% CO₂ atmosphere.

There was a correlation between increasing CO₂ level and inhibition of microflora.

Lactic acid bacteria were not evident as part of the microbial population.

Storage life was limited by organoleptic characteristics rather than microbial spoilage.

There were changes in the CO₂ level in the MAP pouches which correlated with pH changes in the flesh and drip loss.

3.9 pH Measurement	63
4 RESULTS AND DISCUSSION	64
4.1 Preliminary Results and General Discussion	64
4.2 Microbiological Results and Discussion	65
4.2.1 Effects of MAP on Viable Counts (quantitative changes)	65
4.2.1.1 Aerobic Counts at 25°C, 5°C, and 37°C on Plate Count Agar Medium	65
4.2.1.2 Total Microaerophilic and Anaerobic Counts on Plate Count Agar Medium at 25°C	70
4.2.1.3 Total Viable Counts at 25°C and 4°C (gas flushed) on PCA Medium	74
4.2.1.4 Total Viable Counts on Lactic Acid Bacteria Medium (LAB)	77
4.2.1.5 Total Viable Counts at 30°C on Acetate Agar (AcA) Medium	81
4.2.1.6 Summary of Total Viable Counts	81
4.2.2 Effects of MAP on Qualitative Changes of Microorganisms	82
4.3 Organoleptic Results and Discussion	89
4.4 GR Torrymeter Results and Discussion	94
4.5 pH Measurement Results and Discussion	98
4.6 Drip Loss Results and Discussion	100
4.7 Atmospheric Changes Inside the Pouch during Storage Results and Discussion	102
5 CONCLUSIONS	110
6 RECOMMENDATION FOR FURTHER WORK	113
APPENDICES	114
REFERENCES	117

CONTENTS

Page

1	INTRODUCTION	1
2	LITERATURE SURVEY	4
2.1	Quality Deterioration of Fresh Fish	4
2.1.1	Meaning of Quality	4
2.1.2	Factors Influencing Quality of Fish	5
2.1.3	Characteristic of Fresh and Spoiling Fish	8
2.1.4	Stages in Spoilage Process of Fresh Fish	9
2.1.4.1	Microbial Spoilage	9
2.1.4.2	Enzymatic Spoilage	11
2.1.4.3	Chemical Spoilage	12
2.1.5	The Spoilage of Freshwater Fish	14
2.1.6	The Quantitative and Qualitative Flora of Fresh and Spoiling Marine and Freshwater Fish	16
2.1.6.1	Quantitative Aspects	16
2.1.6.2	Qualitative Aspects	21
2.2	Means of Preventing Deterioration in Fish	25
2.2.1	Preservation of Fish by Drying	25
2.2.2	Fish Preservation by Salting	26
2.2.3	Preservation of Fish by Smoking	27
2.2.4	Preservation of Fish by Chilling Methods	28
2.2.5	Preservation of Fish with Modified Atmosphere	29
2.2.5.1	The Role of the Principle Gases used in Modified Atmosphere Packaging of Fresh Fish and Other Muscle Foods	30
2.2.5.2	Microbial Flora of Fish Stored in Modified Atmosphere Packaging	33
2.2.5.3	Extension of Shelflife Resulting from Modified Atmosphere Storage	35
2.2.5.4	Effect of Modified Atmosphere Packaging on Growth of Pathogens	39

2.2.5.5 Applications and Current Usage of Modified Atmosphere Packaging	43
2.3 Methods of Assessing Freshness in Fish	45
2.3.1 Sensory Methods	45
2.3.2 Chemical Methods	47
2.3.2.1 Proximate Analysis	47
2.3.2.2 Total Volatile Bases (TVB)	48
2.3.2.3 Hypoxanthine (Hx)	48
2.3.2.4 Peroxides	48
2.3.3 Physical Methods	49
2.3.3.1 pH	49
2.3.3.2 Torrymeter	49
2.3.4 Microbiological Methods	50
2.4 Aims of the Present Work	51
3 EXPERIMENTAL	53
3.1 Materials	53
3.2 Equipment	53
3.3 Sample Preparation	54
3.3.1 Packaging Procedure	55
3.4 Microbiological Methods	56
3.4.1 Standard Plate Count (SPC)	56
3.4.2 Identification Methods	59
3.4.2.1 Gram Stain	59
3.4.2.2 Motility Test	59
3.4.2.3 Catalase Test	60
3.4.2.4 Oxidase Test	60
3.4.2.5 Sugar Fermentation Test	60
3.4.2.6 Oxidative/Fermentative Test	60
3.5 Organoleptic Assessment	61
3.6 Gas Analysis	61
3.7 Torry Meter Reading	63
3.8 Drip Loss Measurement	63

1 INTRODUCTION

Little is known of the total potential sustainable yield of fisheries resources in Kenya. The country has 40 kilometres of coastline on the Indian Ocean, a number of lakes and dams, thousands of freshwater fish culture ponds and a few rivers, all of which contribute to the fisheries resources.

According to the statistics compiled by the Fisheries Department, approximately 90% of all the fish landed in the country comes from the freshwater fisheries. Lake Turkana and the Kenya waters of Lake Victoria accounts for about 80 - 85% of the total freshwater fish landings, while the remainder comes from the smaller lakes, dams, ponds and rivers.

The fishing operations in all the freshwater lakes and dams are all artisanal in nature. Fish caught by fishermen using gill nets, longlines, beach seines and ring nets are landed and taken to the "Banda" (shed), where the primary sale of fish from fishermen to the traders takes place.

Out of all the fish caught, less than 40% is sold and consumed in the wet form, while the remainder is processed in one way or another. Several factors determine whether fish is sold in wet or processed form, the most important being the species, quality of the fish, market demand and location from the fishing area.

All the small sized fish such as *Engraulicypris* spp. and *Haplochromis* spp. are sun-dried and sold in the dry form.

Fishermen would prefer to sell all the large sized fish such as *Tilapia* spp., Nile Perch *Lates niloticus*, *Bagrus* spp., *Clarius* spp. and the others in the wet form, for fresh fish command a premium price. Further processing is usually expensive and even when it is cheap, is accompanied by heavy losses due to fragmentation, insects and rodents.

In Kenya, a tropical country where ambient temperatures are always above 25° C, maintaining the quality of fresh fish is not possible.

Traditionally, fresh fish have been marketed in towns near areas where the fish are landed, while distribution to distant towns and the city where there is higher demand for fresh fish

is done by use of refrigerated vans. In addition, ice is used to aid in cooling and keeping the fish moist. This makes the fresh fish sold in distant towns very expensive and losses due to bacterial spoilage very high.

From an economic point of view, the ability to preserve the quality and nutritional value of wet (fresh) fish for extended periods of time could be rewarding in terms of reduced waste, increased value of the product and increased sales.

This study has focused on the feasibility of using "Modified Atmosphere Packaging" for the preservation of freshwater fish at low temperature achieved by mechanical refrigeration. The work is confined to one species of freshwater fish, rainbow trout, *Salmo gairdneri*, which is readily available at the place where the work is carried out.

The rainbow trout is often referred to as finger trout or simply as trout. The colour of the rounded body is variable, but the upper part is predominantly olive green, with many rounded dark spots on the body, head and fins. The spots on the tail, unlike those on the native trout *Salmo trutta*, extend to the extreme edge. There is an iridescent band of colour, mainly pink, along the lateral lines, and the lower part of the body is silver. The mouth is large and set obliquely, with well developed teeth in the jaws, on the palate and in the throat. The area of the upper jaw is in line with the rear edge of the eye. The skin, covered with very small scales and slime, feels smooth.

The rainbow trout is native to the rivers of North America, but has been introduced to all continents (Mills, Torry Advisory note No. 74). It occasionally lives wild in Europe in modest numbers but is principally stocked artificially, often in landlocked waters or in man-made ponds.

In Kenya, the Fisheries Department imports disease-free eggs from Denmark and hatch them in a hatchery in the mountains where the river water is very cold. The fingerlings are then moved to outdoor ponds with running clear and cold water, and reared on pellet food until they reach about six centimetres, the size at which they are sold to the fish farmers or stocked into the rivers for sport fishing.

The proximate analysis of the raw flesh of Rainbow Trout is, water 70-80%; fat 0.7-8 per cent; and protein 18-20%. Fat and water content vary with season and with diet (Mills, Torry Advisory Note No. 74).

2 LITERATURE SURVEY

2.1 QUALITY DETERIORATION OF FRESH FISH

2.1.1 Meaning of Quality

In relation to fishery products, the term "quality" has different meanings to different people. Whether a fish is deemed of "good" or "bad" quality may be in relation to its size, the individual species, or the freshness of the product, as well as other considerations. Expensive species may be called "quality fish", the term referring to the price it may bring on the market, whereas lower priced species may be thought as being of lower quality. Suitability of a species for a certain product or process gives another meaning to quality, for example, to be used as fillets or to be processed into sticks. Because of biological variations within the same species, some individuals in the catch may be of poor quality because they are out of condition owing to spawning activity, unfavourable diet, seasonal variations or many other reasons.

Most often, and this is the meaning of quality that is of interest in this study, the term "quality" is synonymous with freshness, or the degree of microbiological spoilage or chemical degradation to which the raw product has progressed. The degree of spoilage is dependent upon many factors, some intrinsic, meaning the sum of attributes inherited in the raw material. Most of the fish consumed throughout the world are caught in the wild where no control or manipulation of intrinsic quality is possible (Wheaton, 1985).

Other factors affecting quality are related to environmental conditions and handling during and after harvest. More often than not, undesirable environmental conditions cannot be avoided, except in cases where extreme pollution is evident or during different seasons of the year. In these cases, the fishing ground affected can be avoided until such time that more favourable conditions exist. Even after harvest, care and speed during handling and the processing and preservation methods used may affect quality. These factors can largely be controlled by man (Wheaton, 1985).

2.1.2 Factors Influencing Quality of Fish

The kind and number of factors influencing quality of fish are numerous, making the process of fish spoilage very complicated.

The degree to which certain factors affect quality varies and may be manifested as an off-flavour or an odour change. In some cases, the keeping quality and storage ability under chill temperatures may be affected.

The rate of spoilage or deterioration is species dependent. It is a well known fact that, when chilled or frozen, fatty species such as mackerel will spoil more rapidly than lean species like cod. Further, ungutted cod will spoil more rapidly than certain other species, such as halibut and flounder.

Differences in composition within a species may be the cause of secondary influences on quality. When placed in refrigerated storage, lean fish in poor condition spoil much more rapidly than specimens of the same species in good condition. This may be explained by the glycogen content of the flesh. In lean fish of poor quality, a lower glycogen content causes a correspondingly higher flesh pH. Soon after death the glycogen in the flesh is converted to lactic acid which determines flesh pH. The lower the lactic acid the higher the pH (Connell , 1980).

Species of fish caught in warmer waters keep longer on ice than those caught in cooler waters. The reason for this, however, is more related to the bacterial flora growing on the surface of the fish rather than the fish themselves. The bacteria growing on the surface of cold water species being psychrotrophic and hence more tolerant to low temperatures and are capable of producing off-flavours and off-odours at low temperature. Bacteria on fish from warmer waters are inhibited by ice temperatures.

The size of fish is also a factor that influences quality. It is an established fact that large fish keep better than small fish (Connell , 1980). One of the main mechanisms of spoilage is the penetration of microorganisms from the surface to the interior of the fish. Large fish have a smaller surface area to volume ratio that, in the same time and period, less of the interior of the larger fish is affected. Also large fish such as cod, tuna, mackerel and others are

generally eviscerated aboard the fishing vessel, while those species too small or numerous for evisceration aboard the vessel may simply be stored in the round.

Small fish of a given species tend to have a higher post-rigor pH than larger fish of the same species, thus giving rise to greater bacterial action (Connell , 1980).

How quickly the fish are eviscerated and placed into cold storage may be related to the distance the vessel must travel from its home port to the fishing grounds. Evisceration generally takes place swiftly aboard the factory-type of vessels which travel great distances from port and may be out at sea for weeks at a time. But aboard the smaller vessels which do not have the capabilities of proper eviscerating and subsequent storage, the fish may simply be stored on ice in the round until the vessel reaches port. Often this time period may be several days, thus allowing the bacteria and enzymes in the gut sufficient time to do their work.

The problem of distance from fishing ground to port is more pronounced in tropical and sub-tropical regions than it is in the colder climates. Warmer air temperatures increase the rate of quality loss, particularly if the catch is piled on deck with little or no ice to keep it cool. The heat from the sun's rays quickly overheats the fish and accelerates post-mortem changes. The rate at which changes occur depend upon the length of time stored in this manner, the temperature and the species.

What a fish eats has a significant effect on the flavour of its flesh. The influences of diet on flavour of pond reared catfish was demonstrated by (Maligalig *et al.*, 1973) cited by (Wheaton, 1985). Catfish fed on a diet of turkey liver developed a liver-like flavour within 19 days which became more pronounced as feeding continued. Those fed on a cereal diet developed cereal flavour within 33 days.

Freshwater fish such as *Tilapia* spp. and catfish occasionally suffer from a muddy odour and flavour which may reduce consumer acceptance.

A condition which produces a flavour described as 'blackberry', 'weedy', 'diesel', 'iodine', or 'sulfide' has been known to develop in cod and other gadoids, mackerel, chum, and salmon. This is a natural occurrence which has been attributed to the presence of dimethyl

sulphide (DMS) (Connell , 1980). Except for some bivalves, DMS does not naturally occur in fish or shellfish. The precursor occurs in the food of the fish in the planktonic bivalve mollusc known as pteropods. The pteropods contain dimethyl - B - propiothetin, which is converted to DMS in fish. The odour of this compound at low concentrations may be noticeable in flesh fillets on ice. However, if the odour increases, the fish becomes unacceptable. The odour becomes more pronounced in flesh that is canned, such as salmon.

Thaysen and Pentelow (1936) cited by (Wheaton, 1985) attributed an "earthy" taint found in salmon to the species of actinomycetes found growing in the same waters as the salmon. Actinomycetes have also been known to cause an iodine-like odour in shrimp and prawns (Connell, 1980).

The location of the fishing grounds play an indirect role ^{in the} quality of the fishery product, (James, 1965) cited by (Wheaton, 1985). Within species, flavour can vary from one season to the next depending upon the nature of the food and physiological condition of the species of interest.

Winds, tides, water conditions and migratory patterns also influence the condition and quality of fish before harvest. These factors have some influence on the type and abundance of food organisms available which could affect the health and condition of the fish (Wheaton, 1985)

Sex plays a large role in quality soon after spawning. The females of certain species may be in such poor physical condition soon after spawning that they are of very poor quality. However some species such as salmon, both sexes, may be in poor condition after spawning. Just prior and during spawning, food reserves in the flesh are transferred for the development of gonads. During spawning and for some period afterward, most fish do not feed (exceptions are the elasmobranchs). The result is that the flesh becomes severely depleted of fat, protein and carbohydrates and the fish are in poor condition.

The effect of spawning on the quality of fish manifests itself in different ways. When cooked, white fishes in poor condition becomes soft and gelatinous. In extreme cases, the depletion of protein results in a jelly-like state which renders the flesh useless. In fatty, pelagic species like sardines, herring and mackèrel, the quality changes brought about by

spawning are even more pronounced. Between the starvation period after spawning and resumption of full condition, the fat content of herring can vary from less than 1 per cent to over 25 per cent (Connell, 1980). During this change in fat content, the overall weight of the fish is maintained somewhat constant by a corresponding decrease in water content. Since a high fat content is desirable in these species for canning and kippering, fish in the post spawning condition are often undesirable for these processes.

2.1.3 Characteristics of Fresh and Spoiling Fish

Freshly caught fish have a shining, iridescent surface covered with nearly transparent, uniformly and thinly spread slime. The eyes are protruding, bright, with a jet black pupil and transparent cornea. The gills are generally bright and free from visible slime. The flesh is soft and flabby, tending to retain finger indentation (Bramsanaes, 1965). Soon after death, however, when the body stiffens (rigor mortis) the flesh becomes hard, and elastic and does not readily yield juice under pressure. The odour of the flesh is generally described as "marine", "fresh seaweedy", or "laky" (for freshwater fish). The flesh of fatty fish has, in addition, a pleasant, margarine-like odour.

As the fish spoils and finally becomes putrid the surface loses its bright sheen and colour, and becomes covered with a thick slime which grows increasingly turbid and lumpy. Finally, the colour of the slime becomes yellow and brown. The eyes gradually sink and shrink, the pupil becoming cloudy and milky and the cornea opaque. At first the gills assume a bleached, light pink colour and finally turn to greyish brown at which point they become covered with a thick slime. The flesh gradually softens until it is very easily stripped from the backbone and exudes juice under light pressure. Simultaneously the elasticity disappears. During this breakdown, the flesh changes from its original translucent sheen to a dull, milky appearance. If originally coloured, as in salmon, the tint often fades to greyish yellow. Along the backbone above the belly and spreading back toward the tail, a reddish brown discolouration penetrates from the main blood vessel into the flesh (Bramsnaes, 1965)

Newly caught fish generally carry feed in their digestive tracts. If they are not gutted soon after being caught, the powerful digestive enzymes attack viscera and belly walls, causing discolouration, so called "belly burn" or disruption, giving rise to so called "torn bellies". The latter phenomenon can occur in a few days at ice temperatures, even if the digestive tract

is nearly empty, but much more rapidly in the case of feeding fish.

As spoilage proceeds, there is a gradual change in odour of the raw fish. Initially it is fresh; it then becomes "sweetish", sometimes "fruity", latter "ammoniacal" or "fishy" odours dominate, until finally the well known putrefaction odours become evident.

When cooked, very fresh fish exhibit delicate, pleasant odours and flavours. As spoilage proceeds, these odours and flavours generally become first "flat" and uninteresting, then "fishy" before the putrid flavours begin (Bramsnaes,1965). In case of fatty fish, an oily and later a rancid taste will be noted.

At lower temperatures 0-2°C, this rancidity may develop so rapidly that rancid flavour becomes limiting factor of keeping quality (Sigurdsson,1965) cited by 'Bramsnaes(1965).

2.1.4 Stages in Spoilage Process of Fresh Fish

As soon as a fish is caught and dies, changes of various kinds begin to occur, leading in many instances to spoilage and deterioration. The spoilage of fresh fish is a complex process for which no single factor is responsible, but rather, it is a combination of several inter-related processes. Among these processes is the degradation of protein with subsequent formation of hypoxanthine, trimethylamine and other products, and processes that result in gradual developments of undesirable odours and flavours, softening of flesh, and loss of cellular fluid containing fat and protein. The three basic modes of spoilage in fish are microbial, enzymatic, and chemical.

2.1.4.1 Microbial Spoilage

Aided by enzymatic activity, microbial spoilage is by far the main mode of spoilage of chilled fish. There are several types of micro-organisms that may cause spoilage, but the major concern is bacteria. Large numbers of bacteria are normally present in the surface slime, on the gills, and in the intestines of live fish. They are not normally harmful to healthy, living fish since the fish's natural defenses keep them at bay, but soon after death, bacteria and the enzymes they secrete begin to invade the tissue through the skin, and through the lining of the belly cavity. Bacteria may also enter the flesh through any punctures or open wounds.

Bacteria secrete digestive juices, enzymes that break down and dissolve the tissues they attack. It is these enzymes that cause the breakdown and spoilage of fish. The bacteria in the flesh result in odour and flavour changes which initially, may be described as "sour", "grassy" or "acidic". The odours and flavours may gradually change to "bitter", or "sulphide" and may change to ammonia in the final changes. In addition to flavour and odour changes, bacteria are responsible for changes in the appearance and physical properties of fish. The slime on the skin and gills may change from normal, clear, and watery appearance to cloudy or discoloured. The skin loses its iridescence and becomes dull and readily detaches from the internal body wall.

Bacterial spoilage of fish does not begin until the passage of rigor mortis when the juices are released from the muscle fibres. Any delay in rigor will, therefore, prolong the keeping time of the fish. Rigor is hastened by struggling of the fish, lack of oxygen, and higher temperature. Low pH and proper cooling will delay the onset of rigor. The pH of the fish flesh is also important because the lower the pH the slower the bacterial decomposition. Fish flesh pH is lowered by the conversion of muscle glycogen to lactic acid (Connell, 1980).

Fish can spoil from both the inner and outer surfaces. The most common inner surfaces through which bacteria enter are the gills. The gills are soft and moist, making ideal places for bacteria to grow. Here the bacteria grow rapidly causing off-odour and discolouration. The condition of the gills is often used as an indicator of the stage of deterioration of the fish. From the gills, the bacteria pass along the vascular system, through the kidney, and into the flesh.

If the fish is "feedy" when captured, that is, the stomach and intestine contain large amounts of food, the intestine soon become loaded with spoilage bacteria attacking the food. In due time, obnoxious compounds begin to develop from the decomposing mass and begin to diffuse into surrounding flesh, resulting in nasty odours and discolouration.

Bacteria on the outer surfaces of the fish are closer to the parts that are normally filleted than are the bacteria in the intestines. Therefore, the flesh may be invaded by surface bacteria long before bacteria penetrate through the gut wall. Also, the skin has a much greater area of contact with the fillet areas than does the gut wall. The numbers of bacteria in the slime and

on the skin of newly caught fish may be in millions per square centimeter. Washing the fish often reduces the surface count of the bacteria by 80 to 90 percent (Wheaton, 1985).

Fish that have already been landed may become contaminated with their own faeces. This is especially a problem with "feedy" fish during the warmer times of the year. Faeces are often contaminated with large numbers of the worst types of bacteria that causes deterioration.

Fish may also become contaminated from the outside by chilling them using unclean ice which may contain millions of bacteria per gram of ice. Fish may also pick up considerable bacterial loads from decks of vessels, from the fishermen handling them, and from the pens in which they are stored in the hold of the vessel. They pick up additional load during evisceration from the work surfaces and from the personnel.

Fish flesh contains a large amount of non-protein nitrogen. The fish's natural enzymes produce autolytic changes such as amines and amino acids, and glucose for bacterial growth. The bacteria then convert these compounds to TMA, ammonia, amines, and aldehydes. The end products may be hydrogen sulphide and other sulphides, mercaptans and indole, products indicative of putrefaction. In the many marine species containing the odourless compound TMAO, one distinct reaction is its reduction to TMA. This reaction is characterised by an ammoniacal odour, but in combination with other compounds may give off a "fishy" odour. Freshwater fish do not contain TMAO. In addition to off-flavour and odours, discolouration of the flesh may occur during spoilage. *Pseudomonas fluorescens*, yellow micrococci, and others cause flesh to turn yellow to greenish-yellow colour (Frazier, 1967) cited by (Wheaton, 1985). Red and pink colours are caused by the growth of *Sarcina*, *Micrococcus* and *Bacillus* species as well as by moulds and yeasts (Frazier, 1967) cited by (Wheaton, 1985).

2.1.4.2 Enzymatic Spoilage

Enzymes are protein-like substances present in the flesh and stomach of fish and shellfish, which initiate or speed up chemical reactions. When the fish is alive, enzymes are usually kept in balance with the help of digestive or blood systems. They remain active after the death of the fish and are particularly involved in flavour changes that take place during the first few days of storage before bacterial spoilage becomes significant. In a short time,

enzymatic activity can also alter texture and appearance of the fish.

When captured or harvested the fish usually contain food in their gut, and powerful enzymes are present upon death of the fish, the enzymes penetrate the gut wall and surrounding flesh, weakening and softening them. The gut and flesh may then be invaded by spoilage bacteria.

Enzymes play a role in the development of rigor mortis, which is the progressive stiffening of the muscles several hours after death. The stiffening effect is as a result of coagulation of muscle protein (Wheaton, 1985). The duration and intensity of rigor depends upon the species, temperature, and condition of the fish. It usually passes before bacteria invade the muscle, leaving the flesh soft and limp. Following rigor the self digestion process commences, as a result of enzymatic activity. Self digestion or autolysis means that the fish literally consumes itself. It can take place rapidly, especially in the smaller fatty fish which may be full of food at the time of capture. The gut enzymes are particularly active at this time. A phenomena known as "belly burst" can occur in just a few hours in some fish such as sardine and herring, and is caused by weakening of the belly wall due to self-digestion. The rate of self digestion is dependent upon temperature and can be retarded, but not halted completely, by chilling the fish to just above the freezing point.

2.1.4.3 Chemical Spoilage

Fish fats contain a high proportion of unsaturated fatty acids, which are subject to attack by atmospheric oxygen, leading to deteriorative changes, especially in fatty fish. The effect of oxidation on odour and flavour is called rancidity. In herring oil, rancidity is characterised in the early stages by a marked fish odour and flavour followed by an unpleasant taint, described as like linseed oil or paint. Similar odours and flavours appear in fatty fish themselves. Lean fish have in the flesh only small amounts of fat of a different kind from that in the flesh of fatty fish. Lean fish do not become markedly rancid during storage but develop a recognisable cold store odour and flavour (Hobbs, 1982).

Fat oxidation has little effect on the rate of deterioration of iced fish, where microbiological spoilage dominates. When bacterial action is prevented or slowed, most importantly by freezing but also by drying or by irradiation, fat oxidation is an important cause of loss of eating quality (Hobbs, 1982).

The species is undeniably the most important factor determining the rate of rancidity. Fish that have a high fat and oil content have a relatively short frozen storage life because of the susceptibility of the fish to oxidative rancidity. Tuna, mackerel, herring and some species of Salmon fall in this category (Wheaton, 1985). However, there are certain species, such as sablefish, which are quite resistant to oxidative rancidity despite a high oil content (Wheaton, 1985).

In fish having a low fat or oil content, the development of rancidity is not severe. Lean fish like cod and haddock may be kept in frozen storage for a much longer period without much quality loss due to rancidity. Fresh water trout have a relatively high lipid content, therefore, are more susceptible to oxidative rancidity than most of other freshwater species (Wheaton, 1985).

Other factors contribute to the susceptibility of a given species to oxidative changes. Even within the same species, small fish tend to spoil more rapidly than large fish. It is felt that this is because surface bacteria are able to exert more influence on smaller specimen (Merritt, 1969) cited by (Wheaton, 1985).

Oxidation rates may be affected by the condition of the fish when caught, diet, season, fishing ground and sexual development, as well as techniques used after being caught, bleeding, gutting, chilling and storage (Merritt, 1969) cited by (Wheaton, 1985).

Brown discolouration, often called rusting, of fatty fish is believed to be due to the combination of some products of fat oxidation with substances in the flesh that contain nitrogen. Bleaching of pigments of the flesh of salmon and redfish accompanies fat oxidation in these species. The effect on taste and appearance concern frozen fish, while nutritional effects of fat oxidation relate mainly to fish meal. Oxidation can reduce the availability of lysine, and essential amino acid, in fish meal made from fatty fish, though seldom to a serious degree.

Vitamin A may be partly lost during fat oxidation. Some of the products of fat oxidation are toxic in large amounts. However, there is no evidence that there is any risk to health from the consumption of fatty fish meal or cold stored fish (Hobb, 1982).

2.1.5 The Spoilage of Fresh Water Fish

Although the spoilage pattern in any particular species of fish generally follows much the same pattern regardless of the origin of the fish, there are wide differences in patterns of different groups. The cartilaginous fishes, such as dog fish and skate, are well known to produce large quantities of ammonia even within eight days in ice. There are also wide differences within the bony fishes. For example, mackerel spoil very rapidly while redfish (*Sebastes*) will keep better than cod but not as well as halibut (Burgess, 1985). There are furthermore wide differences between freshwater and marine bony fishes. At the present time the difference between the two can only be attributed in an overall way to differences in some components of the tissues, in enzyme systems, and bacterial flora.

The most striking problem confronting those concerned with freshwater fish quality is the scarcity of information available on these fish and their spoilage process.

In contrast to marine fishes, the environment from which the freshwater fish are caught can vary from a cold clear arctic lake to the opposite extreme of a grossly contaminated tropical canal or pond. These marked differences and variations in water quality together with varying kinds of climatic conditions have a pronounced influence on consumer quality of freshwater fish products (Bligh, 1971).

It is well known that the composition of fish is affected by their environment. This is particularly true for fat content and composition. The ways in which a fish may be processed, and its storage potential are both very dependent on fat content. Karrick *et al.*, (1956) cited by Bligh (1971) illustrated the large differences that occur in fat content of freshwater fish. He reported that the fat content of fillets of sheepshead *Aplodinotus grunnius* from Lakes Erie and Redlake ranged from 1.17 to 10.30 percent. The same fish from Mississippi River contained 1.45 and 20.36 percent fat, whereas clearwater lake fish had only 0.72 to 1.67 percent fat.

Thurstan (1962) cited by Bligh (1971) found the fat content to be extremely variable in two sub-species of lake trout *Salvelinus namaycush* taken from Lake Superior. Fillets of the lean variety contained 1.9 to 22.5 percent fat and those of the more fatty ones had 21.3 to 67.2

percent fat. Bligh (1971) has quoted many other researchers who found large variations in fat content of some species of freshwater fish from different areas. Spoilage pattern for a particular freshwater species will therefore differ from one area to another.

Freshwater fish taken from warm eutrophic waters are sometimes unpalatable due to an earthy odour and flavour. This earthy odour comes principally from actinomycetes which are known for sometime to produce earthy odours. Thaysen (1936) and Thaysen and Pentelow (1936) cited by Bligh (1971) demonstrated that, odouriferous substances from actinomycetes were rapidly picked up by fish through the gills or mouth and the flesh become tainted. Lastly the widespread pollution of the natural environment greatly affects the quality of freshwater fish.

Bacteriological studies on freshwater fish are quite rare in contrast to the voluminous literature on marine fish. Bligh (1971) considers that some studies carried out on freshwater fish indicated that the bacteriology of freshwater fish is not grossly different from that in marine fish. However, he states that the results have some implications that were important for the handling of freshwater fish both in terms of keeping quality and of public health. The results showed that pseudomonads were virtually absent, relatively few of the isolated organisms would fit into the genus *Aeromonas*, and cocci of all types were a very small proportion of population. Those cocci found were large and of unusual type. The major group of organisms isolated while varying considerably in size and morphological detail had certain overall characteristics in common. Young cultures stained gram positive to gram variables and grew as filaments or "trichomes". The overall characteristics of the major group indicated that they probably fit into the "coryneform" group until it was discovered that over half of the total number of organisms isolated were heat resistant and contained bodies that stained as spores. This supports Kreuzer's concept that freshwater fish have a special spoilage microflora.

Although fish muscles are generally considered sterile, Mattschewsky and Partmann (1981) cited by Bligh (1971) isolated a number of bacteria from the flesh of several freshwater fish such as lake bream, roach, tench, goldfish, river barbel, northern pike and rainbow trout. Generally, however they did not belong to typical water bacteria. They would rather be classified as air, soil, plant or waste bacteria. This made Mattschewsky draw the conclusion that these bacteria were related to the feed of the respective fish.

Another important factor in fish spoilage is rigor mortis. The onset and duration of this phenomenon depend upon a number of factors such as the glycogen ATP relationship. The pH, reaching acid values after death, enhances the hydrolytic activity of some enzymes and the splitting of proteins (Bligh, 1971).

Tarr (1954) cited by Bligh (1971) compared the occurrence and duration of rigor mortis in marine and freshwater fish, mackerel, red snapper, and carp. The rate of glycolysis decreased in the order; mackerel, red snapper, and carp.

2.1.6 The Quantitative and Qualitative Flora of Fresh and Spoiling Marine and Freshwater Fish.

Although the flesh of newly caught healthy fish is sterile, the skin, gills and, in fish which have recently been feeding, the intestines can carry considerable bacterial loads. All the available evidence appears to show that both quantitatively and qualitatively, the microbial flora is a function of the environment in which they are caught.

2.1.6.1 Quantitative Aspects

Tables (I and II) from Shewan (1977) shows that there are higher loads of bacteria on newly caught marine and freshwater fish from tropical and sub-tropical areas. This as Shewan (1971) quotes, confirms the findings of Kriss (1971) of greater contamination in water from hotter areas than in colder regions. More important is the fact that psychrotrophs are more abundant in fish from colder areas than in the tropical or subtropical ones. Thus only about 5% of the total flora of fish from the North Sea could grow at 37°C (Shewan, 1944), compared with 55% in fish caught off the Mauritania coast (Kochanowski and Maciejowska, 1964, 1969) cited by (Shewan, 1977). Table III shows changes in bacterial counts on tropical freshwater fish during storage on ice. Figure 1 shows that there are more gram negative bacteria from the marine fish than from the fresh water fish, both from the temperate water and tropical water.

Table 1. Bacterial loads on newly caught marine fish.

Area	Species	Temp.	Skin per (cm ²)	Gills per (g.)	gut contents per (g or cm ³)	Author
Temperate Zone	North Sea fish	20°C	10 ² - 10 ⁵	10 ³ - 10 ⁷	10 ³ - 10 ⁸	Shewan, 1962
Tropical and Sub-Tropical	Indian Sardine	30°C	10 ⁵ - 10 ⁷	10 ⁶ - 10 ⁸	10 ⁷ - 10 ⁹	Karthiayani and Iyer, 1967, 1971
		37°C	10 ⁴ - 10 ⁷	10 ⁸ - 10 ⁹	10 ⁶ - 10 ⁸	
	Japanese Flat Fish, Australian Mullet etc	20°C	10 ⁴ 10 ⁴	10 ³ - 10 ⁵	10 ³ - 10 ⁷	Simidu et al 1969 Gillespie and Macrae 1975

Table II Examples of bacterial loads on newly caught freshwater fish

Area	Species	Skin ₂ per cm ²	Gills per (g)	Guts per (g or ml)	Author
Temperate waters	UK Salmon: in freshwater in sea	10^2 - 10^3 4.4×10^2		10^1 - 10^2 10^3	Harsley 1973
	CANADA Salmonids			10^2 - 10^7 or 10^7	Trust & Sparrow 1974
	USA 10 Species	10^2 - 10^5			Potter and Baker 1961
Tropical & Subtropical	BANGLADESH Hilsa	10^3 - 10^4	10^6	10^4 - 10^6	Joarder 1974
	AFRICA Kariba	10^3 - 10^5 over 10^4			Watanabe 1971
	Tanganyika	10^3 - 10^5 over 10^3			Watanabe 1971

Table III

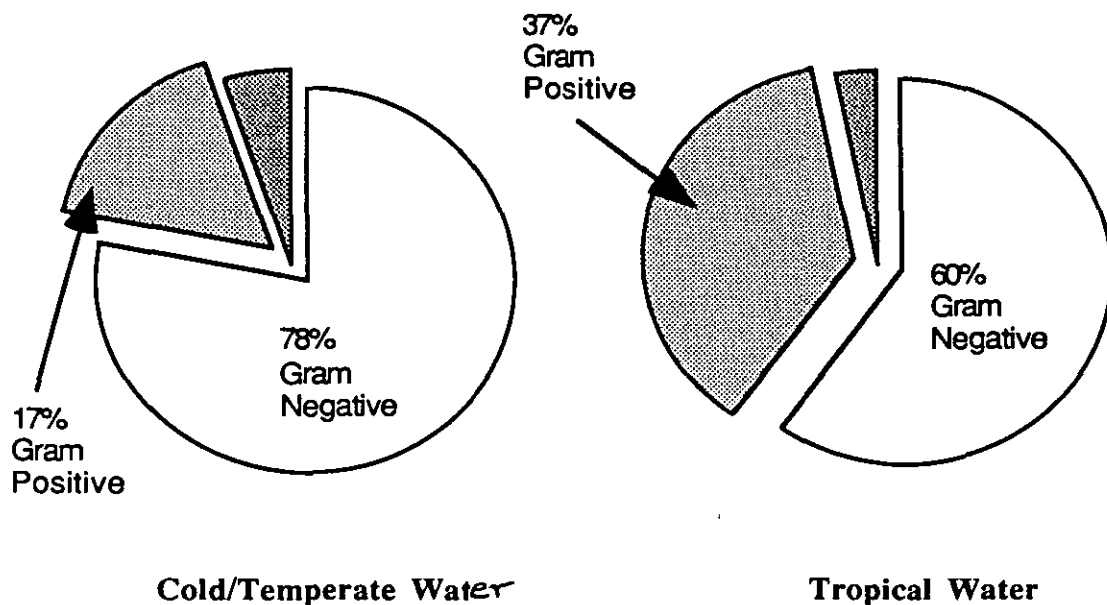
Changes in the bacterial counts on tropical freshwater fish skin (cm⁻²) during storage in ice (*Lima dos Santos - unpublished*)

SPECIES	DAYS ON ICE						AUTHOR
	0 - 2	5 - 7	8 - 9	11 - 12	14 - 16	19 - 22	
TILAPIAS <i>Sarotherddon</i> spp	10 ²	10 ³	-	-	10 ⁶	10 ⁶	Adebona 1981
<i>S. esculentus</i>	10 ³	10 ³	10 ⁴	10 ⁵	10 ⁵	10 ⁶	Hoffman et al 1974
<i>S. niloticus</i>	10 ³	-	10 ⁵ - 10 ⁶	-	10 ⁷	10 ⁷	Hoffman et al 1974
<i>S. mossambicus</i>	10 ⁴	10 ⁵	10 ⁶	10 ⁷	10 ⁷	-	Watanabe 1965 - 66
CAT FISH <i>Brachyplatystoma</i> <i>vaillanti</i>	-	10 ⁵	10 ⁷	10 ⁷	10 ⁸	10 ⁸	Lima dos Santos 1981
<i>Chrysichthys</i> spp.	10 ²	10 ³	-	-	10 ⁴	10 ⁵	Adebona 1981

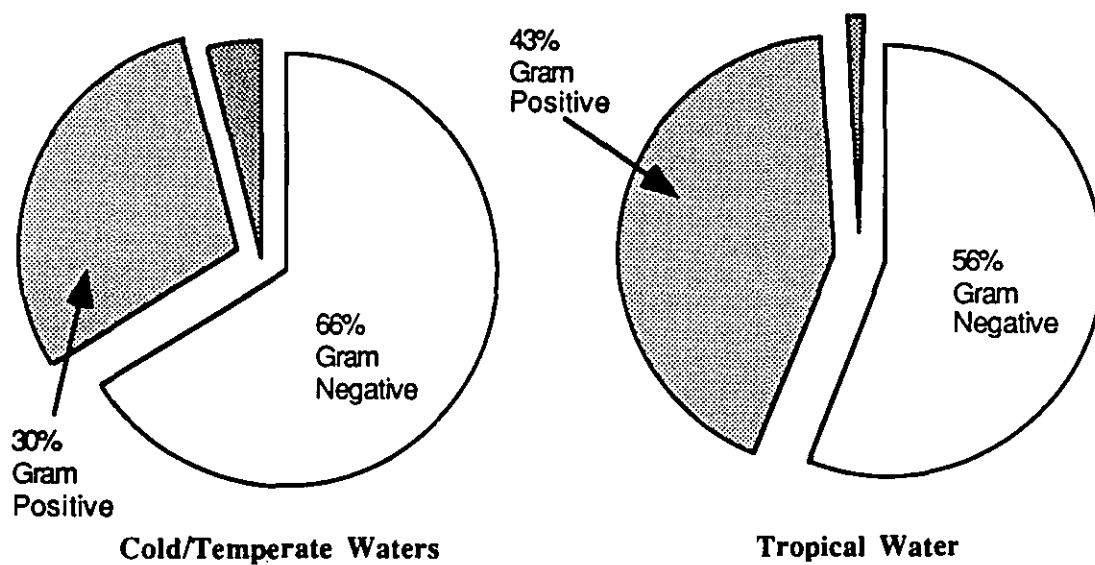
Figure I

Distribution (%) Of composition of gram positives and gram negatives in the micro-flora of newly caught fish from tropical and cold temperate waters, Lima dos Santos (1978).

MARINE FISH



FRESHWATER FISH



Quantitative changes occurring during the ice storage have been reported by many researchers. Shewan (1971) indicated that in cod there is a lag period of 2–3 days followed by a logarithmic increase in bacterial numbers until the tenth day when there is a count of 10^8 organisms per square centimetre of skin or per gramme of muscle. Other quantitative studies on temperate and cold water species such as trout (*Salmo trutta*) and herring (*Clupea harengus*) (Hansen, 1972), plaice (*Pleuronectes platessa*) and haddock (*Melanogrammus aeglefinus*) (Huss, 1972) confirm this general pattern, although counts as high as 10^9 per gramme of flesh have been reported (Disney *et al.*, 1974).

Freshwater fish appear to follow a different pattern. Surface slime seems to promote bacterial growth up to 10^{10} organisms per gramme after 6 days at 3°C (Bligh, 1969; Gillespie and Ostava, 1971) cited by (Disney *et al.*, 1974), whereas flesh counts remain relatively low. These findings would seem to support the view that freshwater fish have a different type of spoilage microflora (Bramstedt and Auerbach, 1961). With tropical iced fish, total viable counts appear to be considerably lower than in cold water species. Valankar and Kamasastri (1956) cited by Disney *et al.*, (1974) found that microbiological deterioration was slower and less pronounced in several species of Indian marine fish than reported for cod. Also the pattern of microbial growth in freshwater fish does not seem to be much different from that of marine species.

An Indian study of freshwater carp by Balakrishnan *et al.*, (1971) reported total viable counts of 10^6 or 10^7 organisms per square centimetre of skin surface and 10^6 organisms per gramme of flesh after 22 days storage in ice.

A study of West African marine fish by Amu and Disney (1973) cited by Disney *et al.*, (1974) found total viable counts of 10^5 to 10^6 organisms per gramme of muscle at the limit of acceptable storage, 20–26 days.

2.1.6.2 Qualitative Aspects

Qualitatively, the flora, particularly of the skin and gills, differs considerably in fish from varying areas (Shewan, 1976). Table IV and Table V from Shewan (1976) shows the bacterial flora of the slime of fish and the changes in the bacterial flora of fish after storage at chill temperatures respectively.

Although the methods of identification and general taxonomic situation are not generally regarded as entirely satisfactory, qualitatively, bacteriological changes during ice storage have been studied in both tropical and cold water species of fish, and the groups of organisms involved in spoilage appear to be similar (Shewan, 1971; Listen; 1969; Da Silva and Mendez, 1963; Karthiyani and Mahadeva, 1963; Velanker and Kamasastri 1956; and Wataniabe, 1965-1966) cited by (Disney *et al.*, 1974).

Disney *et al.* (1974) cited the following qualitative changes in fish stored in ice. On spoiling cod, *Pseudomonas* spp. constitute 90% of the total flora after 12 days in ice (Shewan, 1974). Qualitative studies carried out on West African sea bream (Tropical Products Institute, unpublished) showed that the predominant organisms on the skin are *Pseudomonas* spp. but the dominant species in the flesh throughout storage were *Moraxella* spp. Similar studies on *Tilapia* spp. found that the early predominance of *Micrococcus* spp. and *Staphylococcus* spp. is replaced by *Pseudomonas* spp.. Valenkar and Kamasastri (1956) after investigating bacterial spoilage in several Indian marine fish, found that the generic succession during the course of spoilage reflected the pattern recorded by Disney in one of their experiments. This was attributed to the influence of environmental factors on the bacterial flora of the fresh material . A generic succession of the type found in cod was reported by (Da Silva and Mendez, 1963) and (Wood, 1949) although the latter found that the dominant species during spoilage of elasmobranchs were gram-positive organisms of the type which are only present in significant numbers in teleost fish during the early stages of spoilage.

Table IV. The bacterial flora of the slime of fish (after various authors).

Area	Species Of Fish	Gram Negative Rods %	Coryneforms %	Micrococci %	Bacillus %	Others %
North Sea	Cod	88.3	8.7	1.1	-	1.9
	Skate	81.8	3.4	-	-	12.8
	Lemon sole	83.2	-	-	-	16.8
	Various Species	88.0	-	5.6	1.6	5.0
Norway	Herring	82.2	-	16.7	-	1.1
Adriatic	Hake	43.7	-	33.6	4.2	19.3
Canada	Cod	86.9	-	8.7	-	4.4
West Africa	10 Different Tropical Species	65.6	12.2	17.7	-	4.2
South Africa	West Coast Hake	86.9	8.2	3.3	-	1.6
	East Coast Hake	8.4	33.3	37.5	-	20.8
India	Mackerel	18.3	-	27.0	50.8	3.0
	Sardine	86.0	7.0	6.0	-	-
Japan	Flat Fish	66.7	29.6	3.7	-	-
		82.6	10.4	-	-	6.9
Australia	Elasmobranchs	11.0	61.0	17.0	2.0	-
	Teleosts	16.0	12.0	60.0	8.0	9.0
	Teleosts (4 Species)	35.0	12.0	51.0	2.0	4.0

Table V Changes in the bacterial floras of fish after storage at chill temperatures

	DAYS ON ICE							
	(1)		(2)		(3)		(4)	
	0	7	0	8	Fresh	Spoiled	0	8
<i>Pseudomonas 1</i>			14	3			-	8
<i>Pseudomonas 2</i>	20	53	14	53	18	57	16	53
<i>Pseudomonas 3</i>			3.5	31			-	-
<i>Achromobacter</i>	29	13	-	-	-	-	42	25
<i>Acinetobacter</i>	-	-	32	8	8	26	-	-
<i>Moraxella</i>	-	-	19	3	1	4	2	1
<i>Flavobacterium</i>	25	1	18	0	8	-	-	-
Coryneforms	16	2	-	-	12	1	28	10
<i>Micrococcus</i>	1	-	-	-	49	7	10	2
<i>Vibrio</i>	13	3	-	-	-	-	-	-

(1) Simidu et al., (1969)

(2) Lee and Harrison , (1968)

(3) Gillespie and McRae, (1975)

(4) Shewan et al., (1961)

2.2 MEANS OF PREVENTING DETERIORATION IN FISH

In Section 2.1, the mechanisms that occur in fish flesh after death and which produce changes making the fish unacceptable to the consumer were discussed.

The principal aim of fish preservation is to delay or inhibit the microbial spoilage (Hansen, 1979). In the case of fatty fish the preservation may also aim at reducing or inhibiting oxidation and other undesirable changes in the fish oils, which are highly unsaturated and capable of going rancid at various stages of processing.

Fish preservation by freezing, chilling and canning are widely used in all parts of the world, especially the more developed nations. There are however, several other preservation techniques used. Some of these such as salting, smoking, fermenting and drying, were in use long before canning and or refrigeration were used. Others such as gas exchange, are of a more recent origin and have not yet been fully developed to commercial application (Wheaton, 1985). Techniques for shelf life extension of fresh aquatic products such as Modified Atmosphere Storage, are also relatively new but have been commercialised to at least a limited extent.

Methods such as canning and curing change the character of the fish substantially, while the sequence of freezing, cold storage and thawing normally aim at retaining the fresh fish character as much as possible. This is also the aim of chilling and modified atmosphere storage, which are short term methods for the preservation of wet fish. The mechanisms of some of these methods will be briefly discussed in the following sections. Modified atmosphere packaging which is the principal method used in the present work will be discussed in detail.

2.2.1 Preservation of Fish by Drying

Fish are typically 73-85% water. At atmospheric temperatures of 5°C or higher, fish products are rapidly attacked by bacteria and moulds, causing rapid quality deterioration. Since moulds and bacteria require water to support their life processes, drying fish and fish products to a moisture content below that required for growth of bacteria and moulds greatly reduces the deterioration rates and extends shelf life.

Fish or fish muscle with low oil contents are usually more suitable for drying than fish with large contents of oil, which may rapidly oxidise and become rancid (Hansen, 1972).

Drying of whole fish is most often carried out using sun drying or mechanical tunnel dryers. In many areas where climatic condition permits Fish are simply laid on the beach or rocks in the sun and allowed to dry. Since thickness is a critical factor in drying, the larger fish are often split or filleted. This technique is inexpensive, but results in considerable loss due to bacterial spoilage, rodents and insects. The resulting products are also very variable.

Mechanical air dryers which are widely used range in sophistication from a simple enclosure with a fan to total air temperature and humidity control. Air drying is a lengthy process taking weeks or months, whereas the controlled atmosphere air drying plants are quick and overcome the problems of the natural drying mentioned earlier. However, they are highly energy consuming, a fact which in the future may prohibit their use (Hansen, 1979).

2.2.2 Fish Preservation by Salting

The size, and the oil content of the fish are the main factors governing the technology of salt preservation. Small fish and thin flat fish may be salted whole. Larger fish have to be eviscerated, split, filleted or opened before salting otherwise the salt does not penetrate fast enough to prevent spoilage in the centre part of thick whole fish. With oily fish, contact with air must be avoided to prevent oxidative rancidity during and after salting. Fish low in oil do not need such protection.

There are three distinct types of salting processes, i.e. dry salting, brine salting and rapid salting (Wheaton, 1982). These general types are known by various names depending on the geographical area, the amount of salt used for their production or the fish preparation prior to salting. Dry salting is carried out by stacking fish and dry salt in alternate layers. The water extracted from the fish is allowed to drain off as soon as it is freed from the fish. Brine salting is the process of immersing the fish into a salt brine and keeping it in contact with the brine, at least until it has absorbed the desired amount of salt. Rapid salting refers to one of several processes by which salt absorption rate is increased, for example, when fish is boiled in salt brines.

Regardless of whether dry or brine salting is done, there are several levels of salting, the primary difference being the amount of salt added to the product.

The salt content of light cures is not high enough to prevent growth of normal spoilage bacteria, thus lightly cured fish are highly perishable and must be kept refrigerated prior to drying. Medium salted fish have salt content in the 8-12 percent range (wet basis), the range in which most normal spoilage bacteria experience stress or have ceased to grow. Heavy or hard cured salted fish have salt contents above 12 percent, generally in the 20 percent or higher range (Wheaton, 1985). Salting alone does not stabilise fish products sufficiently to allow long-term storage. After salting therefore, most products are further dried.

2.2.3 Preservation of Fish by Smoking

Smoking is a preservation process combining drying and deposition of the chemical constituents produced by the thermal decomposition of organic materials, usually wood of some type.

Although smoking is a very old preservation process, it is still widely used today in many under-developed countries, whereas in developed countries, smoking is generally used more to impart flavour and colour than for preservation purposes.

Smoking may be divided into four basic techniques, (1) cold smoking, (2) hot smoking, (3) liquid smoking and (4) electrostatic smoking.

Cold smoking is drying and smoking at temperatures below 30° C. The resulting product is not cooked and unless heavily salted, must be treated as a perishable product.

Hot smoking is carried out at temperatures above 30° C, typically in the 70 to 80° C range. The resulting product is stable and can be stored for a long period of time.

Liquid smoking and electrostatic smoking are both used as means of imparting smoked flavour and colour to the product and not for preservation purpose (Wheaton, 1985).

2.2.4 Preservation of Fish by Chilling Methods

Chilling is an extremely effective way of reducing spoilage if fish are chilled quickly and kept chilled and also handled carefully and hygienically. The objective in chilling is to cool the fish as quickly as possible to as low a temperature as possible without freezing them.

Chilling can never prevent spoilage but, in general terms, the colder the fish are, the greater the reduction in bacterial and enzymatic action. To chill fish, they must be surrounded by a medium which is colder than the fish themselves. The medium can be liquid, solid or gaseous.

Ice is an ideal cooling medium. It has a very large cooling capacity for a given weight or volume, and can cool the fish quickly through intimate contact with the fish. For effective chilling, the ice must be allowed to melt. The melting ice keeps the fish moist and glossy (Clucas, 1981).

An alternative method of lowering the temperature of fish is to immerse them totally in some chilled liquid medium, held in tanks. The two basic systems used to hold fish in tanks are refrigerated sea water (RSW) System, where some mechanical means is used to chill sea water down to about -1°C , or the Chilled Sea Water (CSW) System, where fresh water ice is mixed with sea water to lower the temperature. Seawater can be chilled down to -2°C before it freezes, and as long as the fish do not freeze, the extra chilling margin of mechanical chilling over ice is beneficial (Wheaton, 1985).

By using ice and other chilling techniques fish can be kept in fresh condition from a few days up to four weeks or so depending on the species of the fish. In many situations, it is desirable to be able to keep the fish fresh for longer than a few weeks, e.g. for export to distant countries, to even out supplies because of seasonal variations in catch and when grounds are a long way from port. Chill storage does not keep the fish fresh for prolonged periods. However, it is possible to produce a product which resembles fresh fish by using freezing and cold storage.

2.2.5 Preservation of Fish with Modified Atmospheres

Fish is one of the most perishable of foods. In unfrozen aquatic foods, bacterial activity is responsible for the most pronounced offensive changes in odour and flavour. Lipid oxidation in fish with high fat content results in rancidity. Endogenous enzymes adversely affect the texture of fish, but their activity is relatively insignificant during commercial storage periods (Liston, 1965) cited by (Wilheim 1982).

Refrigeration has for many years been successfully used to retard spoilage of fresh fish. However, at 0°C the shelf life of a lean fish such as atlantic cod, *Gadus morhua*, is about fourteen days and at 5°C the shelf life is only about six days, after which the product must be discarded (Ronsivalli and Charm, 1975) cited by (Wilheim, 1982).

Fresh aquatic food products as well as other muscle foods are savoured by many consumers. Moreover, the fresh forms of these foods are generally considered to be superior in quality to their frozen or processed counterparts in terms of cooking preparation, texture, flavour and odour.

As a supplement to refrigeration, variations in the pressure and gaseous composition of storage conditions have been proposed as methods of extending the fresh storage life of fishery products (Statham, 1984).

In literature, the term "modified atmosphere" is usually limited to those storage conditions where the atmospheric gas concentrations are altered before storage.

In "controlled atmosphere" systems, the selected atmospheric concentrations of gases are actively maintained throughout storage. However, in a general sense, atmospheric modification may include any deviation from normal atmospheric pressure or composition. The pressure is reduced under hypobaric storage conditions and increased under hyperbaric storage conditions.

Packaging of products in a modified gas atmosphere involves the replacement of air by other gases, usually carbon dioxide (CO₂), nitrogen (N₂) and oxygen (O₂), alone or in

combination. The aim is to inhibit physical, chemical, and microbiological changes that lead to product deterioration (Statham, 1984). The composition of the modified atmosphere depends on the type of product being packed. In order to maintain the correct atmospheric composition around the product, it must be wrapped in a packaging film with low gas permeabilities.

2.2.5.1 The Role of the Principle Gases Used on Modified Atmosphere Packaging of Fresh Fish and Other Muscle Foods

Several gaseous mixtures have been studied for use in food preservation. Haraguchi *et al.* (1969) cited by Wilhelm (1982) found ozone to have a preservative effect on fish, while ammonia was reported to effectively preserve fish for two months at ambient temperatures (Subrahmanyam *et al.*, 1965) also cited by (Wilhelm, 1982). Ethylene oxide, nitrous oxide and other bactericidal or bacteriostatic gases have been investigated, but they are generally not considered practical for the preservation of fresh fish because of their inherently toxic properties. Low levels of carbon monoxide, have also been included in modified atmospheres to prevent discoloration of flesh caused by the production of metmyoglobin. High levels of carbon monoxide (CO) 10-30% have inhibitory effects on the growth of some bacteria (Gee & Brown, 1981) cited by (Statham, 1984). However the level proposed for use in packaging fresh foods (1% CO) appears to have negligible effects on bacterial growth (Brown *et al.*, 1980).

The principal gases that have been used with success in modified atmosphere systems are, nitrogen, oxygen and carbon dioxide. They are produced inexpensively being natural components of the environment. Oxygen and hydrogen are obtained by taking air, cleaning all the pollutants out of it, drying it and passing it through a compression/expansion refrigeration cycle, so that it may be separated on a refractive distillation column into its constituent gases (Leeson, 1984). Carbon dioxide may be obtained as a by-product from many chemical processes. The most abundant source is the ammonia process which produces over one ton of carbon dioxide to every ton of ammonia (Leeson, 1984).

Nitrogen has a negligible effect on bacterial growth and on shelf life of fresh foods (Coyne, 1932; Enfors, Molin and Termstrom, 1979; Fey, 1980; Tiffney and Mills, 1982). It serves as an inert filler to balance a gas mix. However, nitrogen inhibits lipid oxidation and mould

growth by the exclusion of oxygen. The presence of as little as 0.1% unsaturated fatty acid in a product being packed is enough to cause the development of rancid flavours, when oxygen is present.

Reduction of the oxygen levels in the pack to less than 2% greatly retards the formation of rancid flavours. This is usually achieved by flushing the pack with an inert gas so that the normal atmosphere is displaced. Nitrogen is normally preferred to carbon dioxide in the context that it does not dissolve into the fatty tissue of the food as does carbon dioxide, and that it also permeates packaging films far more slowly as shown in Table VI.

Oxygen sustains the basic metabolism of freely respiring foods, such as fruits and vegetables and oxygenates the pigments haemoglobin and myoglobin in red meats to form the desirable bright, cherry-red colour (Leeson, 1984). High levels of oxygen have been used in modified atmosphere storages. Using white fish, Tiffney and Mills (1982) showed some extension of quality as judged by organoleptic assessment, but the storage life in 100% oxygen did not exceed that of similar fish in vacuum or overwrap packs. In general, the proportion of oxygen used in modified atmosphere storage systems is either equal to or lower than that present in atmosphere.

Of the methods for modification of atmospheric composition, the use of carbon dioxide has been investigated the most thoroughly. Carbon dioxide is effective for extending the shelf life of perishable foods by retarding bacterial growth. The overall effect of carbon dioxide is to increase both the lag phase and the generation time of spoilage microorganisms (Daniels *et al.*, 1985).

However, the specific mechanism for the bacteriostatic effect of CO₂ is not known.

Displacement of oxygen and intracellular acidification were possible mechanisms that were proposed then discounted by earlier researchers. Valley and Rettzer (1927) cited by Statham (1984) suggested that CO₂ acts by lowering substrate pH. However, Coyne (1932) and Haine (1933) demonstrated that pH alone does not account for the inhibitory effect of CO₂.

There are two main hypotheses that have been put forward (Parkin and Brown, 1982). The

first considers the inhibitory effect of CO₂ to be on enzyme systems necessary for growth.

King and Nagel (1975) cited by Statham (1984) showed that a 50% CO₂ atmosphere had a mass action effect on certain enzymatic decarboxylation reactions. *Pseudomonas aeruginosa* was found to have decreased isocitrate dehydrogenase and malate dehydrogenase activity.

The second theory suggests that CO₂ acts on the cell membrane, altering contact between the cell and its external aqueous environment by redistribution of lipids at the interface. This phenomenon has been demonstrated using a model system. (Sear and Eisenberg, 1961) cited by (Statham, 1984). This theory has also been proposed as the mechanism by which CO₂ inhibits bacterial spore germination (Enfors and Molin, 1978) cited in (Enfors and Molin, 1980).

Table VI Typical Gas Permeabilities Of Packaging Materials

Packaging film	Oxygen	Carbon Dioxide	Nitrogen
PVDC	0.05 (5)	0.29 (29)	0.01 (1)
PET	0.3 (6)	1 (20)	0.05 (1)
PVC	3.6 (3)	23.6 (23)	1.05 (1)
POLY PROPYLENE	23 (5)	92 (21)	4.4 (1)
POLY AMIDE	0.38 (3)	1.6 (11)	0.15 (1)
POLY ETHYLENE	35 (3)	161.5 (14)	11.65 (1)
ETHYL CEL.	265 (3)	410 (5)	84 (1)
CEL. ACETATE	5.9 (2)	102 (31)	3.3 (1)

$$\times 10^{-10} = \text{cm}^3 \cdot \text{mm} \cdot \text{Sec}^{-1} \cdot \text{cm}^{-2} (\text{cm Hg})^{-1}$$

Source - Gas encyclopaedia L'AIR LIQUIDE

cited by Leeson, (1984).

(The numbers in bracket gives a relationship of oxygen and Carbon dioxide permeation through the packaging material in relation to Nitrogen.)

2.2.5.2. Microbial Flora on Fish Stored in Modified Atmosphere Packaging

The initial flora of meat or fish taken directly from the slaughter line or aquatic environment respectively consists of a wide spectrum of different micro-organisms. However, whether certain bacteria will grow and how fast they grow is dependant on the environment. The micro-organisms best suited to the environment will be the most successful ones in

competition and will hence be the ones that will outgrow the others and dominate the spoilage flora.

High concentrations of CO₂ (20-100%) in the gas atmosphere around the meat (as used in the modified atmosphere packaging) have been proven to greatly influence development of the spoilage flora of refrigerated meat. Storage in CO₂ does not only prolong the shelflife of meat by retarding microbial growth, it also selects for lactobacilli and inhibits *Pseudomonas* spp. (Blickstand *et al.*, 1981; Enfors *et al.*, 1979; and Erichsen, 1981). Banks *et al.* (1980) with their study on shelf life of CO₂ packaged fin fish from the Gulf of Mexico speculated in their conclusion that, the presence of CO₂ not only limits microbial growth but also causes changes in the type and distribution of micro-organisms. They reported at least a log reduction in bacterial counts after 2 days in atmospheres containing CO₂ compared to fish held in air. They also reported that the microbial population on the fish changed from predominately gram-positive (coryneforms, and *Micrococcus* spp.) to gram-negative (*Pseudomonas* spp. and *Moraxella* spp.) during storage in air, whereas during storage in CO₂, the fish exhibited the opposite distribution in bacterial types. As the storage proceeded in CO₂ they found an increase in gram-positive organisms, mainly *Lactobacillus* spp.

However when fish stored under CO₂ atmosphere were removed and put into a typical supermarket retail case, the trend exhibited in the modified atmosphere were immediately reversed. The inhibition^{effect} of CO₂ on bacterial numbers was terminated upon opening the package and immediate acceleration in the growth of gram-negative common type of spoilage organisms took place.

There has been conflicting data on the type of micro-organisms that grow in CO₂ enriched atmospheres. Wolfe (1980) cited by Richter *et al.* (1982) reported that spoilage of fresh fish due to aerobic psychrotrophic bacteria such as oxidase-positive Pseudomonads, remain a primary problem. On the other hand Banks *et al.* (1980) observed a higher percentage of gram-positive bacteria (*Lactobacillus* spp.) in CO₂ packaged fish than fish packaged without CO₂. However, Gill and Tan (1980) cited by Richter (1982) reported that this was an

oversimplification because species from both groups may be insensitive to CO₂. The findings of Richter *et al.* (1982) suggest that the Pseudomonads (oxidase-positive psychrotrophs) were present in high numbers throughout the shelf-life studies and in some instances were equal or exceeded all other types. Gill and Tan (1980) stated that the dominant position of Pseudomonads, in the spoilage flora of CO₂ - packaged meat was maintained although some enrichment of unaffected species did occur.

Stenstrom (1984) working on cod fillets reported that the time needed for CFU's (colony forming units) to reach 1x10⁶ g was about 5 times in the atmosphere containing 50% of CO₂ than in air, while with 90% and 100% CO₂ it was 10 and 14 times respectively. He reported that while the initial flora of cod fillets were dominated by organisms in order of *Flavobacterium*, spp, "Coryneforms", *Pseudomonas* spp. and *Moraxella* like organisms, the spoilage flora of the fillets stored in air was dominated by *Alteromonas putrefaciens* (62%) and non-fluorescent *Pseudomonas* spp. (28%). In 50% CO₂/50% O₂ he reported equal percentages of homofermentative *Lactobacillus* spp. and *Alteromonas putrefaciens*. In 50% CO₂/ 50% N₂, the flora to be dominated by *Alteromonas putrefaciens* 75% and Enterobacteriaceae 12%, while in gas atmospheres with 90-100% CO₂, homofermentative *Lactobacillus* spp dominated the spoilage flora (62 - 85%) with lower incidences of *Alteromonas putrefaciens* (12-30%).

2.2.5.3 Extension of Shelf Life Resulting from Modified Atmosphere Storage

Shelf life is a difficult term to define since it may be based on criteria derived from chemical, microbiological or organoleptic assessment employed singly or in combination. The ultimate measure of shelf life is a consumer satisfaction, which is more significant than defining the end point as, when arbitrarily defined levels of spoilage components or bacterial numbers are reached (Regenstein and Regenstein, 1981).

The definition of the time at which shelf life begins is also uncertain. It may be taken as the time of catch or death or the time at which processing is completed. These inconsistencies make comparisons between sets of data of different origin very difficult.

As early as 1930, Killefer showed that the shelf life of both meat and fish could be extended up to 2-3 times as long in pure CO₂ as in air at the same temperatures.

In Callow (1932) cited by Kirk *et al.* (1981) found that the use of 100% CO₂ extended the shelf life of pork and bacon beyond that observed in these products stored in normal air atmospheres.

Stansby and Griffiths (1935) reported that freshly caught haddock held in ice kept twice as long in CO₂ as in air.

Recently, Cann *et al.* (1983) on their further studies on marine fish stored under modified atmosphere packaging concluded that, packaging of cod in gas atmosphere 40:30:30% / CO₂:N₂:O₂ extends its shelf life by 40-80% depending on the quality index used. They also concluded that extension of shelf life of herring when packaged either in 40:30:30% / CO₂:N₂:O₂ or 60:40% / CO₂:N₂ is less certain. Their observations were that, in terms of bacteriological and chemical criteria, the results are similar for cod. However, sensory analysis showed no extension over vacuum packaged controls.

They further concluded that no extension of shelf life was found for hot-smoked mackerel when stored in either 40:30:30% / CO₂:N₂:O₂ or 60:40% / CO₂:N₂. In all cases, the temperature of storage was critical, whereby fish stored at 5°C suffered marked reduction in shelf life in comparison with fish stored at 0°C.

Cann *et al.* (1984) in their report on the Studies of Salmonids gave the following conclusions:

- 1 There was no clear extension of shelf life for fresh whole trout when compared with the controls (vacuum packaged).
- 2 Using sensory assessment of flavour as the measure of spoilage, there was no extension of shelf life at a 95% confidence level.
- 3 Bacteriological and chemical analysis indicated that there was an overall trend of delayed

spoilage in modified atmosphere packaging with the effect being greater at 0°C.

- 4 The results for salmon steaks were similar to that of trout, but at 0°C MAP fish had a slightly extended shelf life when compared with the vacuum packaged controls, using sensory assessment of flavour as a measure of spoilage. However, there was a considerable scatter amongst the data. This slight extension of shelf life was lost at the higher storage temperatures of 5-10°C.

Cann (1985), recommended that for a white fish, scampi and scallops, a mixture of 40% CO₂ 30% N₂ and 30% O₂ gives the best results, while for Salmon, trout, and fatty fish such as herring, mackerel and the smoked products, a mixture of 60% CO₂ 40% N₂ would give better results.

An interesting aspect of the CO₂ effect was shown by (Ogilvy and Ayre, 1951) cited by (Mead, 1983). They speculated that transferring CO₂ - stored poultry meat to normal atmospheric conditions of cold storage would still give an extended shelf life which tends to be intermediate between the keeping time in air alone and that in the CO₂ atmosphere. A similar after-effect was reported for poultry meat by Bailey *et al.* (1979) and by Silliker and Woulfe (1980) cited by Mead (1983).

Blickstad *et al.* (1983) in their study stated that storage in 100% CO₂ at 0°C gives the meat a shelf life of about 3 months, while combination of curing and storage in 100% CO₂ at 0°C gives the meat a shelf life of more than 5 months.

Table VII from Statham (1984) shows the shelf life obtained in studies of modified atmosphere storage of various types of fish by different authors.

Table VII Shelflife of Modified Atmosphere Storage of Various Types of Fish

Author	Atmosphere	Species	Storage temp(c)	Shelf-life (days)	Shelf-life extension (%)
Coyne, (1933)	100% CO ₂	Cod, Whiting, Haddock	0	10-12	50-100
Stansby & Griffiths, (1935)	25% CO ₂	Haddock	0	25	100
Fey, (1980)	60%CO ₂ :21%O ₂ : 19%N ₂	Red hake	0-1	>27	
	60%CO ₂ :5%O ₂ : 35%N ₂	Red hake	0-1	>27	
	20%CO ₂ :21%O ₂ : 59%N ₂	Red hake	0-1	>27	
Lee, (1982)	20%CO ₂ :20%O ₂ : 60%N ₂	Dover sole	0		100
	60%CO ₂ :20%O ₂ : 20%N ₂	Dover sole	0		200
Stier et al., (1981)	100% CO ₂	King salmon	4.4	12	100
	100% CO ₂	King salmon	22	<2	<100
Barnett et al., (1982)	90% CO ₂ : 10% air	Chum & Coho			
		Salmon	0	>21	
Haard & Lee, (1982)	100% CO ₂	Salmon	3	>20	>100
Mills & Tiffney, (1982)	40% CO ₂ :30% N ₂ : 30% O ₂	Various White fish	0	15	70
			2	8	60
Cann , Smith & Houston, (1983)	40% CO ₂ : 30% N ₂ : 30% O ₂	Cod	0	8.5-12.5	40-80*
Wang & Brown, (1983)	80% CO ₂ : 20% air	Freshwater crayfish	4	>21	>50

* Depending on quality criterion used.

2.2.5.4 Effect of Modified Atmosphere Packaging on Growth of Pathogens

Both modified atmosphere and vacuum packaging, (which is technically a form of modified packaging), of frozen fish and other fresh foods have been criticized as hazardous because of the anaerobic environment usually created in packages. Anaerobic conditions are classically associated with the growth of *Clostridium botulinum*, and *Clostridium botulinum* type E is known to be present on many aquatic foods.

The effect of CO₂ on the growth of various pathogens at both chill and abuse temperatures has been studied widely.

According to Silliker and Wolfe (1980), Goodfellow (1982), Lee (1982) and Silliker (1982) cited by Statham (1984) modified atmosphere storage does not appear to increase the hazards from *Salmonella* spp. *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Enterococcus* spp. above those expected for air stored product. Silliker (1982) showed that CO₂ caused some reduction in numbers of staphylococci at temperatures up to 20°C, whereas contrary to these findings, (Tomlins *et al.*, 1982) cited by (Statham, 1984) showed that levels of CO₂ from vacuum packaging up to 60% stimulated the growth of *Salmonella enteritidis*.

The pathogen of most concern in fish is the anaerobe *Clostridium botulinum* type E. This organism is known to occur naturally in fish as it is found in marine and lake sediments. It is unusual in that it has an ability to grow and produce toxin at low temperatures > 3.3°C and has a relatively heat resistant spore, which are more sensitive to heat at low pH (Harrigan *et al.*, 1976).

The non-proteolytic organism only becomes a hazard in mishandled processed products, for example canned and smoked products, and more recently, in vacuum packaged products. The occurrence of *Clostridium botulinum* in canned and smoked products primarily depends on the quality control of thermal processing whereas hazardous vacuum packaged products are more likely to have been subject to consumer abuse. Vacuum packaged products held under proper refrigeration normally spoil long before *Clostridium botulinum* can grow to a

dangerous level (Hardy and Hobbs, 1968) cited by (Boone, 1986).

With the unprocessed fish, there is an increased margin between spoilage and toxin production as the temperature of storage is lowered below 10°C. At high temperatures toxin can be produced before the fish is spoiled, but below 10°C, putrefaction will generally be evident before detectable amounts of toxin are produced (Hobbs, 1976) cited by (Boone, 1986).

When a new process is introduced to prolong the shelf life of fresh fish, it can be assumed that the rate of growth of the spoilage bacteria will be altered in some way. Refrigeration causes a substantial delay in microbial growth. Additional preservation techniques such as modified atmosphere storage will affect the growth of the naturally occurring microflora. By inhibiting the growth of the spoiling micro organisms, poor competitors such as *Clostridium botulinum*, *Staphylococcus aureus* and lactobacilli, if present, may be allowed to grow.

According to Lindsay (1982) some believe that the inclusion of some oxygen with nitrogen or carbon dioxide in modified atmosphere will prevent hazards from botulism in fresh packaged fish is a misconception and a false sense of security. He states that it is a well known fact that *Clostridium botulinum* type E will grow in fresh fish if it is abused sufficiently at high temperatures (20° - 30°C) regardless of the presence of oxygen or carbon dioxide.

Table VIII summarises the results of a number of studies undertaken to assess the botulism risk of modified atmosphere storage of fisheries products. From it the following conclusions can be drawn:

- 1 The temperature of product storage is very critical. At 10°C, toxin production was evident between 6 and 11 days both in vacuum packaging and modified atmosphere packaging of cod. However, in all cases the product was spoilt before toxin production.
- 2 At higher temperatures (15° - 20°C) toxin production period shortened, depending on temperature as well as the CO₂ concentration. For example, at 20°C and with modified atmosphere of 60/40% CO₂/N₂, toxin was produced within 2 days. With modified atmosphere of 40/30/30% CO₂/N₂/O₂, toxin production was evident within 2 days at

15°C and 1 day at 20°C.

- 3 Overall, it is evident that conditions associated with toxin production by *Clostridium botulinum* in modified atmosphere packaged fish are complex and require further research.

From a practical viewpoint, temperature abuse appears to be a highly significant feature associated with toxin production. The presence of oxygen as part of the gas mixture in the pack does not give any safeguard against toxin production particularly as the temperature of storage increases.

Although in most instances where data is available, spoilage occurs prior to demonstrable toxigenicity, this does not take into account the possibility of inadvertent ingestion by a consumer in spite of the presence of off odour and/or off flavour.

TABLE VIII Growth and toxin production by Clostridium botulinum in MAS fish products

Author	Species	Storage temperature (°C)	Atmosphere	Inoculum	Toxicity (storage time)	Spilled before being toxic
Anon. (1979)	Salmon	25	Stored in 7% CO ₂ for 17 days then inoculated and stored at 25°C or 10°C	Types A & E — method of inoculation not specified		+
		10	As above	As above		+
		25	7% CO ₂	Type E	+ (24 h)	-
		25	7% CO ₂	Type A	+ (48 h)	-
		10	7% CO ₂	Type E	+ (14 d)	+
		10	Air	Type E	+ (7 d)	+
		10	CO ₂ or air	Type A		+
		5	CO ₂ or air	Type A		+
Huss <i>et al</i> (1980)	Smoked herring	15	99.5% CO ₂	Type E 10 ⁷ spores/g. surface inoculum	+ (4 d)	-
		15	46.1% CO ₂ :53.8% N ₂	As above	+ (4 d)	-
		15	23% CO ₂ :77% N ₂	As above	+ (4 d)	-
		15	90% N ₂	As above	+ (4 d)	-
		15	Vacuum	As above	+ (4 d)	-
		15	48% CO ₂ :52% O ₂	As above	+ (10 d)	-
		15	99% O ₂	As above	+ (9 d)	-
		15	Air	As above	+ (6 d)	-
Lindsay (1982a)	Rockfish	27	Unsealed vacuum, partial CO ₂ and 100% CO ₂	Mixed type E strains 5 & 50 spores/g	+ (24 h)	-
		21	As above	Site of inoculum not specified	+	-
		7.2	As above	As above	- (29 d)	-
		4.4	As above	As above	- (29 d)	+
		1.7	As above	As above	- (29 d)	+
Sher <i>et al</i> (1981)	Salmon	22.2	60% CO ₂ :35% O ₂ : 15% N ₂	Types A, B and E spores — type E cells 10 ⁷ /g. surface inoculum	+ (2-3 d)	+
		4.4	As above	As above	- (28 d)	+
		22.2	Air	As above	+ (2-3 d)	+
		4.4	Air	As above	- (28 d)	+
Eklund (1982)	Salmon	25	60% CO ₂ :35% O ₂ : 15% N ₂	Type E, 10 ⁷ spores/ 100 g — site of inoculum not specified	+ (48 h)	-
		25	90% CO ₂ :10% air	As above	+ (48 h)	-
		25	Air	As above	+	-
		10	60% CO ₂	Type E 10 ⁷ spores/ 100 g	+ (10 d)	-
		10	90% CO ₂	As above	+ (10 d)	-
		10	Air	As above	+ (10 d)	+
		5	60% CO ₂	Type E 10 ⁷ spores/ 100 g	+ (21 d)	-
		5	90% CO ₂	As above	+ (21 d)	+
Cann. Smith & Houston (1983)	Cod	10	40% CO ₂ :30% O ₂ :30% N ₂	Types B & E 10 ⁷ spores/g deep muscle	11 d	+
		10	Vacuum	Surface	10 d	+
		10	Vacuum	Site of inoculum not specified	8 d	+
		20	40% CO ₂ :30% O ₂ :30% N ₂	Deep muscle	2 d	-
		20	40% CO ₂ :30% O ₂ :30% N ₂	Surface	2 d	-
		20	Vacuum	Site of inoculum not specified	2 d	-
	Herring	10	40% CO ₂ :30% O ₂ :30% N ₂	Deep muscle	7 d	+
		10	40% CO ₂ :30% O ₂ :30% N ₂	Surface	9 d	+
		10	Vacuum	Site of inoculum not specified	7 d	+
		10	60% CO ₂ :40% N ₂	Deep muscle	6 d	-
		10	60% CO ₂ :40% N ₂	Surface	8 d	-
		10	Vacuum	Site of inoculum not specified	6 d	+
		15	40% CO ₂ :30% O ₂ :30% N ₂	Deep muscle	2 d	-
		15	40% CO ₂ :30% O ₂ :30% N ₂	Surface	2 d	-
		15	Vacuum	Site of inoculum not specified	1 d	-
		15	60% CO ₂ :40% N ₂	Deep muscle	2 d	-
		15	60% CO ₂ :40% N ₂	Surface	2 d	-
		15	Vacuum	Site of inoculum not specified	1 d	-
		20	40% CO ₂ :30% O ₂ :30% N ₂	Deep muscle	1 d	-
		20	40% CO ₂ :30% O ₂ :30% N ₂	Surface	1 d	-
		20	Vacuum	Site of inoculum not specified	1 d	-
		20	60% CO ₂ :40% N ₂	Deep muscle	2 d	-
		20	60% CO ₂ :40% N ₂	Surface	2 d	-
		20	Vacuum	Site of inoculum not specified	1 d	-
	Smoked mackerel	10	40% CO ₂ :30% O ₂ :30% N ₂	Deep muscle	-	-
		10	40% CO ₂ :30% O ₂ :30% N ₂	Surface	-	-
		10	Vacuum	Site of inoculum not specified	-	-
		10	60% CO ₂ :40% N ₂	Deep muscle	-	-
		10	60% CO ₂ :40% N ₂	Surface	-	-
		10	Vacuum	Site of inoculum not specified	12 d	-
		15	40% CO ₂ :30% O ₂ :30% N ₂	Deep muscle	5 d	-
		15	40% CO ₂ :30% O ₂ :30% N ₂	Surface	-	-
		15	Vacuum	Site of inoculum not specified	6 d	-
		15	60% CO ₂ :40% N ₂	Deep muscle	6 d	-
		15	60% CO ₂ :40% N ₂	Surface	-	-
		15	Vacuum	Site of inoculum not specified	4 d	-
		20	40% CO ₂ :30% O ₂ :30% N ₂	Deep muscle	3 d	-
		20	40% CO ₂ :30% O ₂ :30% N ₂	Surface	4 d	-
		20	Vacuum	Site of inoculum not specified	3 d	-
		20	60% CO ₂ :40% N ₂	Deep muscle	2 d	-
		20	60% CO ₂ :40% N ₂	Surface	3 d	-
		20	Vacuum	Site of inoculum not specified	2 d	-

+ Propagation in CO₂ not stated
- Not used

2.2.5.5 Application and Current Usage of MAP

Carbon dioxide has been used by the food industry for many years, particularly for the preservation of highly perishable, and certain high-value commodities. For instance, controlled atmosphere warehousing of fresh fruits has been practised since the 1920's (Brecht, 1980) cited by (Daniels *et al.*, 1985). This application of carbon dioxide has the benefit of retarding the ripening that is caused by a mass action effect on respiration of stored fruits. This technique is still widely used and has been extended to the long distance transport of different fruits and vegetables in air tight rail-cars, trucks and sea-board containers (Kadar, 1980) cited by (Daniels *et al.*, 1985)

Though not yet as widespread, carbon dioxide has also found application in other foods such as meat and fish where bacterial spoilage must be controlled. This effort has been spurred by recent advances in the packaging industry that has led to development of highly specialised polymeric packaging films, and so have brought about a renewed interest in use of carbon dioxide and other gases for modified atmosphere packaging (MAP) (Daniels *et al.*, 1985).

In the case where carbon dioxide application is expected to give bacteriostatic effects its concentration to produce optimum inhibition of bacterial growth and its potential for the growth of pathogenic bacteria under high concentration is paramount.

With regard to gas concentration, there is considerable ambiguity among various researchers as to optimum values, as well as methodologies for approaching the question.

Valley (1982) cited by Daniels *et al.* (1985) reported that concentrations only slightly above atmospheric can actually stimulate bacterial growth, but that in still higher concentrations bacterial growth is inhibited. Concentrations as low as 10% and as high as 100% CO₂ have been used and recommended by different authors. There is some evidence to support the observation that the bacterial inhibition increases with the concentration of carbon dioxide present in the system. King and Nagel (1967) cited by Daniels *et al.* (1985) controlled the various growth factors for pure cultures of *Pseudomonas aeruginosa* and found a linear relationship between generation time and carbon dioxide level. This relationship was more recently confirmed by (Bliskstad *et al.*, 1981), who concluded that the

bacteriostatic/preservative effect of carbon dioxide increases with increasing concentration. With regard to the growth of pathogens there is evidence that there is no risk involved so long as proper sanitation and temperature controls are employed.

Bell (1982) cited by Statham (1984) suggested three methods by which modified atmosphere packaging may be employed for transportation and distribution. The first method involves bulk transportation in refrigerated seavans, railcars or trailers. The container is loaded with pre-cooled material and the atmosphere injected and sealed in. This kind of system allows continuous monitoring and management of temperature during transport and minimises risks of temperature abuse. Using this method Pacific salmon and Alaskan salmon have been successfully transported (Veranth and Robe, 1979; Bell, 1980; and Schwartz, 1982) respectively cited by (Statham, 1984).

The second method is the master pack concept, which employs permeable over-lap packs of the type used in supermarkets, placed in a large impermeable master pouch flushed with CO₂. This method is said to hold promise in that it provides shelf life and convenience since the masterpack is maintained under strict control during transport and retailers open the pack as required. The system eliminates the need for re-packing in the supermarket and protects the consumer from any misconceptions about the stability of the product. This system has been successful for a Gulf-coast area processor in the USA (Banner, 1979) cited by (Statham, 1984).

The third type of package is the individual consumer pack intended for direct retail sale. Bell (1982) sees this system as hazardous since each pack is a potential 'leaker', susceptible to mishandling and abuse. However, in view of the low degree of contamination, modified atmospheres are being used for pre-packed cod, haddock, plaice, mackerel, dover and lemon soles, crab, lobster and scampi (Tiffney and Mills, 1982a) and smoked fish lines, (Anon, 1983) cited by (Statham, 1984). The development of adequate processing procedures (Cann and Tylor, 1979) also allowed the safe use of modified atmospheres with trout products.

It is likely that with continuing development of packaging materials and equipment, and further research into optimising the techniques effectiveness, carbon dioxide and other gases

will see increased use for preservation of food quality in the food manufacturing and marketing industries.

2.3 METHODS OF ASSESSING FRESHNESS IN FISH

Different methods are used to assess freshness in fish, the term freshness being used rather than spoilage because measurement of freshness implies that the product may still be edible when it has lost freshness whereas spoilage implies that the product is no longer edible once it has started to spoil.

There is no single index of quality or one that can accurately predict the storage life of fresh fish. Objective methods provide value for quantities that can be compared with figures previously reported, and the use of particular methods vary among different species. These methods may be discussed under four headings namely, sensory, chemical, physical and microbiological methods.

2.3.1 Sensory Methods

These are defined as those wholly dependant upon the human senses of taste, smell, sight and touch. As the consumer only uses senses in deciding what he likes, sensory methods as opposed to non-sensory methods, offer the best opportunity of getting a valid idea of what the consumer wants (Connell, 1980). Sensory methods also have a great advantage in that human beings are very adaptable and can switch easily from, for example testing odours to visual inspection for defects. Furthermore, for some tasks, human senses are better at recognising complexities and are more discriminatory than instruments. Their main disadvantages are, that responses can vary, and that using people can be expensive and inconvenient.

When carrying out storage trials it is necessary to make a precise estimate of the quality and to assign a value to it. The scale used assumes that quality ranges from absolutely fresh (just caught) to absolutely putrid (Lima dos Santos, 1982). Freshness can therefore be related to quality along a continuous line between the opposite ends of the scale. For example, one can use a 10 point scale from 1-10 which, for the purpose of statistical analysis, the intervals between each score mark are assumed to present equal differences in quality of the attribute

in question i.e. colour, odour, flavour, texture or any other attribute being measured.

The most commonly used organoleptic testing methods have been described by many writers, (Amerine *et al.*, 1965; Ellis, 1966; Larmond, 1967) cited by (Larmond, 1971). They could be divided into two groups; those which do not require intensive panel training and those which do.

The first group includes:

- 1 triangle test where three coded samples whereby two are identical and one is different and the judge is asked to select the different sample.
- 2 Paired comparisons, where samples are presented in pairs for comparison on the basis of some definite criteria.
- 3 Duo-trio, where a judge selects from two unknown a sample identical to the reference.
- 4 Multiple comparison where the judge rate samples according to difference from a reference sample.
- 5 Finally , ranking, where the judge ranks samples according to the intensity of a particular characteristic.

In order to be effective the judges must be trained to some extent before using these test methods.

In the second group, scoring and descriptive tests are included. In scoring, the judges record reactions to a particular characteristic of samples on a graduated scale, whereas descriptive sensory analysis involves describing the flavour of samples in terms of individual notes, their intensity and order of perception (Larmond, 1971).

Scoring tests are adaptable and can be used to evaluate different characteristics by modifying the descriptive terms. Several workers have used scoring methods for evaluating fish. For example the following researchers cited by (Larmond, 1971), (1) Connors and Steinberg (1964) used a 5 point scale for fried clams, (2) Ostover *et al.* (1967) scored white fish for odour, appearance, texture, flavour and composite grade using 5 point scale, (3) Power *et al.*, (1964 a and b) developed a scoring system for grading taste, texture and overall acceptability of fish, (4) Baines and Shewan , (1965) developed scales by selecting points representing distinguishable characteristics that appear during spoilage of fish. There has

been suggestions that descriptive terms in scoring quality should exclude value judgements such as "excellent", "good" and "bad" and also "strong", "weak" or "mild" (Ehrenberg and Shewan, 1953).

In many cases, it is sufficient to have a few grades rather than detailed scores. This is the basis of the EEC (European Economic Community) grading system where batches of fish are graded E, A, B or unfit. Grade E is very fresh, A is good quality fish and B is acceptable but not good quality.

TFRU system is another grading method that uses very few scores, (0,1,2,3), (0,1,2) or even (0,1) for some attributes. The system was based on experimental observations and experience in that any attribute which visibly altered was incorporated into the scheme (Bremner, 1985). This system gives scores of zero (or near zero) for very fresh fish while increasingly larger totals result as fish deteriorate (Branch *et al.*, 1985).

2.3.2 Chemical Methods

A variety of chemical components or groups of compounds accumulate post mortem in fish flesh. These chemical components are intermediates or end-products of biochemical changes occurring in the muscle of fish after they have died or result from the action of exogenous bacterial enzymes released by the proliferating bacteria on muscle metabolites (Lima dos Santos, 1982).

There are a number of chemical methods as shown below, but taking into account the specific storage trials involved, one should decide which of them to employ if any (Lima dos Santos, 1982).

2.3.2.1 Proximate Analysis

Because of the influence of chemical composition in keeping quality, it is useful to determine the proximate chemical composition, that is, moisture, fat, protein and ash content of the fish samples under investigation. This determination need only be done at the first day of sampling a storage trial.

2.3.2.2 Total Volatile Bases (TVB)

TVB components consist of ammonia (NH_3) and trimethylamine (TMA) and reflects the action of spoilage organisms on non-protein nitrogen. TMA [$\text{N}(\text{CH}_3)_3$] smells like ammonia and is chemically similar to ammonia. It is produced by many spoilage micro-organisms form a compound known as trimethylamine oxide (TMAO), $\text{O}=\text{N}(\text{CH}_3)_3$. TMAO is found in marine species at a level related to the salinity of the habitat (Lima dos Santos, 1982).

For most species the TVB content in the flesh is low during the edible storage period and, increasing amounts of TMA and TVB are found only when the fish is near rejection level (Lima dos Santos, 1982).

2.3.2.3 Hypoxanthine (HX)

This substance accumulates in the fish flesh as a result of nucleotide degradation. ATP (adenosine triphosphate) is degraded via inosine to HX mainly due to autolytic processes, but in the latter phase a bacterial action may also be involved. The advantage of using HX as an indicator of freshness is that there is no lag phase in its formation and that it reflects autolytic as well as bacteriological deterioration. Its disadvantage however, is that, great species differences have been demonstrated.

2.3.2.4 Peroxides

The often highly labile fat in fish is susceptible to oxidation. In the early phases peroxides are formed, and these compounds, being odour-less and flavour-less, can often be detected chemically before any rancidity becomes apparent. These peroxides are eventually further oxidised to aldehydes and ketones, which have a very disagreeable "fish" or "rancid" odour and taste.

2.3.3 Physical Methods

2.3.3.1 pH

The early post mortem changes in fish are associated with a drop in pH from about 7.07 or 7.2 to about 6.2 or 6.5 due to formation of acid compounds. The pH in the muscle increases again during the later phases of spoilage when volatile amines and other basic compounds predominate. However, the pH changes depends on a variety of factors, which makes its determination a very unreliable index of freshness (Lima dos Santos,1982)

2.3.3.2 Torrymeter

Based on the principle that electrical resistance in tissue decreases progressively after death of fish, instruments have been designed for the rapid measurement of the degree of freshness of wet fish.

The Torrymeter is an electronic instrument which measures changes in the dielectric properties of fish as spoilage proceeds. A method based on differences between the ratio of two electrical resistances of natural cell tissue as measured by two electrical currents of different frequencies was developed by (Hannings ,1963) cited by (Fey,1980). Wittfogel and Schlegel (1969) cited by Fey (1980) found that the cell walls of tissue became more permeable after death and during storage, and slowly lose their capacitance, or dielectric properties. Hanning claimed that this property could be directly correlated with freshness. International Electronics Company Ltd, Hamburg - Liksted, working with Hannings developed the Interelectron Fish Tester V to measure freshness in fish (Whittfogel and Schlegel, 1965; BCFTL 1965) cited by (Fey ,1980). The instrument, however, proved inadequate for it was heavy and cumbersome to use and the results not always significantly producible (Cheyne, 1975). GR International Electronic Ltd, Almondback, Scotland, through the research effort of the Torry Research Station in Scotland improved the Interelectron Fish Tester IV; the result was the GR Torrymeter (Fey,1980).

The Torrymeter differs from the Interelectron Fish Tester in that it measures the decrease in both the resistance and capacitance of spoiling fish. The values of both properties are dependant on the orientation of the electrodes with regard to the muscle and geometry of the

sample and would be difficult to obtain. However, Gr International Electronic Ltd, resolved the problem by measuring the power factor of intact fish, which is a fraction of the product of the resistance and the capacitance, and found it to decrease uniformly as the fish spoiled (Cheyne,1975) cited by (Fey ,1980). Since the power factor is dependant on temperature, the meter contains an automatic correction thermistor that results in readings corrected to 0°C, the reference temperature. The test is non-destructive and can yield immediate results.

The manufacturer emphasises that although both sensory scores and the power factor decreases during storage of fish in ice, their correlation can be affected by season of catch, fishing grounds, pelagic fish of high fat content, bruising fish, freezing or brining fish. For example, a previously frozen fish will always give values between 0 - 3, and thus, the readings may be used as a way to indicate if samples have been frozen and rethawed. Since the quality of a batch of fish may vary considerably, the meter has an added built-in function which can give the user either a single value or an average of 16 separate determinations.

The relationship between Torrymeter values and various species of fish at different stages of spoilage has been given by (Cheyne ,1975) as cited by (Fey ,1980). Each species requires the establishment of standard set of values that correspond to sensory quality. For example, for whiting, a score of between 4 and 5 would indicate spoiled fish according to the grading system of the European Economic Community.

2.3.4 Microbiological Methods

It is generally accepted that bacterial growth predominates over other causes of deterioration of fish.

The use of direct bacterial counts, total viable counts, and other microbiological assays in the quality assessment of fresh fish was reviewed by (Martin *et al.*, 1978; Farber, 1965; and Reay and Shewan ,1949). The most frequently used tests are the standard plate counts and the enumeration of indicator bacteria of the coliform group such as *E. coli* and toxin producers such as *S. aureus* (Martin *et al.*, 1978).

Castel *et al.* (1948) reported bacterial counts at 37°C as valueless in measuring the degree of spoilage in fresh cod fillets. However, a close correlation was reported to exist between

psychrophilic gram negative ^{bacteria} and keeping time. Mathen *et al.* (1965) as cited by Fey (1980) claimed the bacterial count to be the best index of quality in fishery products, but (Baines *et al.*, 1969) found direct bacterial counts and accelerated bacterial tests too imprecise to predict shelf life.

Standard plate counts have been performed over a wide range of incubation temperatures, but plate incubation at 20° or 25°C for three days is most popular. Although growth rate is most rapid near 20°C, the maximum total crop of bacteria is obtained at 5°C (Martin *et al.*, 1978). There is a general agreement that the psychrotrophic plate count shows the closest correlation with keeping time.

While the determination of the total count gives the quantitative aspect of the bacterial load, the gram stain, motility test and the biochemical analysis of the bacteria that follows gives the qualitative aspect of the bacterial load.

Fish samples for bacteriological analysis may be collected from different parts of the fish, for example, skin, flesh, guts and gills.

2.4 Aims of the Present Work

Fresh fish is not only favoured by many consumers but is generally considered to be superior in quality to the frozen or processed fish. However, due to its high perishable nature, only about 42% of the world's catch of fish is marketed fresh for human consumption (FAO, 1978). The greatest portion of this percentage being consumed in the towns near the landing areas, while the people living in the distant towns have to do with the processed products or pay dearly for the little fresh fish that manage to reach them. If a shelf life were long enough, it would be possible to transport fresh fish to those distant towns or to export fresh fish to overseas countries.

Since it's a well known fact that fresh fish spoilage is caused by aerobic gram negative bacteria at chill temperatures or in melting ice, and the MAP is known to retard the growth of these bacteria as well as to select the type of bacteria that will grow, the specific aims of this study are:

1. To identify an appropriate gas mixture for the storage of fresh, freshwater fish in relation to microbial growth inhibition and organoleptic assessment.
2. To intensively and objectively assess the benefits of modified atmosphere packaging over those of ice storage by use of a semi-trained taste panel.
3. To compare the microbial flora of ice stored fish and the modified atmosphere packaged fresh water fish by isolation and identification at various storage intervals.
4. To determinewhether modified atmosphere packaging at chill temperatures provides a significant shelf life extension of fresh freshwater fish over the ice storage.

3 EXPERIMENTAL

3.1 MATERIALS

Fresh rainbow trout *Salmo gairdneri*, were purchased from Grimsby fish market, after being harvested from Louth Trout Farm Ltd, Louth, Lincolnshire.

The gas used for the modified atmosphere packaging of fish was supplied at the required composition by B.O.C. Ltd.

The gas for the standard curve was supplied by the Deeside Industrial park (UK).

Rigid trays made of PVC/PVDE were supplied by the Young Seafood Company Ltd.

Aluminium foil, 12 UM (813) polyester M1S/50 UM LDPE Compack Bilaminate was purchased from Comvac Ltd, (England) for making the pouches.

All the microbiological media used in the experimental work, together with gas generating kits (BR38) for anaerobic system, (BR60) for microaerophilic microorganisms and anaerobic indicator (BR55) were supplied by Oxoid Ltd.

3.2 EQUIPMENT

A multivac A300/22 vacuum/gas flushing sealer (West Germany) - evacuation, gas flushing and sealing of the samples.

A Jencons pH m4 pH/Temperature Meter in conjunction with a Russel glass rod combined pH electrode with piercing teat, (Russel pH Ltd, Scotland) - determination of the pH values of the samples.

Gr Torrymeter (Gr International Electronics Ltd, Scotland) - testing the freshness of the samples.

Stomacher Lab Blender 400 (UAC Ltd) - homogenisation of the samples for microbiological analysis.

Oxoid Anaerobic Jars (HP11) of 3.5 litre Capacity (Oxoid Ltd England) - anaerobic and microaerophilic incubation of bacteria cultures.

Incubators (LTE, England) - Incubators.

Gallenkamp Colony Counter (England) - Colony counters.

IBM Personal Computer (MS microprocessor Services, 77a Beverly Road, Hull) - plotting the data into an x-y line graphs.

DG Roland x - y Plotter (Dxy - 880) - to produce graphs.

Perkin Elmer F11 Gas Chromatograph (Perkin - Elmer Ltd) - analysis of the atmospheric changes inside the pouches.

Trivector Trio Chromatograph Computing Integrator (England) - integration of the peaks as well as calculating the areas under the curve.

Chiller (walk-in-type) supplied and installed by Lincolnd Refrigeration, Scunthorpe (UK) - storage of the experimental samples.

Hamilton Gas tight Syringe of 1.0 ml Capacity (Hamilton Bonaduz AG Switzerland) - gas samples from the pouch and injection into the gas chromatograph.

Mettler Instrumente AG PE 1600 scale (Germany) - Measurement of the mean weight of the samples, the weight of packaging trays and mean weight loss of the samples.

3.3 SAMPLE PREPARATION

After arrival at the laboratory, prior to gutting, washing, packaging and icing, three fish were taken for analysis to act as unprocessed controls. The analysis consisted of, pH of flesh, Torrymeter reading, organoleptic scoring and total viable counts.

For the initial experiment, the storage procedure and analysis were carried out on ungutted fish. Fish were randomly distributed for the appropriate treatment. Control samples were packed directly in ice, in perforated polystyrene tray containers. Experimental samples were gas flushed with the appropriate gas mixtures. The packaged samples were packed on ice in perforated polystyrene containers and covered with a layer of ice. All the samples were stored in a walk-in chill store set at +2 °C. Re-icing was done when necessary during the storage period.

In all the subsequent experiments, fish were immediately gutted, rinsed in cold running tap water to remove adhering debris from the mouth and gills. After a brief draining period the fish were weighed, randomly distributed for the appropriate treatment. Control and experimental fish were packed and stored in an identical manner to the ungutted fish. The experimental samples were packaged in atmospheres of the following composition: 100% CO₂; 60/40% CO₂/N₂; 50/50% CO₂/N₂; or 50/50% CO₂/O₂, according to the packaging procedure given in Section 3.3.1.

3.3.1 Packaging Procedure

Each fish was placed on a pre-weighed rigid tray container, which was then put into a pre-formed pouch of film. In order to obtain an effective seal, it was necessary to prevent moisture from fish making contact with the edge of the pouch. The pouch plus its contents was then placed into the chamber of the Mutivac A300/22 vacuum/gas flushing sealer, in such a manner that the middle of the pouch became level with the sealing bar, and the mouth of the pouch surrounded one or more gas jets. The required cylinder was connected to the gas inlet at the back of the machine via a pressure regulating valve. The gas at the cylinder was turned on and adjusted to 5 bars. The machine was then set according to the manufacturers instruction manual. When the lid of the machine was closed, evacuation occurred and was followed by gas flushing and sealing. The finished package was checked for leakage by pressing the pack and judging the integrity of the seam.

After packaging, the fish samples were placed into polystyrene iced containers and stored in a walk-in chill store set at + 2°C.

Several empty pouches were gas flushed with the same atmosphere as the experimental fish samples, and stored in an identical manner. These were analysed for any changes in the gas composition occurring during storage and which could be attributed to packaging permeability

3.4 MICROBIOLOGICAL METHODS

All microbiological work was carried out aseptically.

3.4.1 Standard Plate Count (SPC)

Viable counts were carried out by spread plate technique (Harrigan and McCance, 1976).

The following media, temperatures of incubation and conditions were employed:

(1) Plate count agar (PCA), ingredients

	<i>grams per Litre</i>
Tryptone	5.0
Yeast extract	2.5
Dextrose	1.0
Agar	9.0
pH 7.0	

PCA plates were incubated at 5°C (aerobically , and gas flushed with the appropriate gas under study) for ten days, 25°C (aerobically, anaerobically, microaerophil ically,and gas flushed with the appropriate gas under study) for three to four days. Some plates were aerobically incubated at 37°C.

(2) L.A.B. medium ingredients

	<i>per 950 ml</i>
Lactose	20g
Tryptone	10g
Yeast extract	10g
Lab-Lemco powder	10g
Tween 80	1ml
Di-potassium hydrogen phosphate	2g
Agar	15g

pH 6.6

LAB plates were incubated at 5 °C for ten days aerobically, microaerophilically , gas flushed with the appropriate gas under study for ten days. Some plates were incubated at 30°C in identical conditions given to incubation at 5°C for five days.

(3) Acetate Agar (AcA), ingredients,

	<i>per litre</i>
Yeast extract	5g
Peptone	10g
Lab-Lemco	4g
Glucose	10g
Lactose	10g
Mn SO ₄	0.14g
Na acetate	21g
Tomato juice	200 ml
Acetic acid (Glacial)	2.6 ml
Tween 80	0.5 ml
Agar	15 g

pH 5.2

Selective medium for the isolation or enumeration of *Lactobacillus* spp. AcA plates were incubated at 30 degrees centigrade for 5 days, aerobically ,and microaerophil ically.

(4) Nutrient agar slopes and nutrient broths, ingredients:

(slope)

	grams per litre
Lab-Lemco powder	1 g
Yeast extract	2 g
Peptone	5 g
Sodium chloride	5 g
Agar	15 g

pH 7.4

(Nutrient Broth)

As for the nutrient agar slope but omit agar. Nutrient agar slopes and nutrient broths were used here for subculturing prior to identification, of microorganisms. They were incubated for 18-24 hours at 25°C after inoculation from appropriate colonies by wire loop.

(5) Ringers solution, quarter strength; ingredients:

	grams per litre
Sodium chloride	2.25
Potassium chloride	0.105
Calcium chloride 6H ₂ O	0.12
Sodium bicarbonate	0.05

pH 7.0

diluent for homogenising skin samples and for making serial dilutions.

The media and diluents were sterilized by autoclaving at 121°C, 1.0×10^5 Newtons/m² for 15 minutes.

Samples of fish skin were excised using sterile scalpel and metal templates of 6.2 cm diameter giving an area of 30 square cm . Placed into a stomacher bag with 100 ml of Ringer's solution, and stomached for five minutes to liberate the micro-organisms. 1 ml of the resulting solution was transferred to 9 ml Ringer's solution to give a dilution of 10^{-1} . Succeeding dilutions were made by pipetting 1 ml of 10^{-1} dilution into a 9 ml Ringer's

solution to give a dilution of 10^{-2} , 1 ml into 9 ml for successive serial dilutions up to 10^{-6} .

Total viable counts by spread plate method (Harrigan and McCance, 1976) 0.1 ml of each dilution was pipetted onto the surface of the appropriate medium in duplicate and promptly spread using a sterile glass spreader. The plates were then left on the bench for a few minutes to allow the inoculum to absorb. The plates were then incubated under the required condition and temperature.

Bacterial colonies on plates corresponding to dilutions showing between 30 and 300 colonies per plate were enumerated. Since the skin samples taken was of 30 cm^2 , the colony forming units per cm^2 were calculated as follows:

$$\text{Cfu/ cm}^2 = (\text{plate count} \times \text{dilution factor}) / (\text{size of inoculum} \times 30)$$

3.4.2 Identification Methods (Shewan ,1960 a)

3.4.2.1 Gram Stain (Mitruka, 1976)

The bacterial cells from an 18-24 hour old culture were heat fixed, stained with ammonium oxalate crystal violet for two minutes, washed with tap water and treated with Lugols iodine (mordant) for one minute. The preparation was then washed with iodine acetone for thirty seconds, counterstained with safranin for one minute, washed with tap water, blotted dry and examined microscopically under the oil immersion lens.

3.4.2.2 Motility Test (Harrigan and McCance, 1976)

A loopful of a twenty-four hour old culture was transferred to a clean glass cover slip with the aid of a loop wire. Vaseline was applied round the edge of the depression of the cavity slide. The cavity slide was inverted over the glass cover slip so that the drop was in the centre of the cavity and pressed gently but firmly so that the vaseline sealed the glass cover slip in position. The slide was inverted quickly and smoothly to create a hanging drop. This was examined under X400 objective lens for evidence of motility.

3.4.2.3 Catalase Test (Harrigan and McCance, 1976)

Two drops of hydrogen peroxide were placed on the surface of an agar culture. Effervescence caused by liberation of oxygen as gas bubbles indicated the presence of catalase in the culture under test. Negative results were checked by emulsifying a loopful of culture on a microscope slide, covered the emulsion with a glass cover slip and adding two drops of hydrogen peroxide. Positive results were recorded if effervescence was observed under the cover slip.

3.4.2.4 Oxidase Test (Harrigan and McCance, 1976)

Fresh oxidase reagent was prepared by adding 10 ml of distilled water into a bottle containing 0.1 gm of solid N, N, N, N - tetramethyl p - phenylene - diamine hydrochloride. A drop of reagent was added to the surface of bacterial culture previously streaked onto nutrient agar, incubated at 25°C.

Development of bluish colour within ten seconds was recorded as positive.

3.4.2.5 Sugar Fermentation Test (Mitruka, 1976)

A loopful of bacterial culture grown in nutrient broth for twenty-four hours at 25°C was transferred to peptone water containing 1% glucose with bromocresol purple as indicator and incubated for four days at 25°C. Results were recorded for acid production, acid plus gas or no fermentation.

3.4.2.6 Oxidative/Fermentative Test (Mitruka, 1976)

Two tubes of Hugh and Leifson medium containing 1% glucose were inoculated with organisms using a sterile stab wire. One tube was left aerobic while the surface of the second tube was covered with sterile liquid paraffin to provide anaerobic conditions. Production of acid in both aerobic and anaerobic tubes was recorded as fermentive while acid in the aerobic tube only was recorded as oxidative. If the medium in the open tube turned

blue, it was recorded as alkaline.

3.5 ORGANOLEPTIC ASSESSMENT

The organoleptic assessment of fish was conducted using Tasmania Food Research Unit System, a system developed at CSIRO Division of Food Research, Tasmania Food Research Unit, Hobart.

In this system, the fish are inspected and characteristics listed on the score sheet assessed as 0, 1, 2, or 3. The appropriate demerit score is recorded and the quality of the fish determined by the addition of all the demerit scores to give a final total.

The panel consisted of regular contributors, each of whom were familiar with the parameters of fresh rainbow trouts, *Salmo gairdneri*.

Appendix I shows the sensory assessment score sheet as developed by the TFRU for whole and gutted fish. The belly and vent parameters were omitted from the assessment because gutted fish were used in this study. This reduced the total scoring points from 38 to 28. The range of quality scores of gutted rainbow trout used in this study is given in Appendix II.

3.6 GAS ANALYSIS

The method used for the analysis of Carbon dioxide, nitrogen and oxygen concentration changes from the day of packing throughout the storage period was developed by (M. Dillon, 1985 personal communication).

Perkin Elmer F11 Gas Chromatograph, using a thermal conductivity detector and fitted with two columns was used. The first column was of 3' length, 1/8" outside diameter and 2 mm inside diameter. Its support material was molecular sieve, 5/A of 60/80 mesh range. This column was used for the separation of oxygen from nitrogen while the CO₂ in the sample was bound irreversibly by the molecular sieve. The second column was of 6' length, 1/8" outside diameter and 2 mm inside diameter. Its support material was chromosorb 102 of 60/80 mesh range. It was used for the separation of carbon dioxide while nitrogen and

oxygen came out under one peak.(Dimensions given by suppliers - Phase Separation Ltd (UK)).

Carrier gas (helium) was flushed through the column each day experimental run, ensuring that the gas was flowing by allowing the flowstream to pass into a beaker containing water plus a small amount of detergent, before switching on the detector.

The oven temperature was set at 200°C while the detector temperature was set at 132°C for the separation of nitrogen from oxygen and at 165°C for the separation of carbon dioxide.

The injection temperature was adjusted to supply position 4, giving a bridge current of 144 mA. The carrier gas (helium) was operated at a flow rate of 10 ml/min.

The injection of the gas sample from the pouch into the gas chromatograph was carried out using a 1 ml. Hamilton gas – tight syringe throughout the experiment. By sticking two pieces of vinyl insulating tape on the pouch to form a cross, the sample could be withdrawn through the first piece of tape and the pin hole thus created quickly covered with the second piece of tape. This enabled five samples to be taken from each pouch without any significant change due to leakage.

The gas sample was injected into the gas chromatograph and separated into its components according to the column used, the signal from the gas chromatograph was fed into a Trio Chromatograph Integrator for analysis.

The Trio chromatograph computing integrator was programmed to calculate area under the peaks. By determination of retention times from the standards, percentage areas were expressed.

Appendix III shows the information given by the Trio of the analysed peaks. It also gives a general view of the appearance of the peaks.

In every sampling day, three pouches containing fish and three empty pouches containing the same atmosphere were analysed and the mean of 5 readings from each pouch recorded.

3.7 TORRYMETER READING

The operator's handbook containing instructions for using Gr Torrymeter was followed. It has a three position switch giving settings of '16' for average 16 value (highest), '1' for single value and a centre 'off' position. After selecting the required operating mode, ('1' in this case), the sensing head of the Torrymeter was placed in firm contact with the fish skin, with the axis of the electrodes parallel with and adjacent to the lateral line of the fish. After depression of the read button, a red light illuminates and the appropriate reading is displayed in the window (0–16). The reset button was depressed, the meter lifted and replaced in close proximity to the initial position and a second reading taken. If the second reading differed from the first which is possible due to misplacement of the contact electrodes, readings were repeated until two consistent values were recorded as a representation of the torrymeter quality of the fish. Three fish from each sampling day and the mean of the three recorded. After all the fish were analysed, the meter was cleaned with water and re-charged in its carrying case the day before the next analysis, but never for longer than twenty-four hours.

3.8 DRIP LOSS MEASUREMENT

The amount of exudate from fresh fish was measured gravimetrically. Once the fish was removed from the pouch for sampling, the tray and the accumulated drip were weighed. The difference from the preweighed tray determined and compared to the weight of the fish as percentage weight loss.

3.9 pH MEASUREMENT

The pH measurement was taken from just under the skin by use of a Jencons pHm₄ pH/temperature meter, used in combination with a Russell Combined glass rod pH electrode with a piercing teat. The temperature of each fish was taken at the time of measurement, the pH monitoring unit adjusted accordingly and after stabilisation the pH reading recorded. Three fish from each treatment were measured every sampling day, and three readings taken from each treatment. The average of the three readings was recorded as the pH measurement of the fish, for the respective treatment, for that sampling day.

4. RESULTS AND DISCUSSION

The central thrust of this experiment was to determine whether or not MAP was able to extend the shelf life of freshwater fish in comparison to ice stored fish. The packaging film chosen was aluminium coated 12 μm (813) polyester MIS/50 μm LDPE Campack Bilaminate. The choice of this film was based on its virtual gas impermeability. However, it lacks transparent properties that may be considered desirable by a consumer. This film has in addition to its high gas impermeability a significant water repellent property. This combination was necessitated by the manner in which it was considered necessary to store the fish to control temperature fluctuation i.e. in ice. In commercial practise where fish may be stored in chill conditions, this film could be substituted by a film of corresponding gas permeability which would not necessarily be moisture impermeable.

4.1 PRELIMINARY RESULTS AND GENERAL DISCUSSION

An initial objective of the study was to treat the fish in a way that would be acceptable to the traditional Kenyan fish purchaser. The manner in which fish was accepted in Kenya was therefore considered. Most consumers in Kenya prefer to purchase their fresh fish in an unmutilated and unprocessed form. This means that in practice, fish is stored in ice without gutting or scaling. Preliminary results involved using whole ungutted fish. The results of these experiments showed that by the seventh day, 65% of whole ungutted fish stored using MAP (100% CO_2) had belly bursts, in comparison with the control (ice packed fish) which did not develop belly bursts, during the period of 14 days storage. Belly burst was not observed in MAP fish immediately after packaging.

Under normal circumstances, belly burst in fish (stored in ice) is attributed to the attack of the belly walls by the digestive enzymes secreted in the fish gut. The rate of attack is enhanced in fish that have been feeding. The condition is most often seen in pelagic species (Connell, 1980).

The reason for belly burst in the MAP fish would appear to be a different form, or an enhanced form, of belly burst observed elsewhere in fish stored in ice. It is possible that the evacuation procedure during the gas packing process weakened the body wall, gut wall or both, so allowing the belly burst to be seen after a shorter storage time than that observed in

non-MAP fish. Gas pockets in the gut, which may not be present in all fish, may contribute to this. It is also possible that pH changes caused by 100% CO₂ used for MAP may alter the activity of gut enzymes or may affect the structural integrity of the flesh.

This phenomenon of belly burst in conjunction with MAP fish was not investigated further in the current study but would merit further investigation.

Storage temperature throughout the study was zero degrees Celsius. This was achieved by icing the control fish directly, icing the MAP packs, and storing both in a chill store which had been set at +2°C. Although the experimental storage was carried out at an ideal temperature, it is appreciated that in commercial practise, temperature abuse may occur during marketing and handling.

Several gas compositions were used, 100% CO₂, 60/40% CO₂ /N₂, 50/50% CO₂ /N₂, 50/50% CO₂/N₂ and 50/50% CO₂ /O₂.

Shelf life extension was determined by microbiological and organoleptic methods.

4.2 MICROBIOLOGICAL RESULTS AND DISCUSSION

4.2.1 Effects of MAP on Viable Counts (Quantitative changes)

4.2.1.1 Aerobic counts at 25°C, 5°C and 37°C on plate count agar medium

The relationship between aerobic counts of bacteria/cm² skin surface and days of storage for rainbow trout stored in different gas compositions, (100% CO₂, 60/40% CO₂/N₂, 50/50% CO₂ /N₂ and 50/50% CO₂/O₂ and in ice (control)) are shown in Figures 2 (Total aerobic count at 25°C), and 3 (psychrotrophic count at 5°C).

The total viable aerobic count at 25° C showed a low initial count (2.3 log count), representing a low level of contamination from the environment that the fish were caught, and hygienic handling after harvesting. After 24 hours of storage, a decrease in the number of microorganisms was noted in all the four experimental treatments and the control. This

decrease could partly be attributed to the effect of washing the fish after gutting, a concept that was demonstrated by (Hunter, 1920) cited by (Georgala, 1957). Hunter showed that washing influenced the course of spoilage of Salmon. His investigations involved bacterial counts on muscle, and found that during storage the counts on the muscle of the washed fish were, with few exceptions, lower than with unwashed fish; and the washed fish had a longer storage life. Lumley *et al.* (1929) also cited by Georgala (1957) obtained similar results with haddock, whereby they found that fish gutted and washed under near sterile conditions had a lower bacterial count (in the free liquid in the gut cavity) and spoiled more slowly than fish handled in the normal commercial fashion. A lag phase of 5 days was common to the control, 100% CO₂, 60/40% CO₂/N₂ and 50/50% CO₂/N₂ treatments but not in 50/50% CO₂/O₂ treatment, where rapid and steady growth of microorganisms up to 4 log counts by the eighth day was evident, followed by a stationary phase. By the end of the storage period, the count for the 50/50% CO₂/O₂ treatment was a log count of 4.3. By the seventh day, the total aerobic counts at 25°C of all the treatments had reached at least 2 log counts (2 log counts for 100% CO₂, 2.4 log counts for 60/40% CO₂/N₂, 3.4 log counts for the control, 3.3 log counts for 50/50% CO₂/N₂ and 3.4 log counts for 50/50% CO₂/O₂). 100% CO₂ showed negligible bacterial growth throughout the entire 21 days of storage, with a maximum log count of 2.8. 60/40% CO₂/N₂ MAP followed the 100% MAP trend of growth very closely up to fourteen days, after which there was an increased growth, giving a maximum log count of 3. From day 8 to the end of the storage period, the trend for 50/50% CO₂/N₂ and 50/50% CO₂/O₂ on the total aerobic counts were very close.

The psychrotrophic aerobic counts carried out at 5°C, (spoilage organisms) as portrayed in Figure 3 are very similar to those of total aerobic count at 25°C. However, the decrease in bacterial counts on the first day is greater than in the total aerobic count at 25°C. In addition there is a slower growth rate at 5°C in all the MAP treatments when compared with the aerobic TVC at 25°C. The 100% CO₂ treatment at 5°C took 14 days (twice as long) to reach 2 log counts, and the 60/40% CO₂/N₂ and 50/50% CO₂/N₂ gave a maximum count of 3.8 and 3.9 log counts respectively at 21 days.

These two Figures (2 and 3) show clearly that bacterial counts made on the fish held in the MAP (100% CO₂ and 60/40% CO₂/N₂) remained low for the full 21 days of test. The results show that the counts for these two treatments never exceeded 10⁴ cfu/cm² skin surface which is normal for very fresh fish. Though treatments with 50/50% CO₂/N₂ and 50/50% CO₂/O₂ permitted higher counts, at no time did they exceed 10⁶ cfu/cm² skin surface generally considered indicative of incipient spoilage. The control fish however gave a maximum of 6.3 log count by twentieth day of storage.

Counts carried out at 37°C for 18-24 hours showed that all samples had less than three hundred colony forming units per square centimetre of skin surface. Counts at this temperature are often taken to indicate mesophilic contamination of possible mammalian origin, including pathogens.

The results from the total aerobic counts at 25°C and psychrotrophic counts at 5°C show significant inhibition of microbial growth by MAP. In ranking order the effectiveness of this inhibition taken at 5°C would appear to be:

100% CO₂,

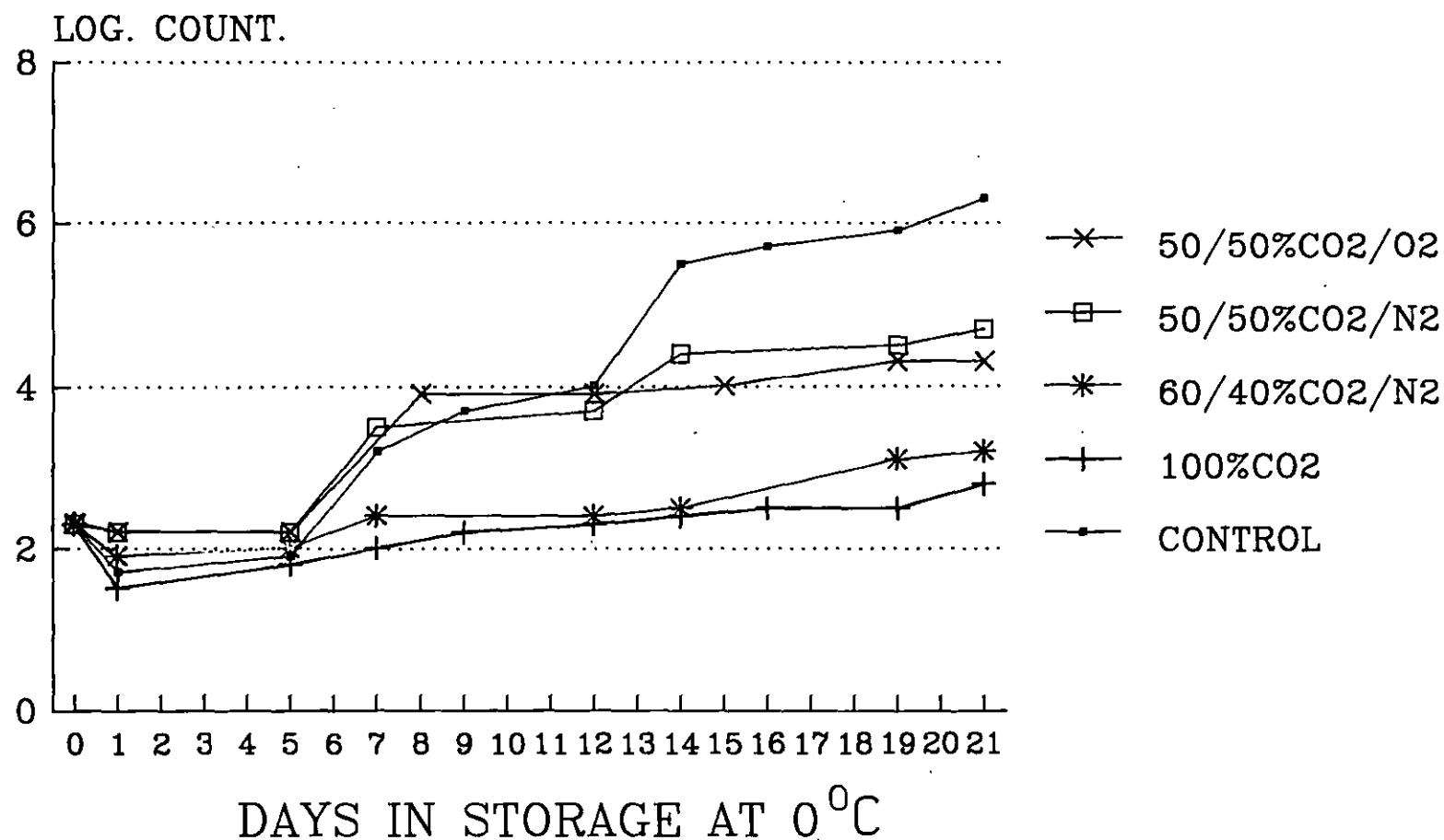
60/40% CO₂/N₂,

50/50% CO₂/N₂

50/50% CO₂/O₂.

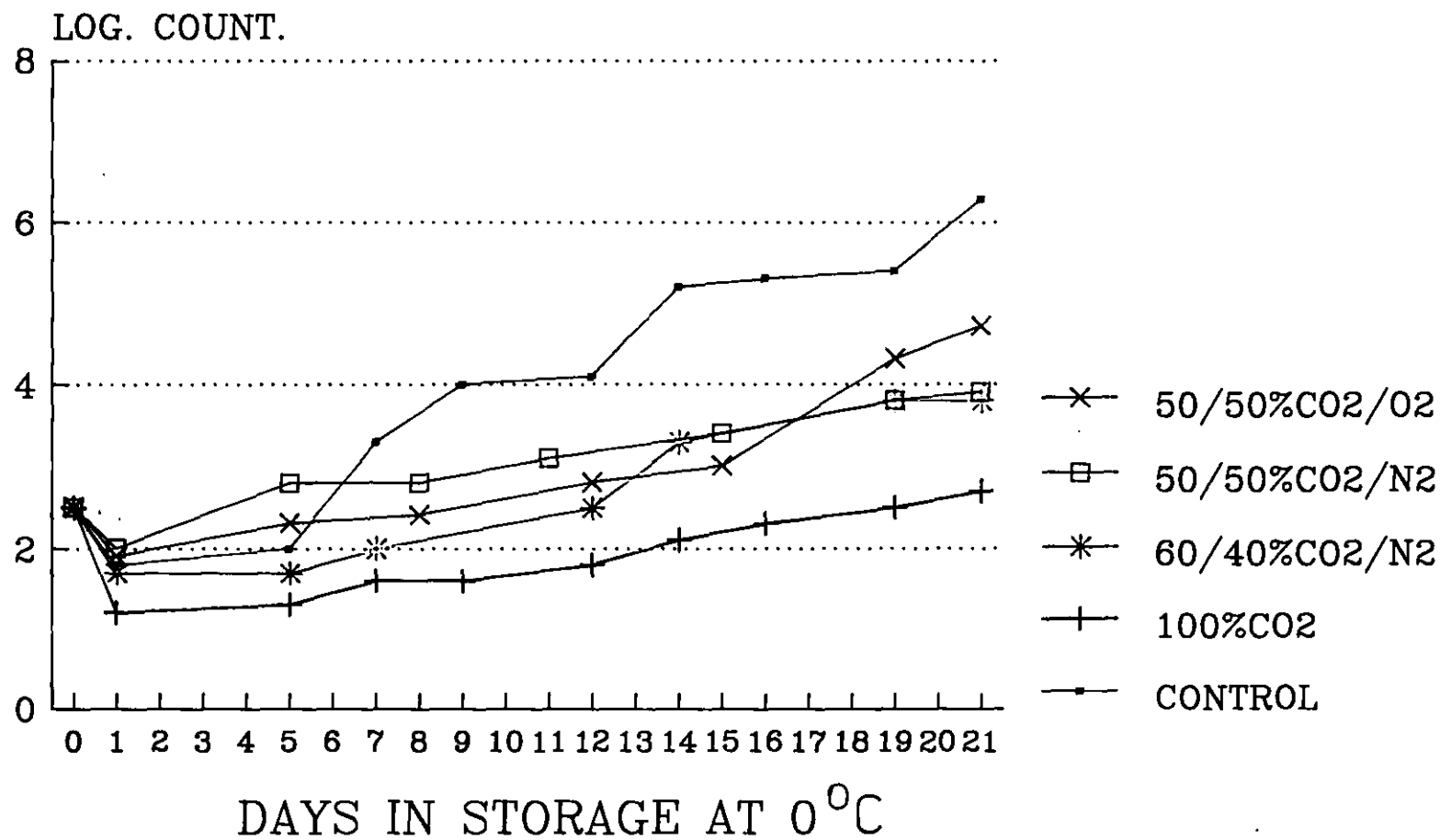
Where bacterial counts are taken to represent the freshness of fish, MAP extends the shelflife of fresh, freshwater fish. However, it is questionable as to whether freshness can be assessed purely on microbial analysis. Therefore, parallel studies were carried out to assess freshness by other means, i.e. organoleptic tests and Torrymeter readings.

FIG.2 TVC @ 25⁰c
PLATE COUNT AGAR (AEROBIC)



Mean of two experiments each of two samples, and duplicate counts per sample.

FIG. 3 TVC @ 5⁰C
PLATE COUNT AGAR (AEROBIC)



Mean of two experiments, each of two samples and duplicate counts per sample.

4.2.1.2 Total Microaerophilic and Anaerobic Counts on Plate Count Agar Medium at 25°C

In fish stored in packs where oxygen has been reduced or removed, it would be expected that anaerobic or microaerophilic microorganisms would be favoured. In order to investigate this possibility, plates were incubated under microaerophilic conditions, achieved by using microaerophilic gas generating kits (oxid BR 60), while anaerobic conditions were achieved by using gas generating kits (oxid BR 38) and the anaerobiosis determined by use of an anaerobic indicator (oxid BR 55). The plates were incubated in the anaerobic gas jars in both cases.

The relationship between total microaerophile/anaerobic counts per cm² of skin surface and days of storage for rainbow trout stored in different gas compositions and the control are shown in Figures 4 and 5. There were no microaerophilic or anaerobic microorganisms detected on the initial day of sampling.

MICROAEROPHILIC COUNTS

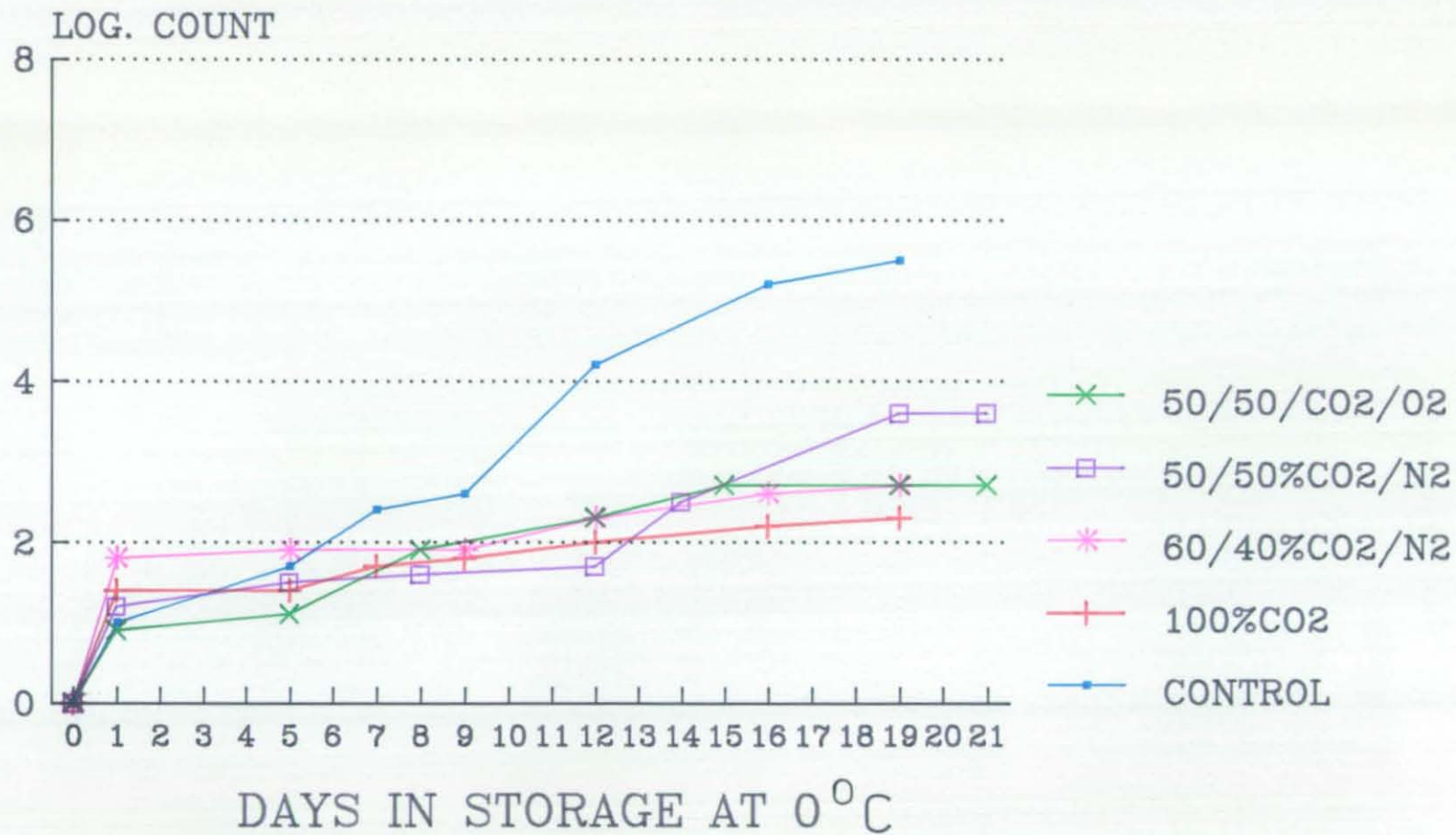
After the first day of storage there was a sharp rise in microaerophilic count in all the treatments, (1 to 1.8 log count) after which, a very slight growth in samples stored in MAP occurred. 100% CO₂ was found to be more inhibiting and gave a maximum log count of 2.3 by the end of the storage period (21 days). The counts for all the other MAP conditions (60/40% CO₂/N₂, 50/50% CO₂/N₂ and 50/50% CO₂/O₂) did not reach 2 log counts, while the count for the control rose to log 5.4 showing a trend similar to the total aerobic count at 25°C and psychrotrophic count at 5°C.

ANAEROBIC COUNTS

After the first two days, the control and 50/50% CO₂/O₂ atmosphere storage showed similar slight growth increases, the control maintaining a slightly higher count than the 50/50% CO₂/O₂ storage. Bacterial growth in 100% CO₂, 60/40% CO₂/N₂ and 50/50% CO₂/N₂ treatments showed no increase till the fifth day of storage, after which there was a significant growth in these three gas packed treatments. On the last few days of storage, there was little difference in bacterial counts between the 50/50% CO₂/N₂ and 50/50% CO₂/O₂ treatments.

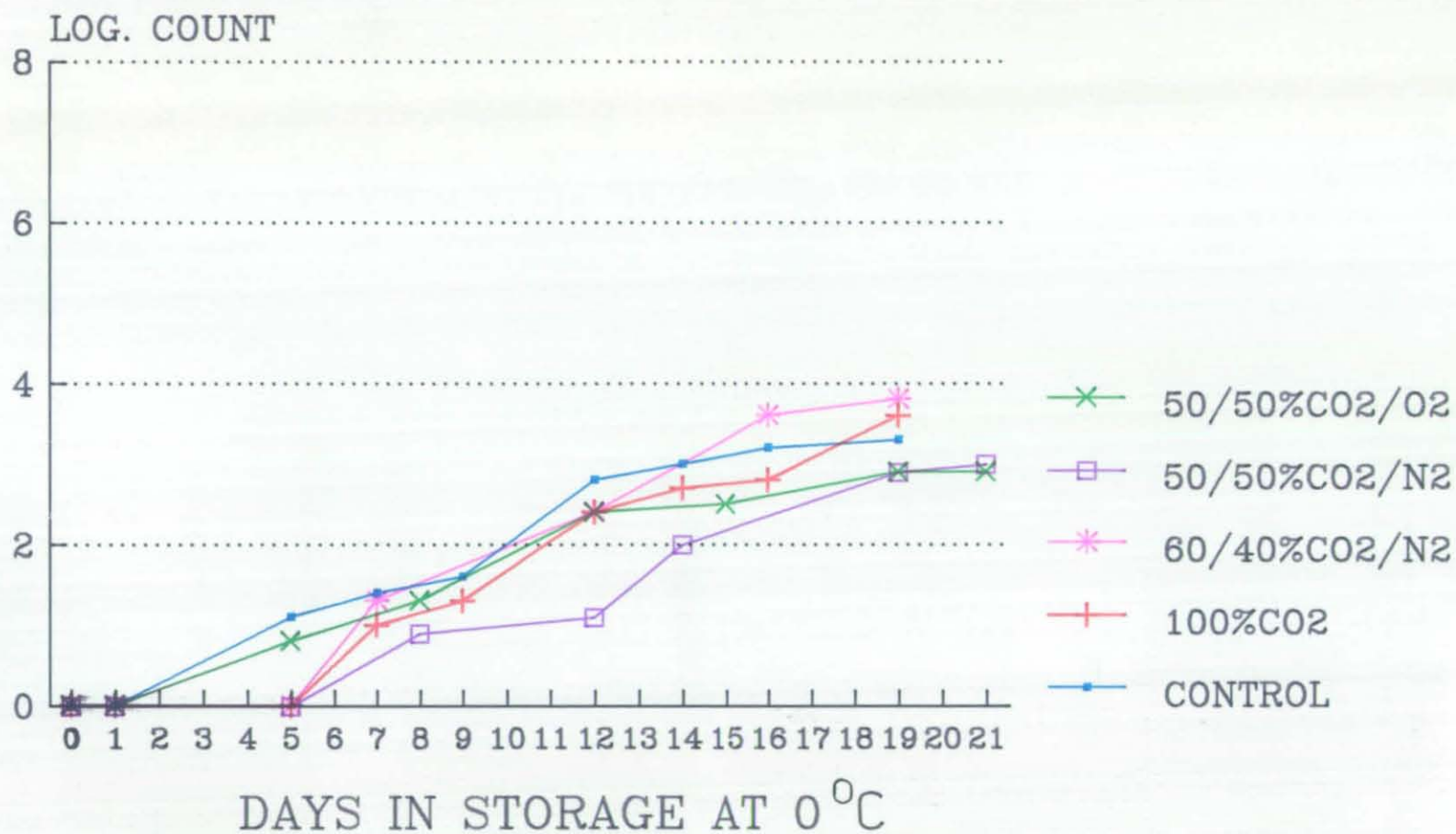
All treatments used in this study appeared to suppress the growth of microaerophiles when compared with the control. However, as far as anaerobic organisms are concerned, apart from a delay of growth in some instances, there appear to be no appreciable differences between the final counts. This is complicated by the fact that some microaerophiles will also grow in the enhanced CO₂ conditions used in the Gas Pack system producing anaerobic conditions.

FIG.4 TVC @ 25⁰C
 PLATE COUNT AGAR (MICROAEROPHILIC)



Mean of two experiments, each of two samples and duplicate count per sample.

FIG. 5 TVC @ 25⁰c
PLATE COUNT AGAR (ANAEROBIC)



Mean of two experiments, each of two samples and duplicate counts per sample.

4.2.1.3 Total Viable Counts at 25°C and 5°C (Gas Flushed) on PCA Media

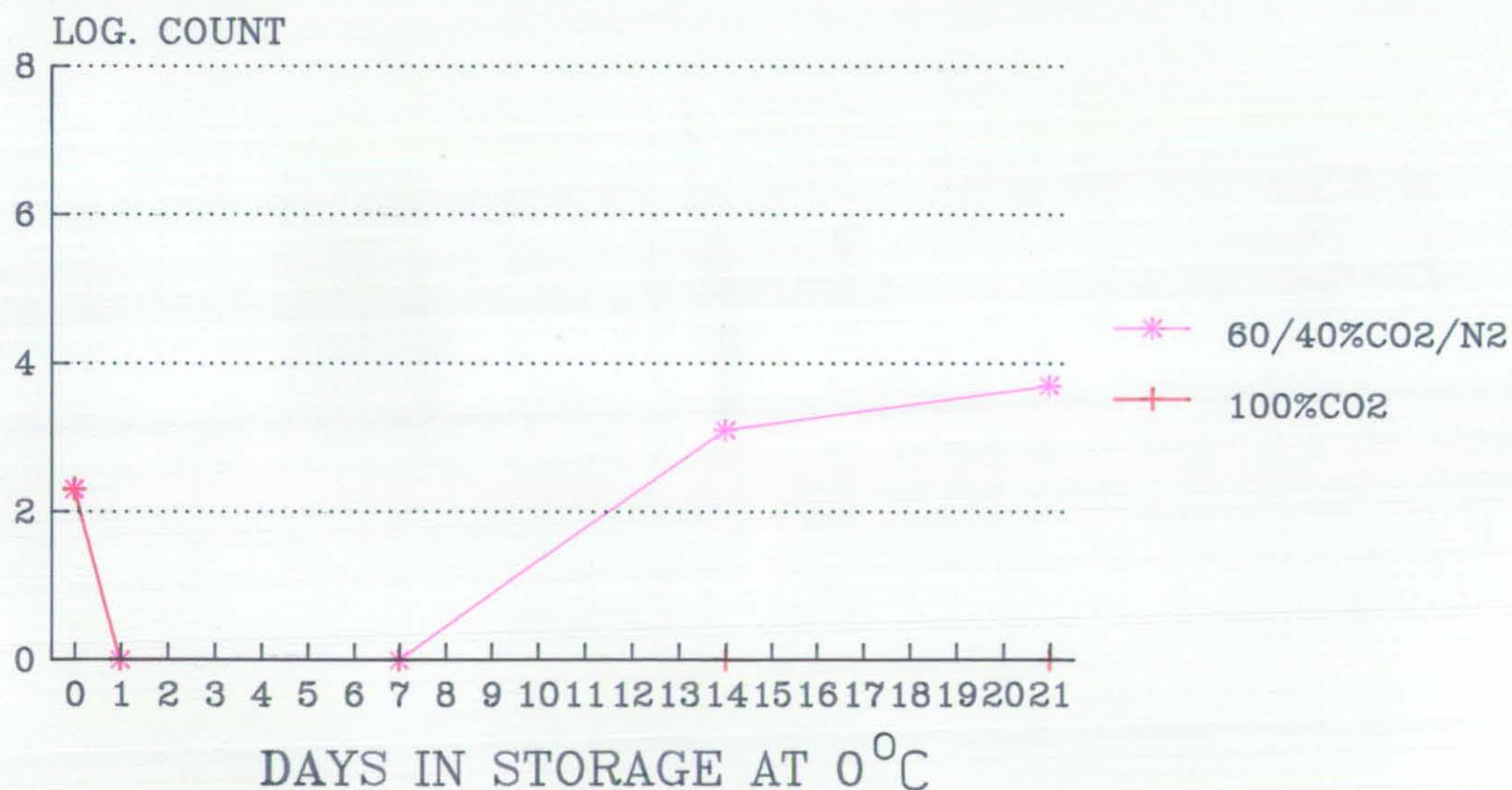
This experiment was carried out, incubating the plates under the same atmospheric composition as that used to store the fish (MAP fish). This was achieved by placing batches of inoculated petri dishes in film pouches and gas flushing them in an identical manner to the experimental fish. The objective of this treatment was to attempt to isolate the microflora actually growing during the storage of each gas mixture.

Figures (6 and 7) shows the mean log counts of viable bacteria, in relation to storage days and gas composition (100% CO₂ and 60/40% CO₂/N₂), at 25°C and 5°C respectively.

Initial counts before gas flushing were approximately 2 log counts. However, when plates were inoculated with samples from 100% CO₂ storage, and then gas flushed with the same atmosphere and incubated at 25°C or 5°C, there was no growth at all up to the end of the storage period (21 days). This correlated with the slight increase shown on microaerophilic incubation. Plates inoculated with samples from 60/40% CO₂/N₂ and gas flushed with this atmosphere then incubated at 25°C and 5°C gave counts up to a log countⁿ of 3 after 14 days. However there was a lag period of upto 7 days, when no increase in counts was seen which again correlates with the microaerophilic incubation counts.

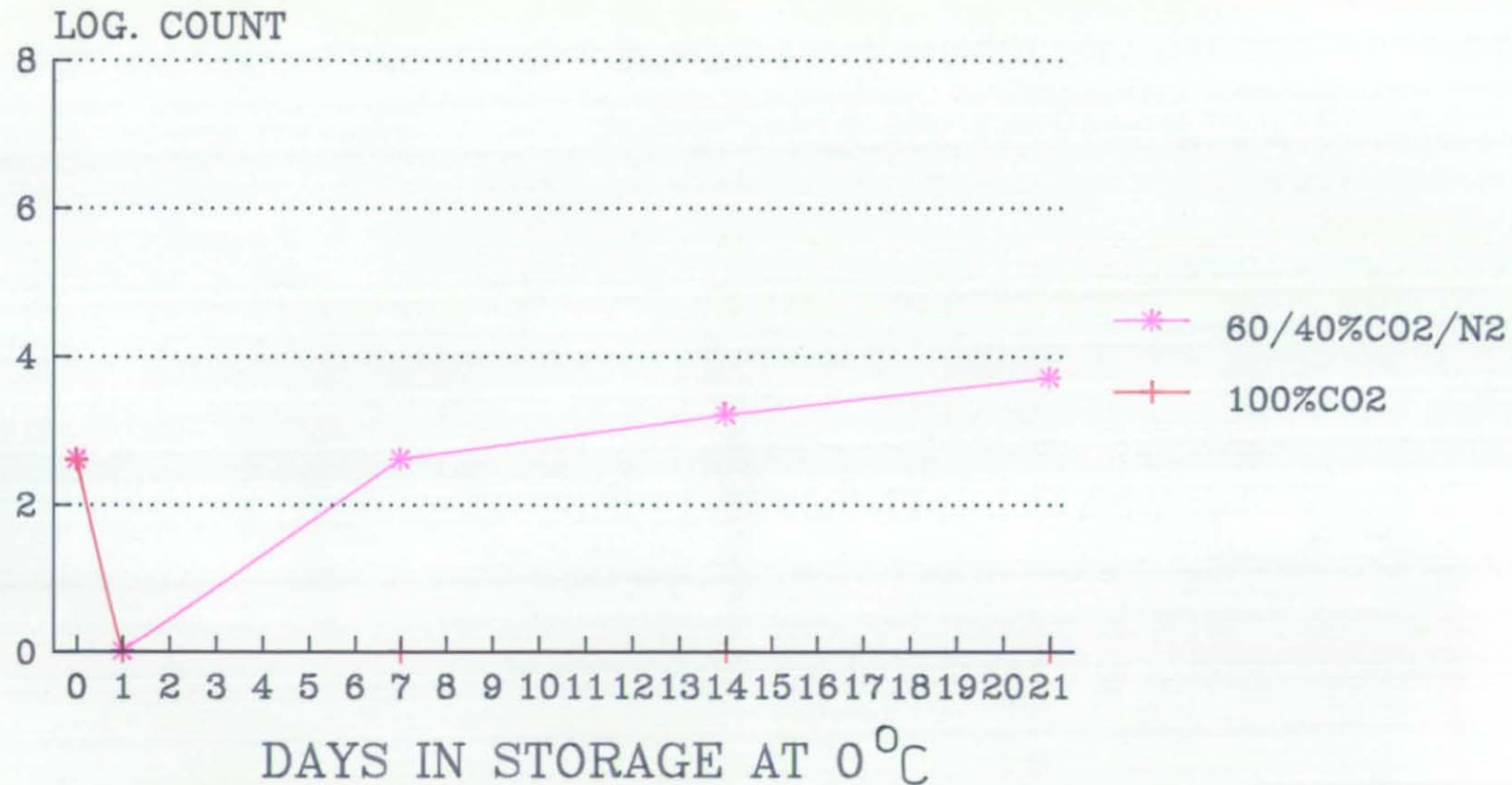
These results indicate that there was negligible increase in microbial growth during storage under these conditions, with the 100% CO₂ environment having a slightly greater inhibitory effect than 60/40% CO₂/N₂.

FIG. 6. TVC @ 25°C
 PLATE COUNT AGAR (GAS FLUSHED
 – 60/40%CO₂/N₂ AND 100%CO₂)



Mean of two experiments, each of two samples and duplicate count per sample.

FIG. 7 TVC @ 5°C
PLATE COUNT AGAR (GAS FLUSHED
– 60/40%CO₂/N₂ AND 100%CO₂)



Mean of two experiments, each of two
samples and duplicate count per
sample.

4.2.1.4 Total Viable Counts on Lactic Acid Bacteria Medium (LAB)

Many researchers conducting MAP studies have reported an increased occurrence of lactic acid bacteria in MAP storage (Molin *et al.*, 1984; Blickstad *et al.*, 1983; Enfors *et al.*, 1979; and Banks *et al.*, 1980). The organisms referred to as lactic acid bacteria include certain species in the genera *Streptococcus*, *Pediococcus*, *Leuconostoc* and *Lactobacillus*.

The first two genera are homofermentative, the leuconostocs are heterofermentative and lactobacilli include both homofermentative and heterofermentative types (Banwart, 1979).

In the MAP studies, *Lactobacillus* spp. especially the homofermentative strains are considered most important, and may become a dominant element in the microflora of MAP stored muscle products including fish (Enfors *et al.*, 1979).

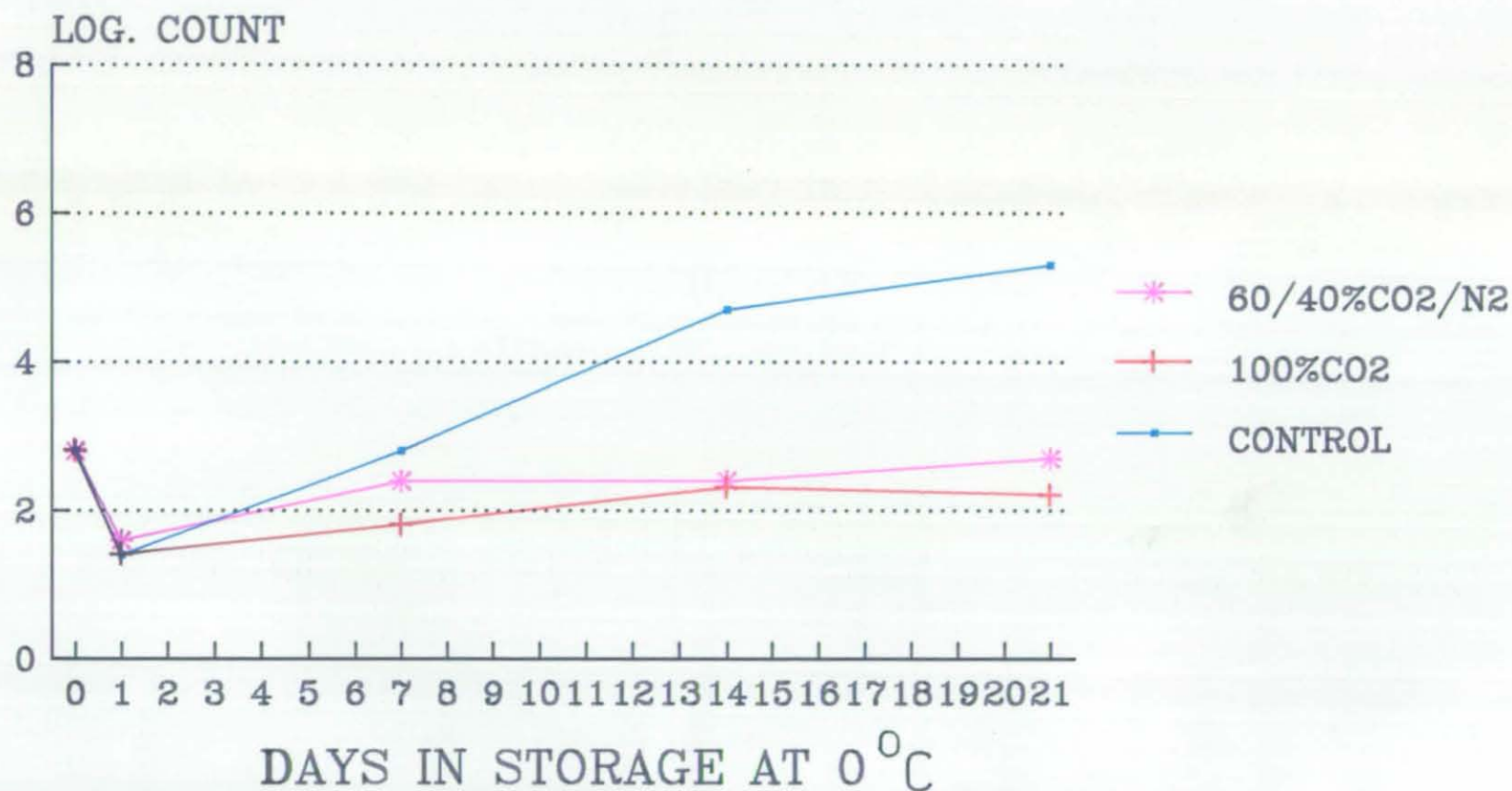
An established medium used to isolate lactic acid bacteria from a wide variety of food products is LAB (lactic acid bacteria medium). The medium is not considered inhibitory but contains stimulatory materials that promote the growth of lactic acid bacteria. Incubating this medium under mesophilic conditions, provide the correct intrinsic and extrinsic parameters for growth. Incubation temperature of 30°C was chosen to further select for mesophilic lactic acid bacteria, whilst 4°C was used to select for psychrotrophic lactic acid bacteria.

Figures (8, 10 and 9) shows total viable counts at 30°C aerobic on LAB, at 30°C microaerophilic on LAB, and 5°C aerobic on LAB respectively.

Comparison of Figure 10 (TVC at 30°C microaerophilic on LAB) with Figure 4 (TVC at 25°C microaerophilic on PCA medium) suggest that the LAB medium grew the same microflora as PCA medium.

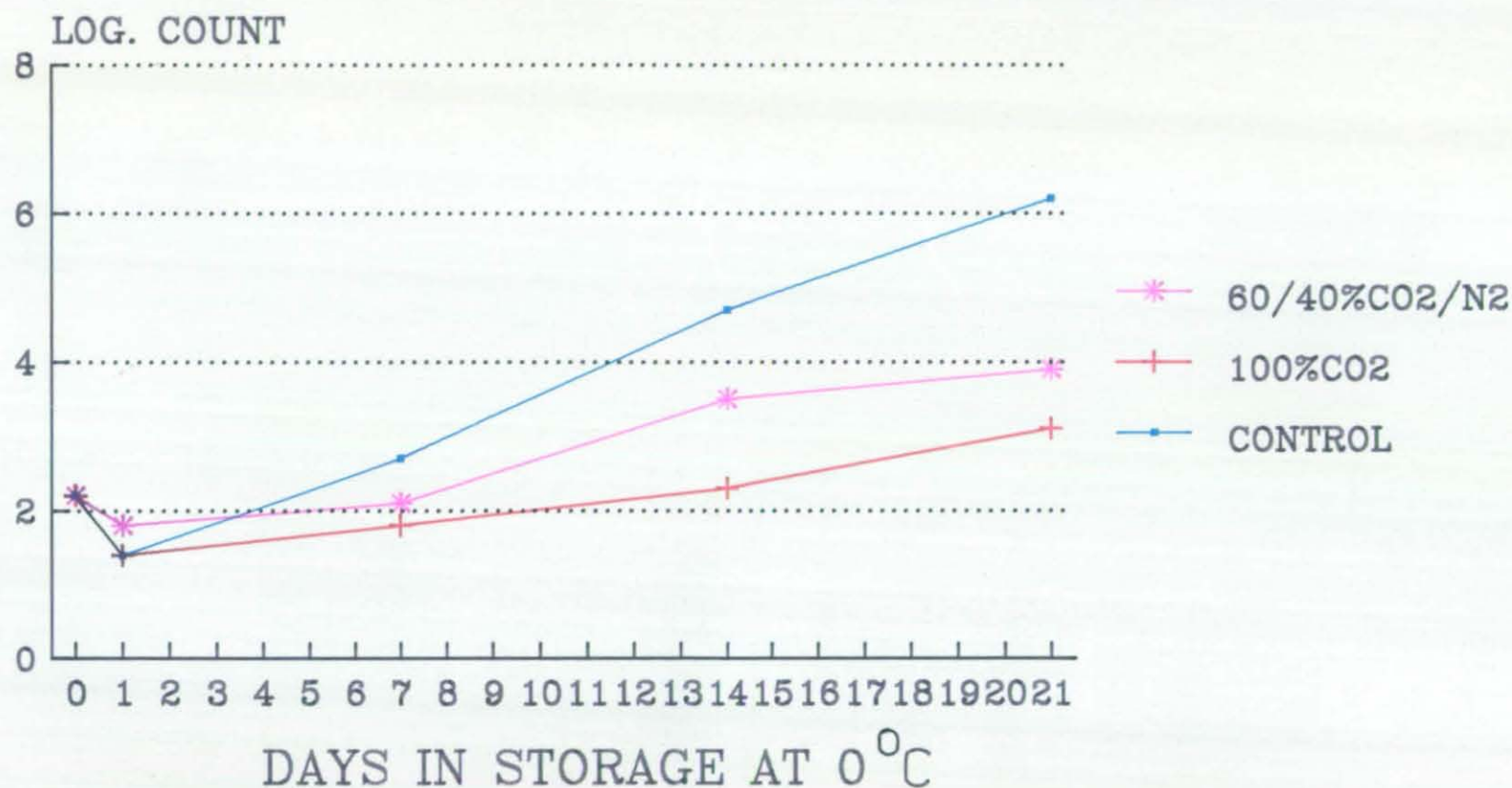
Comparison of Figure 9 (TVC at 5°C aerobic on LAB, with Figure 3 (TVC at 4°C aerobic on PCA medium) suggests that the LAB medium again grew the same microflora. This is substantiated by Gram stain and catalase tests carried out on colonies growing on LAB. No colonies showed evidence of lactic acid bacteria i.e., catalase negative, Gram positive rods or cocci.

FIG.8 TVC @ 30°C
LACTIC ACID BACTERIA MEDIUM (LAB)
(AEROBIC)



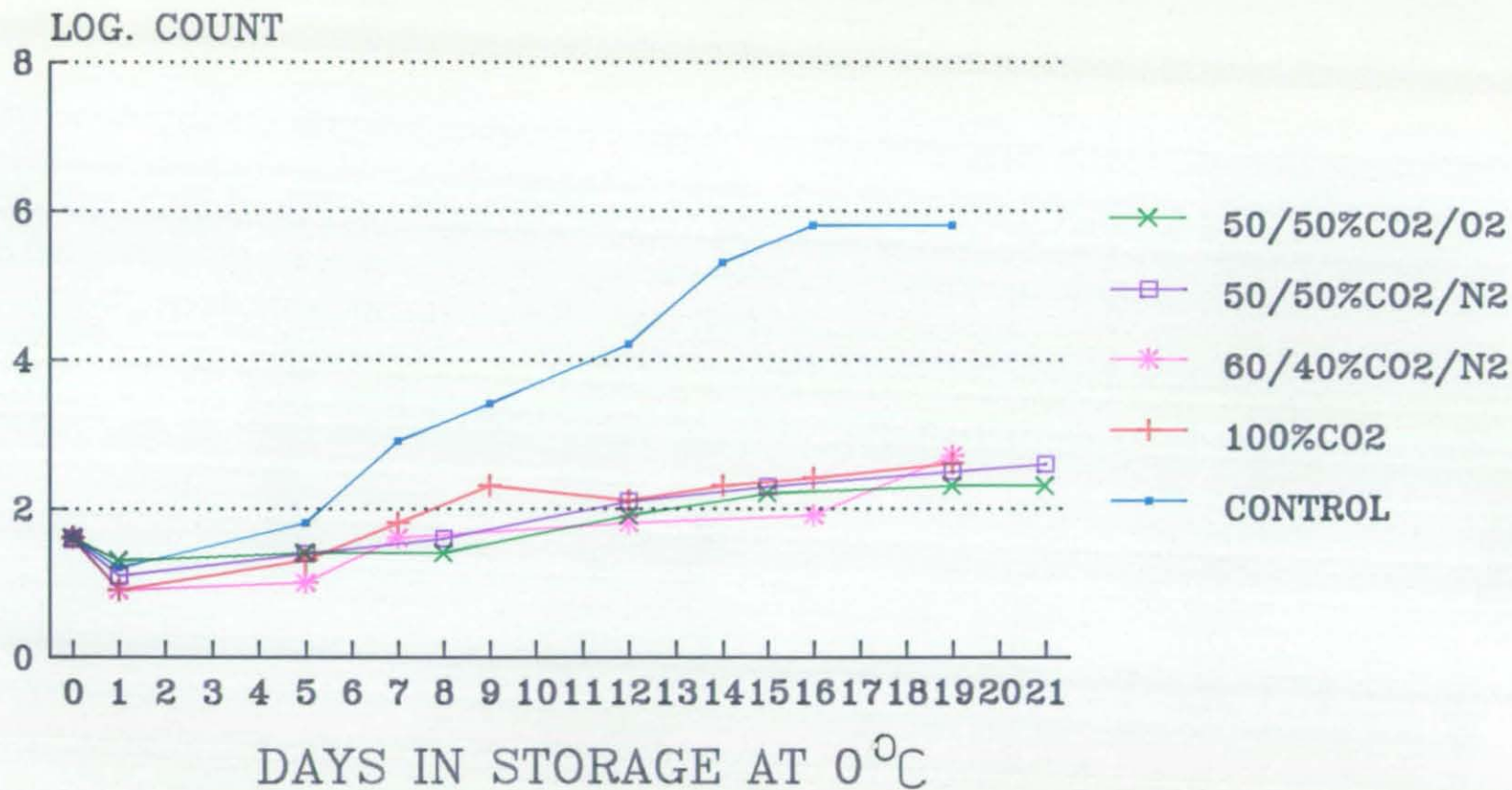
Mean of two experiments, each of two samples and duplicate count per sample.

FIG.9 TVC @ 5⁰ C
ON LACTIC ACID BACTERIA MEDIUM (LAB)
(AEROBIC)



Mean of two experiments, each of two samples and duplicate count per sample.

FIG.10 TVC @ 30⁰C
LACTIC ACID BACTERIA MEDIUM (LAB)
(MICROAEROPHILIC)



Mean of two experiments, each of two samples and duplicate counts per sample.

4.2.1.5. Total Viable Counts at 30° C on Acetate Agar (AcA) Medium

Acetate Agar medium was used in this experiment because of its reported selective properties for *Lactobacilli* spp. (Enfors *et al.*, 1979; Blickstad *et al.*, 1983 ; and Stenstrom , 1985).

Plates were inoculated with samples from MAP storage (100% CO₂, 60/40% CO₂/N₂, 50/50% CO₂/N₂ and 50/50% CO₂/O₂) and from the control (ice storage). Throughout the experiment and storage periods, no colonies were formed on the plates. This result was confirmed through three experimental trials. However, inoculation of pure cultures of *Lactobacillus* spp. grew on the medium.

It could be concluded that contrary to previous studies (Enfors *et al.* 1979; Blickstad and Molin, 1983; Molin *et al.*, 1984; and Stenstrom 1985) lactic acid bacteria including homofermentative lactobacilli did not form part of microflora during storage in any of the gas mixtures. Possible reasons for this may be either the initial contaminatory flora did not contain lactic acid bacteria, the fish under investigation (rainbow trout, *Salmo gairdneri*) could not support growth of these organisms under MAP conditions or dominating microflora inhibited the growth of this bacterial group.

4.2.1.6 Summary of Total Viable Counts

Total bacterial counts made on the fish held in the MAP remained low for the full 21 days of the storage and test. The results show that the counts never exceed 10⁴ cfu/cm² of skin sample. This means that the number remained in the range considered normal for very fresh fish. A total bacterial plate count in excess of 10⁶ cfu/cm² is generally considered indicative of incipient spoilage. Similar results were given by Barnett *et al.* (1982) while (Parkin , 1979) as cited by (Barnett *et al.*, 1982) reported essentially no growth of aerobic bacteria on rockfish fillets stored refrigerated in an atmosphere containing 80% CO₂/20% O₂ for 14 days.

Total aerobic counts obtained on PCA medium at 25°C for 3 days were similar to that at 5°C for 10 days, on all the treatments. The total anaerobic counts showed the longest lag phase, showing less than 30 cfu/cm² skin surface during the early stages of storages. Total aerobic counts on LAB were similar to aerobic counts on PCA. These results plus lack of

growth on acetate agar medium strongly indicate that lactic acid bacteria were not part of the MAP fish microflora in this study.

4.2.2 Effect of MAP on Qualitative Changes of Microorganisms

Identification of microflora was performed on randomly selected isolates from the aerobic plate counts. This isolation and identification procedure was used to follow the changes in microflora on fish samples stored in ice (control), 100% CO₂ and 60/40% CO₂/N₂ up to 21 days. A total of 480 colonies were selected from the plate count agar media of the above mentioned treatments of rainbow trout *Salmo gairdneri* to determine the bacterial composition at various stage intervals and analyse microbial growth patterns. Out of 480 colonies, 120 represented the initial flora of the samples.

As has been reported by a number of investigators (Shewan, 1962; Bramsnaes, 1965; Nair and Lahiry, 1968; and Castell, 1971) the microflora of fish differ between species, area and method of catch, season and other environmental conditions.

In this study, the variation in the microflora during storage in different gaseous composition is given in tables IX, X and XI. These tables also show the variation in proportion of gram positive to gram negative organisms (expressed as percentages) during the course of storage. The initial bacterial population of fresh whole trout was composed of 35% gram positive and 65% gram negative as determined by gram stain (Mitruka, 1976). This indicates a dominance of gram negative bacteria on the samples used. Initially, the dominating type of microorganisms were in ranking order, *Flavobacterium/ cytophaga* spp., *Micrococcus* pp., *Pseudomonas* spp., *Bacillus* spp., and *Aeromonas* spp. All the four groups of *Pseudomonas* spp. contributed to 18% of the initial flora. Of these, 56% were from *Pseudomonas* group II. On the 9th, 15th and 21st days of storage and sampling, *Pseudomonas fluorescens* (group I) were not found in any of the treatments. *Pseudomonas* group II were found at all sampling stages in the control, where they dominated the flora. They were, however, not found on day 9 of sampling from 100% CO₂ and 60/40% CO₂/N₂ storages, but appeared on day 15 and day 21. *Pseudomonas* group III/IV did not form part of the bacterial population of ice stored fish, but it occurred in MAP storage, giving rise to a significant part of the flora in 60/40% CO₂/N₂ atmospheres.

From the initial sampling, *Micrococcus* spp. represented a major group of bacteria in this study. By the 9th day of sampling, *Micrococcus* spp. had risen steadily in number, giving rise to 30% , 50% and 82% of the flora in the control, 60/40 CO₂/N₂ and 100% CO₂ respectively.

Micrococcus spp. is considered a strict aerobe and therefore the proportions found are somewhat unexpected. This may indicate either the ability of *Micrococcus* spp. to grow under these conditions, or its capacity to survive relative to other elements of the microflora. The latter would appear the more likely interpretation but requires further study. In the last two sampling days, the number of *Micrococcus* spp. in all treatments decreased. By day 21, the level of *Micrococcus* spp. on the iced control fish had fallen to 4%. On the 100% CO₂ stored fish , however, they were the dominant group, and on the 60/40% CO₂/N₂ treated fish the second most numerous (after *Bacillus* spp.).

Enterobacteriaceae though not detected in the initial flora, were found in the later storage period in ice and 100% CO₂ storage. These organisms would not appear to have any significance here in determining shelflife. Generally, the MAP supported a varied flora of similar genera to the control, and different only in percentage proportions of the total population.

In this study, gram negative bacteria dominated in the initial flora, contrary to results obtained by (Banks et al., 1980) in which only 20% gram negative were present on fish at time of harvest.

In 100% CO₂ MAP fish, the gram negative bacteria (*Pseudomonas* group II and III, *Acinetobacter* , *Vibrio* , *Moraxella* , *Aeromonas* and *Flavobacterium lcytophaga*) represented 54% of the total microflora at day 21, whilst in the 60/40% CO₂/N₂ MAP fish the gram negatives, (*Pseudomonas* group II and III , *Moraxella* and *Vibrio*) represented 45% of the flora at the same storage time.

The microflora of MAP fish did not show dominance by any one group of organisms. However there was a marked increase in gram positive organisms by the end of the storage

trials (21 days). In comparison, the control fish stored under aerobic conditions (in ice) was dominated by *Pseudomonas* group II organisms. This corresponds with established data (Molin and Stenstrom , 1984; and Castell and Anderson, 1948) cited by (Banks , 1980).

In meat, poultry and fish stored refrigerated in vacuum packages or modified atmospheres containing CO₂, a shift in microbial flora often occurs from normal gram negative flora to a gram positive one consisting of high numbers of lactic acid bacteria. This has been reported by a number of investigators including (Enfors et al ., 1979) for pork, (Sander and Soo, 1978) for poultry , and (Banks et al., 1980) for dressed fish. Sutherland et al.. (1977) reported that *Lactobacilli* were able to grow at any CO₂ concentration from 10 - 100% CO₂ on beef cubes stored at 0 - 5°C. Banks et al. (1980) showed this change in microbial population during refrigerated storage of fish by an increase in the ratio of gram positive to gram negative bacteria.

There were no colonies formed on the acetate agar plates, and in the identification procedure no lactic acid bacteria were identified throughout the storage period of all the treatments. This suggests that there were no *Lactobacillus* spp. or any other lactic acid bacteria in the samples used in this experiment, and that colonies that developed on LAB media did not represent lactic acid bacteria.

The atmospheric composition has been said to have some important effect in providing the optimal oxygen tension to stimulate the growth of lactic acid bacteria. This was demonstrated by (Lee, 1981) cited by (Boone , 1982). When working on Pacific Northwest fish species, Lee showed that 20% CO₂ was more conducive to the growth of lactic acid bacteria than 60% CO₂. This concept may explain the findings of this study but, high levels of CO₂ in MAP gas compositions also have shown an increased growth of lactic acid bacteria as reported by (Stier *et al.*, 1981 and Lannelongue *et al.*, 1981) cited by (Boone , 1982).

The literature regarding lactic acid bacteria, and their growth in MAP especially at different CO₂ levels is contradictory. The present study suggests that lactic acid bacteria are not an important part of the microflora at high concentrations of CO₂.

The growth of *Clostridium botulinum* in MAP stored fish was not determined in this study, however all the samples were stored at 0°C (± 1) which would not permit the growth of *Clostridium botulinum* type E.

Table IX

% Distribution of Different Microorganisms
During Storage - (Control.)

Type of Microorganisms	Days in Storage / % Numbers			
	0	9	15	21
<i>Pseudomonas Group I</i>	2	-	-	-
<i>Pseudomonas II</i>	10	15	30	45
<i>Pseudomonas III/ IV</i>	6	-	-	-
<i>Micrococcus spp</i>	20	30	15	4
<i>Aeromonas</i>	10	5	10	17
<i>Moraxella</i>	5	5	-	3
<i>Acinetobacter</i>	4	-	-	-
<i>Bacillus spp</i>	15	-	10	2
<i>Vibrio</i>	7	10	15	13
<i>Streptococcus spp</i>	-	-	-	-
<i>Enterobacteriaceae</i>	-	-	5	2
<i>Flavobacterium / Cytophaga</i>	21	35	15	14
Total Number	100%	100%	100%	100%

% Distribution / Gram Stain.

	0	9	15	21
	35	30	25	6
	65	70	75	94
	100%	100%	100%	100%

Table X % Distribution of different
microorganisms during storage -
(60 / 40% CO₂ / N₂) MAP

TYPE OF MICROORGANISMS	DAYS IN STORAGE / % NUMBERS			
	0	9	15	21
<i>Pseudomonas Group I</i>	2	-	-	-
<i>Pseudomonas Group II</i>	10	-	10	10
<i>Pseudomonas Group III / IV</i>	6	5	5	15
<i>Micrococcus spp.</i>	20	50	25	25
<i>Aeromonas</i>	10	-	-	-
<i>Moraxella</i>	5	-	15	10
<i>Aerinetobacter</i>	4	5	10	-
<i>Bacillus spp.</i>	15	20	20	30
<i>Vibrio</i>	7	5	10	10
<i>Streptococcus spp.</i>	-	5	-	-
<i>Enterobacteriaceae</i>	-	-	-	-
<i>Flavobacterium / Cytophaga</i>	21	10	5	-
Total Number	100%	100%	100%	100%

% Distribution / Gram Stain

	0	9	15	21
Gram Positive	35	70	45	55
Gram Negative	65	30	55	45
Total Number	100%	100%	100%	100%

**Table XI Percentage Distribution of Different Microorganisms
During Storage - (100%) MAP .**

Type of Microorganisms	Days in Storage		% Numbers	
	0	9	15	21
<i>Pseudomonas Group I</i>	0	--	--	--
<i>Pseudomonas Group II</i>	10	--	10	5
<i>Pseudomonas Group III/IV</i>	6	--	5	7
<i>Micro. ccus spp.</i>	20	82	20	30
<i>Aeromonas</i>	10	--	5	4
<i>Moraxella</i>	5	6	16	10
<i>Acinetobacter</i>	4	--	10	15
<i>Bacillus spp.</i>	15	6	15	16
<i>Vibrio</i>	7	--	9	9
<i>Streptococcus spp.</i>	--	--	--	--
<i>Enterobacteriaceae</i>	--	--	5	--
<i>Flavobacterium / Cytophaga</i>	21	6	5	4
Total Number	100%	100%	100%	100%

% Distribution / Gram Stain

	0	9	15	21
Gram Positive	35	88	35	46
Gram Negative	65	12	65	54
Total Number	100%	100%	100%	100%

4.3 ORGANOLEPTIC RESULTS AND DISCUSSION

The sensory method for freshness assessment of fish is the traditional and really the ultimate method in terms of sensitivity. However Baines *et al.* (1969) cited by Fey (1980) warned of variable standard in taste panels, that make it difficult to relate the results to objective methods. Learson *et al.* (1969) cited by Fey (1980) pointed out that "although organoleptic panels lack precision, they are the only instrument, at present, that can integrate all the factors that affect quality in fish."

In this study, a panel for assessment of gutted rainbow trout, *Salmo gairdneri* according to Tasmania Food Research Unit (TFRU) system was used. Figure 11. shows the scores obtained from the panel in relation to different gas compositions and storage days. Ice storage was the control, against which all the MAP stored fish were compared. A score of 16 in this study coincided with the level at which the fish were considered unacceptable by the members of the panel. Therefore, a score of 16 or more for sensory criteria indicates a product of unacceptable quality in this study.

The control fish (ice storage) remained acceptable up to 14 days. The eyes remained clear and normal in shape for up to 5 days. The skin remained shiny and the colours of the rainbow trout, *Salmo gairdneri* did not change much during the first 10 days of storage. The flesh was firm and normal but becoming tough towards the end of the storage period. This general external appearance of the control was acceptable for several days, giving a low score, well within the acceptance level for 14 days.

The detrimental parameters of the control fish were, (1) the bad odour, from the whole fish but primarily from the gills. (2) The appearance of the belly cavity which became grey after 6 days of storage. (3) Finally the dull appearance of the skin due to microbial growth, the sinking and the clouding of the eyes, and also the bleaching of the gills.

Fish stored in 100% CO₂ were rejected on the 10th day of storage. This means that according to the organoleptic results, 100% CO₂ atmosphere reduced the shelf life of fish by 4 days. This early rejection was associated with the general appearance of the fish. The reaction of high CO₂ concentrations with the fish surfaces brought about conditions that under normal circumstances are associated with the spoilt fish. For example, the cornea of

the eyes of fish stored in this atmosphere became cloudy after 24 hours. Similar results of cornea clouding was reported by (Coyne , 1933) and by (Stansby and Griffith , 1935) working on haddock. The skin appearance and hence the colours of the rainbow trout became bleached within 2 days, causing the fish to look dull and unappetizing. Similar results of bleaching was given by (Stansby and Griffith , 1935).

In addition to these general appearances, the flesh of 100% CO₂ atmosphere stored fish became soft to the touch, and when squeezed between the thumb and the first finger, disintegrated in a manner similar to putrid fish. Softness of the flesh was also reported by (Coyne , 1933). The gills slowly bleached, becoming very bleached by the end of the storage period. However, the belly cavity of the fish stored in 100% CO₂ atmosphere remained translucent, only greying along the cut edges towards the last days of storage (12 - 14). Bad odour was not noted until the last 2 days of storage.

On comparing the total scores obtained from fish stored in 60/40% CO₂/N₂ atmosphere and for the control , the trend in deterioration appears to be similar. However the demerit score on the control was largely achieved from changes in odour and belly flap, whereas the demerit score on the 60/40% CO₂ occurs principally from appearance.

In the 60/40% CO₂/N₂ MAP fish, the cornea became cloudy but not as quickly as in the 100% CO₂ MAP fish. Bleaching of the skin and gills was moderate but quite noticeable. The slime became clotted, gradually milky and no bad odour was noted until the last 2 days of storage. The belly cavity results were similar to 100% CO₂ atmosphere. Finally the flesh was intermediate in firmness between the 100% CO₂ MAP fish and the control.

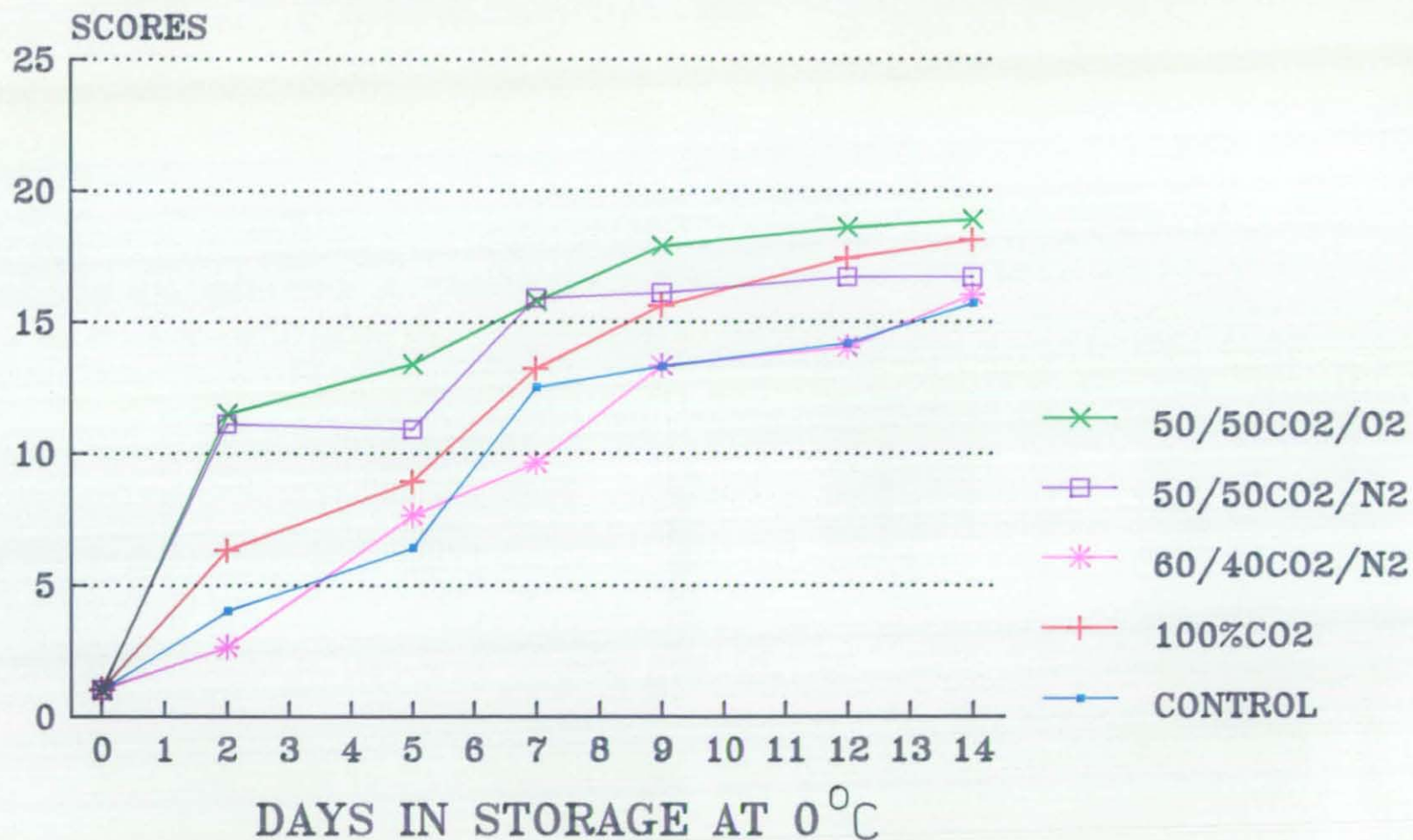
Fish stored in 50/50% CO₂/N₂ and 50/50% CO₂/O₂ were rejected on 10th day and 7th day of storage respectively. On the second day of storage in the 50/50% CO₂/O₂ atmosphere, the score was above 10 points. This very early rejection was due to the development of rancidity, which was so obvious by smell and also by yellowing of flesh that objective chemical measurements were not necessary. In 50/50% CO₂/N₂ atmosphere, the fish were rejected after a similar time as the 100% CO₂ treated fish. The high demerit score was due

partly to the effect of CO₂ on the general appearance of the fish.

Figure 12 shows a comparison between aerobic count and organoleptic rejection time of fish from different treatments. This figure shows that Total Viable Counts are not a satisfactory criterion for determining the shelflife of MAP fish (Rainbow Trout, *Salmo gairdneri*) because although the ice stored fish (control) had the highest count of bacteria, (5.5 log count), it remained acceptable up to 14 days, while 50/50% CO₂/O₂ with a low count of 3.6 log count was rejected at 7 days of storage.

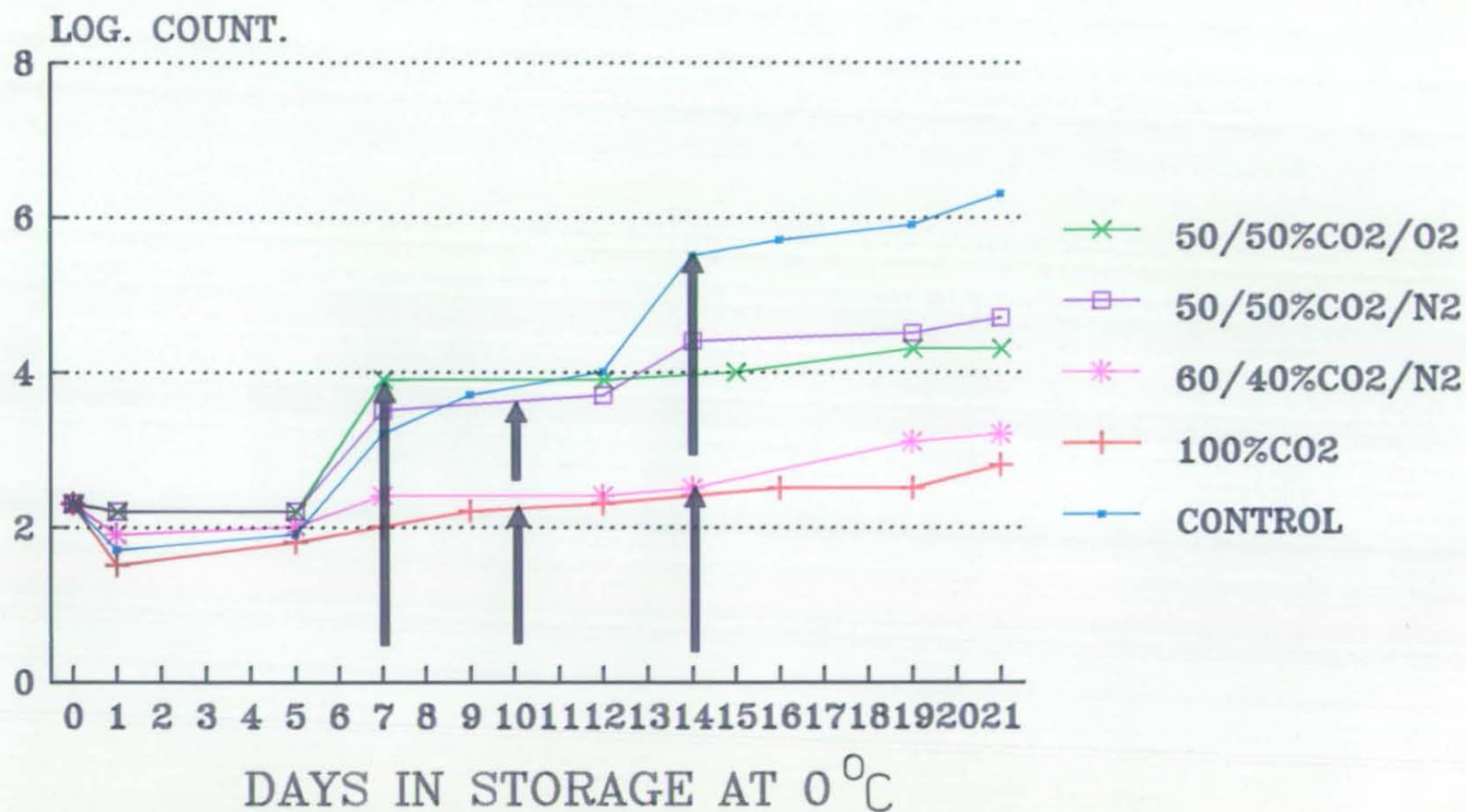
Neither the control nor the treated fish reached a bacterial count of log 6/cm² skin surface, which is reported to be the level of incipient or imminent spoilage, at any stage during the experimental period.

FIG.11 ORGANOLEPTIC MEAN SCORES
TFRU SYSTEM – GRADING METHOD



Mean of two experiments each of two samples.

FIG.12 TVC / ORGANOLEPTIC
TVC AT ORGANOLEPTIC REJECTION
LEVEL



Arrow shows day of rejection while
pointing at the Log. count .

4.4 GR TORRYMETER RESULTS AND DISCUSSION

The GR Torrymeter (GR International Electronics Limited, Almond Bank, Perth, Scotland) was intended to measure the freshness of intact wet fish (Jason and Richards, 1975) cited by (Lees and Smith , 1980). In this study it was used on gutted fish, (Rainbow trout ,*Salmo gairdneri*), the instrument being placed behind the belly cavity, above and parallel with the lateral line. The highest Torry meter number that would be achieved from a very fresh fish is 16, while a fish with no residual shelflife will score 0. The readings decline with age in storage.

Figure 13 shows the results obtained in this study, giving a relationship between Torry meter number and MAP treatments on fish during storage. Fresh rainbow trouts,*Salmo gairdneri* used in this study gave a Torrymeter reading of 14. This is less than might be expected with some fresh cod, but different species of fish vary slightly in their fresh readings. In all experimental treatments including the control, the Torrymeter readings declined during the storage period.

Bacterial numbers were low, and growth minimal during storage of the MAP treated fish, but relatively high in the control. This would suggest that, the reduction in Torrymeter readings was independant of bacterial activity, and that the fall in Torrymeter readings was due primarily to autolytic activity. It is well known that during the storage of marine fish in ice, that minimal growth of bacteria occur during the first 5 to 6 days. In addition, little organoleptic deterioration can be determined, even by a trained panel during this period. However Torrymeter readings fall during this period which is thought to be a result of post-mortem cellular and tissue changes.

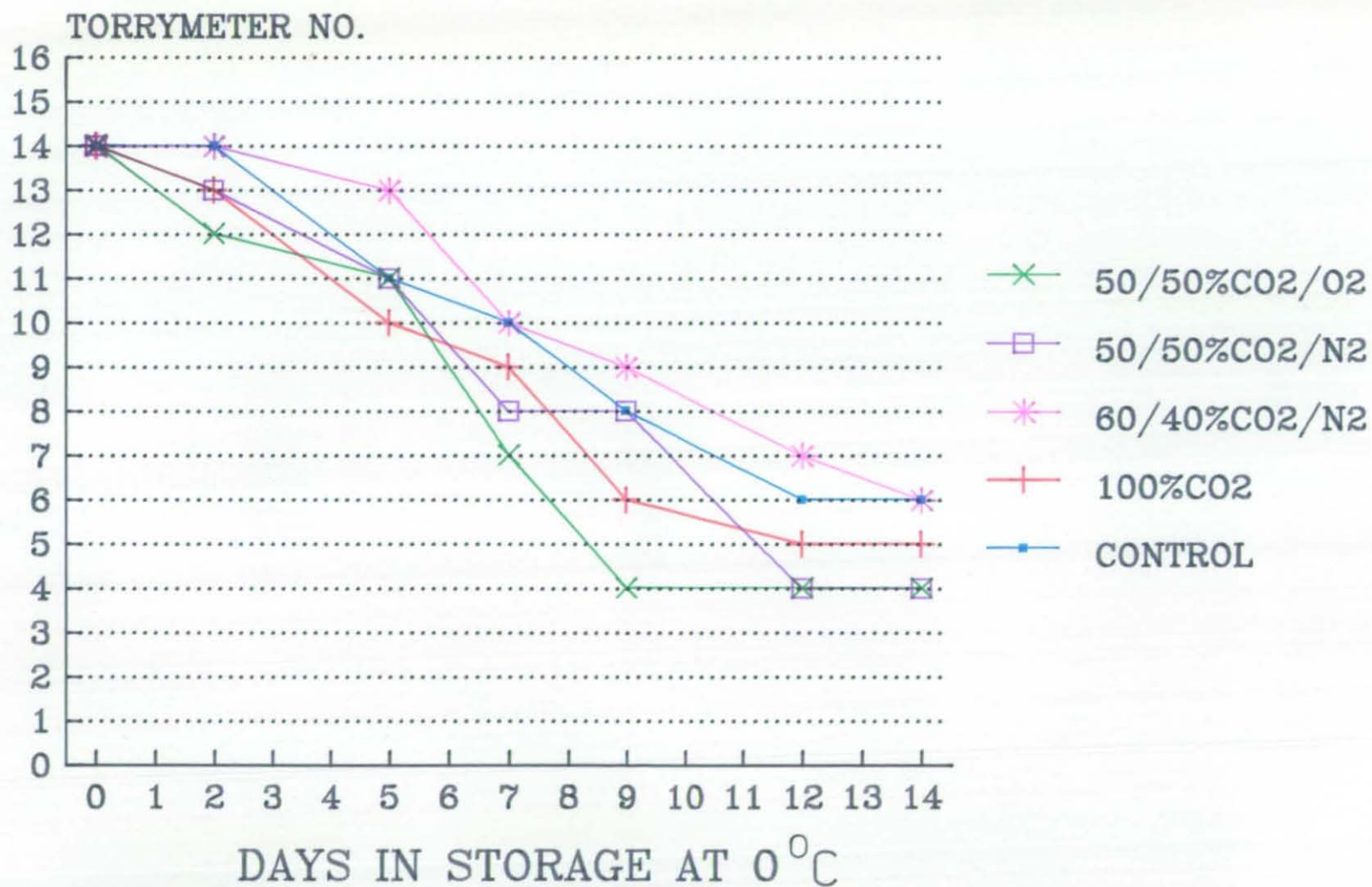
The average torrymeter rejection reading at the TFRU rejection point as portrayed in Figure 14 were as follows:

Control	6
100% CO ₂	5.6
60/40% CO ₂ /N ₂	6
50/50% CO ₂ /N ₂	6.8
50/50% CO ₂ /O ₂	7

The 50/50% CO₂/O₂ MAP fish had high demerit score caused by rancidity changes. With the exception of this treatment, there appears to be a high degree of correlation between Torrymeter results and organoleptic score as given by the TFRU scheme.

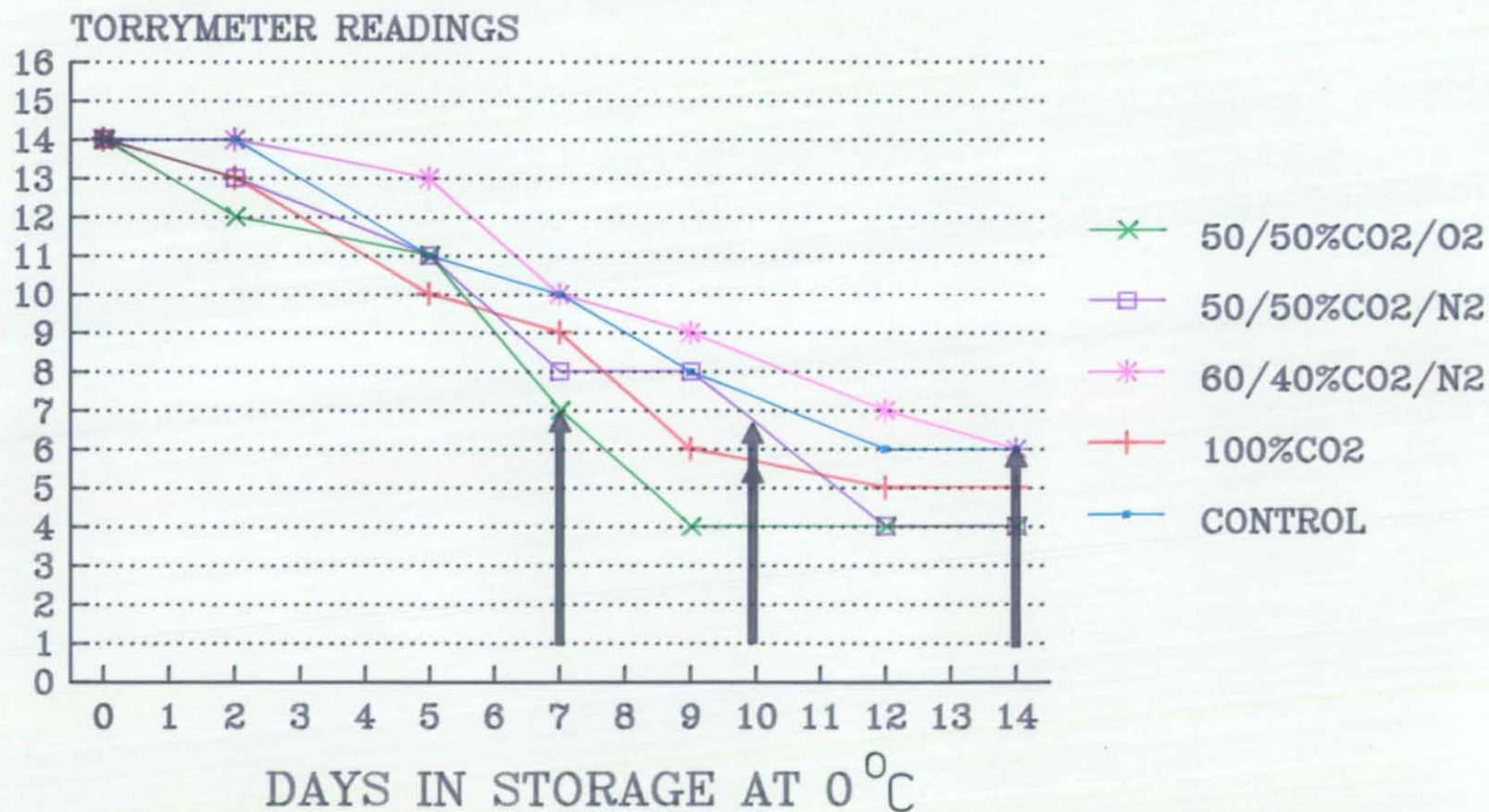
It is suggested that a Torrymeter reading of 6 be adopted as the end of useful storage life of MAP fresh-freshwater fish.

FIG.13 TORRYMETER READINGS



Mean of two experiments each of three samples.

FIG. 14 TORRYMETER/ORGANOLEPTIC
TORRYMETER NO. AT ORGANOLEPTIC
REJECTION LEVEL



Arrow shows the day of rejection
while pointing at the torrymeter number. 97

4.5 pH MEASUREMENT RESULTS AND DISCUSSION

After death, all the glycogen reserves in the fish muscle are depleted via the glycolytic pathway to lactic acid. This ultimately serves to lower the pH. The catching method and hence the amount of stress experienced during the death throes greatly influences the amount of glycogen present at the time of death and therefore the onset and duration of rigor mortis. (Love, 1980). Owing to the small glycogen reserves in fish, the decrease in pH values is not as great as in meat commodities (Huffman et al., 1975) cited by (Boone, 1982).

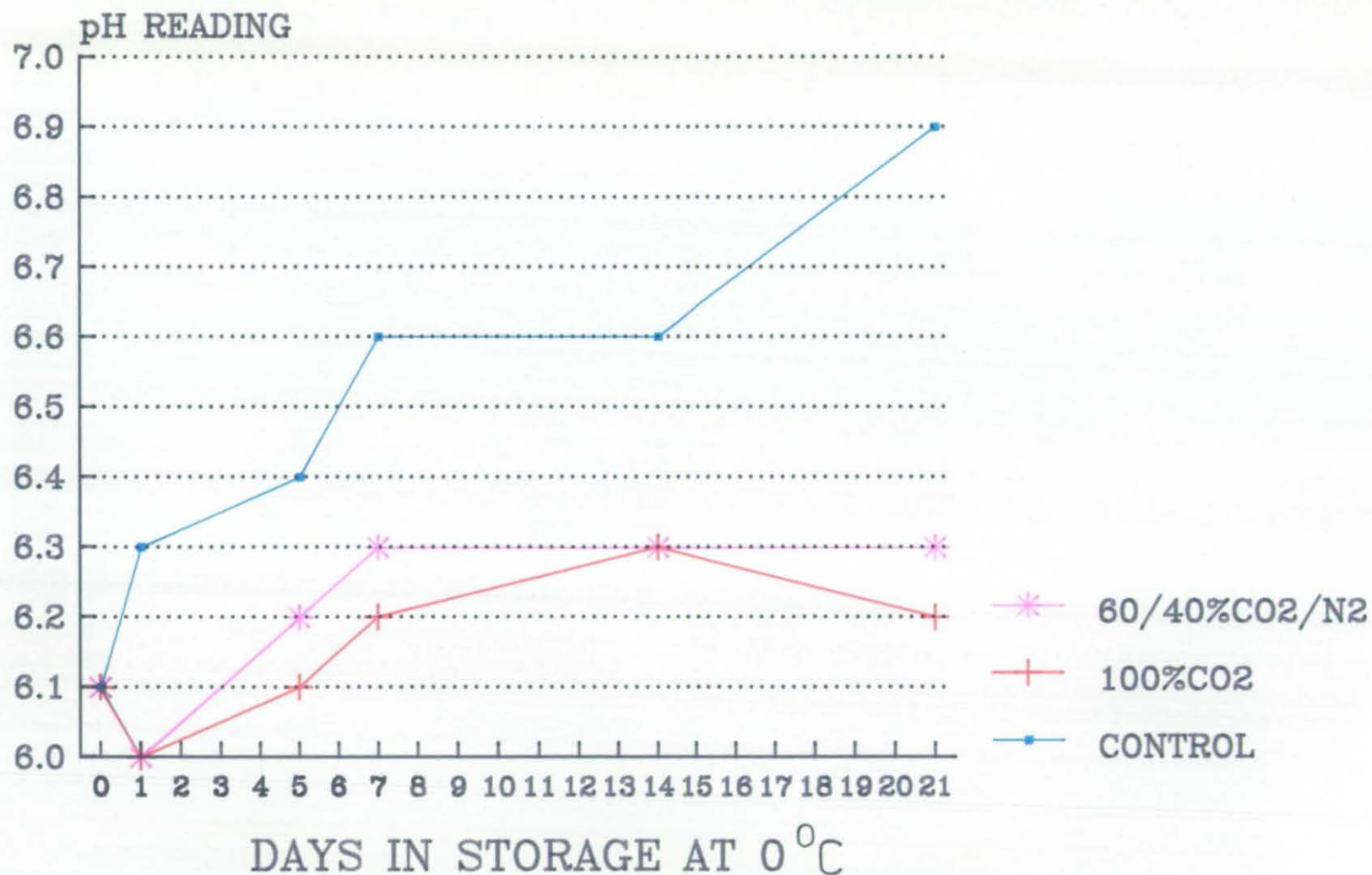
In this study, pH measurements were taken subcutaneously by use of a piercing electrode. The results are shown in Figure 15 which shows the effect of storage time on pH in 100% CO₂ and 60/40% CO₂/N₂ in comparison with the control.

The initial pH of rainbow trout *Salmo gairdneri* used in this study was 6.1. The pH in the control showed a significant increase with storage time to pH 6.9. As fish spoil, bacteria cleave the amino groups of nitrogenous compounds via oxidative deamination to yield ammonia and other low molecular weight nitrogenous compounds (Boone, 1982). These ammonia related compounds increase the pH with storage time. This was clearly apparent in the control fish (ice storage) but occurred at a much slower rate in the MAP fish. This correlates with the results showing microbial activity in the MAP fish during storage period (Figure 2). The 100% CO₂ and 60/40%CO₂/N₂ MAP fish showed an initial decrease in pH from pH 6.1 to 6.0 after one day. This is probably caused by the tissues absorbing CO₂ and consequently lowering the pH. Subsequent to this there is a slight rise in pH reaching a maximum of pH 6.3. This lower pH level compared with the control appears to correlate with the low bacterial numbers recorded under similar storage circumstances.

It may be that this lower pH has a synergistic effect together with the CO₂ in limiting or inhibiting microbial growth. On the other hand, the lack of microbial growth may be the reason why the pH remains relatively stable.

There is no evidence from this study that lactic acid bacteria are responsible for any pH drop or maintenance of a pH level of below 6.3.

FIG.15 pH MEASUREMENTS



Mean of two experiments each of three samples.

4.6 DRIP LOSS RESULTS AND DISCUSSION

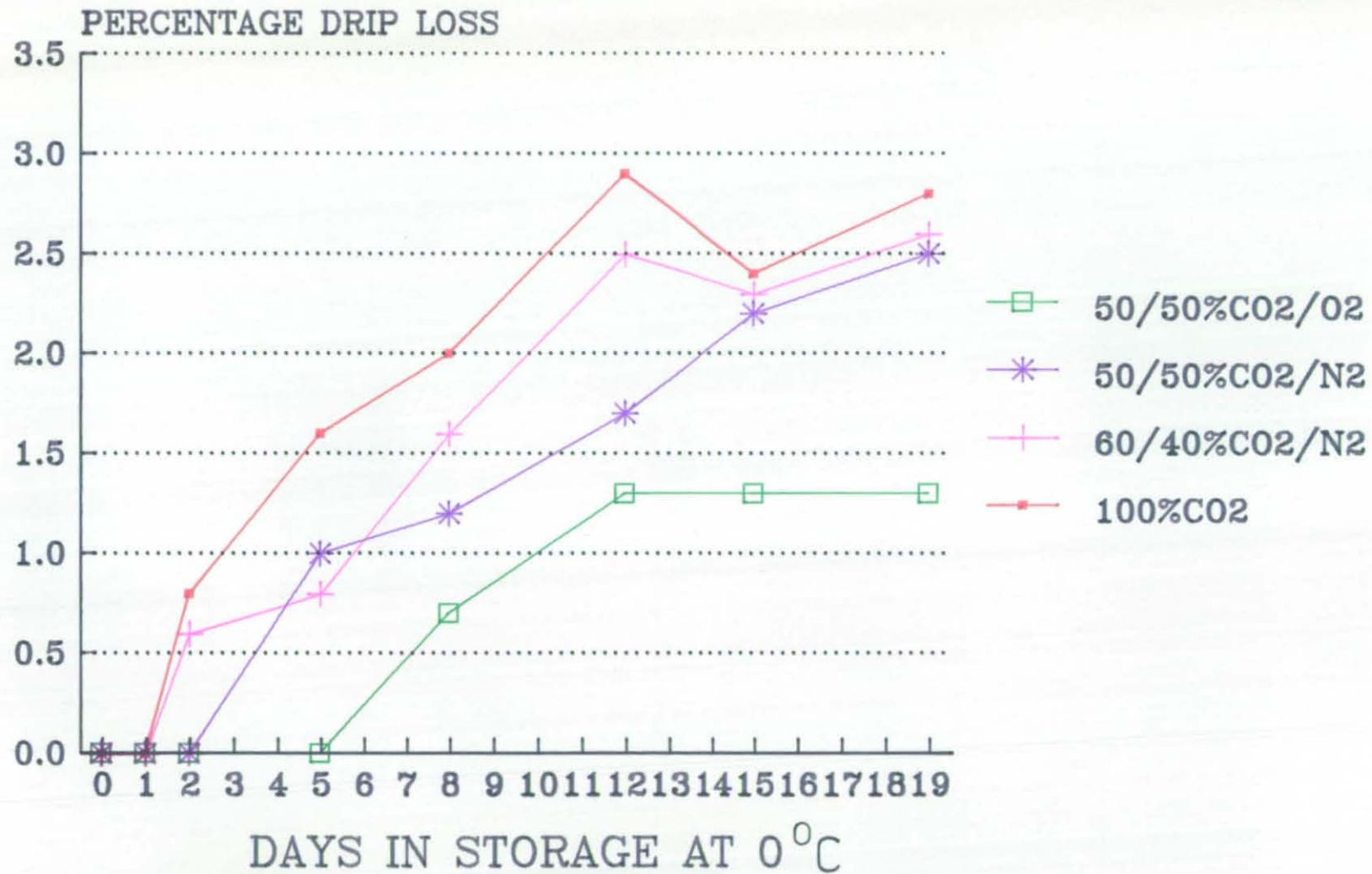
In this study, drip loss was carried out on MAP samples only, because the control samples were packed directly in ice. The experiment was carried out to determine the effect of various gas compositions used in this study on the drip loss. Figure 16 shows the percentage drip loss by weight in relation to different gas compositions and days in storage.

There was a lag period of 1, 2 and 5 days before a measurable drip could collect in 100% CO₂ and 60/40% CO₂/N₂; 50/50% CO₂/N₂ and 50/50% CO₂/O₂ respectively.

The amount of drip loss, as determined in this experiment, is related to the percentage of carbon dioxide in the MAP. The higher the CO₂ percentage the more the drip loss. However, there was a difference between the 50/50% atmosphere, depending on the balancing gas. In the atmosphere where oxygen was the balancing gas, there was a lag phase of 5 days before the drip collected in the tray, and throughout the storage period, the drip was always less than in 50/50% CO₂/N₂ reaching a level of 1.3%. Where nitrogen was the balancing gas, there was a shorter lag phase (2 days) and the drip was much more, reaching 2.5% by the end of the storage period. This experiment shows that inclusion of oxygen in the atmosphere used for MAP of fish would reduce the amount of drip loss. Tifney and Mills (1982) did not find inclusion of oxygen to a level of 20% to consistently affect the pattern of drip production. Boone (1982) found the amount of drip to be proportional to the amount of surface area exposed to the modified atmosphere, giving an example of thin starry flounder fillets having greater area than Pacific Ocean perch fillets. She quoted the moisture loss in 10 days of MAP storage as 10.4% and 7.8-8.4% respectively.

Although the samples were allowed to drain for a short period of time before packaging, rinse water from the fish could have drained and collected in the tray, becoming indistinguishable from the fish muscle drip and hence adding to the percentage drip loss.

FIG. 16 DRIP LOSS



Mean of two experiments each of three samples.

4.7 ATMOSPHERIC CHANGES INSIDE THE POUCH DURING STORAGE

RESULTS AND DISCUSSION

There is no specific information available on the gas permeability properties of the aluminium film (12 μm (813) polyester M1S/50 μm LDPE Campack Bilaminate, used under the conditions of this study. In order to assess the permeability and change in gas composition within the pack, empty pouches were filled with particular gas compositions used for MAP fish and stored in similar conditions, at 0°C for 19 days.

The packs were sampled by means of an airtight 1 ml gas syringe and the gas composition determined by gas chromatograph throughout a storage period of 19 days. Comparative data was determined by assessing the gas composition of the packaged gas for MAP fish stored in a similar manner to the empty pouches. This made it possible to determine the change in gas composition due solely to interaction of internal gases, atmospheric gases, and film during the storage period at 0°C ; and to compare these changes with those occurring in the MAP fish packs stored under the same conditions.

The results are given in Figures (17, 18, 19, 20 and 21). Figure 17 shows the percentage atmospheric changes in the 100% CO_2 packs used for the control (empty) and the fish pouches during storage time. From this figure, it is evident that the deviation from the initial CO_2 atmosphere in the pouches without fish is less than 10% throughout the storage period. There is an initially sharp change of 5% during the first 24 hours, but there is only a further change of 5% for the rest of the storage period. The initial nitrogen level in the control (empty) pouches as well as in the fish pouches was 0%. During the first 24 hours, this rose in the empty pouches to just under 5%, and further rose to 8% during the 19 day storage. The increase in N_2 exactly paralleled the loss of CO_2 from the packs suggesting that initially the pack allowed some exchange of gas with the atmosphere, but that this stabilised at a low level during storage. The fish pouches showed a greater CO_2 decrease and N_2 increase for most of the storage period.

Figure 19 shows the change in CO_2 level expressed as a percentage for empty pouches and pouches containing fish, flushed with the same gas mixture. For the control (empty) pouches, there is an initial drop in CO_2 level during the first day, of approximately 5%,

followed by a further fall of 3% to a level of 52% by the 19th day of storage. There is a corresponding increase in percentage nitrogen during the same period (Figure 20).

In comparison the pouches containing the fish show a loss of CO₂ in the first two days of about 12% followed by a steady decline during the following 17 days to an overall level of approximately 42%. Again there is a corresponding change in the percentage of nitrogen. Figures 18 and 21 show the percentage CO₂ decrease and percentage N₂ increase due to the interaction of fish and the atmosphere around it, without considering changes due to interaction with the packaging material and the external atmosphere. This was achieved by subtracting the percentage change of gas obtained for the control (empty) pouches from the percentage change of gas obtained for the fish pouches at each sampling time. The resultant graph shows changes due only to the influence of the atmosphere on the fish.

The decrease in CO₂ is due to adsorption and/or solubilization of CO₂ in MAP fish. Other researchers (Jensen *et al.*, 1980) cited by Statham, 1984; Mills and Tiffney, 1982 and Boone, 1982) have reported a similar decrease of CO₂, caused by adsorption and/or solubilization into the aqueous phase of the fish tissue.

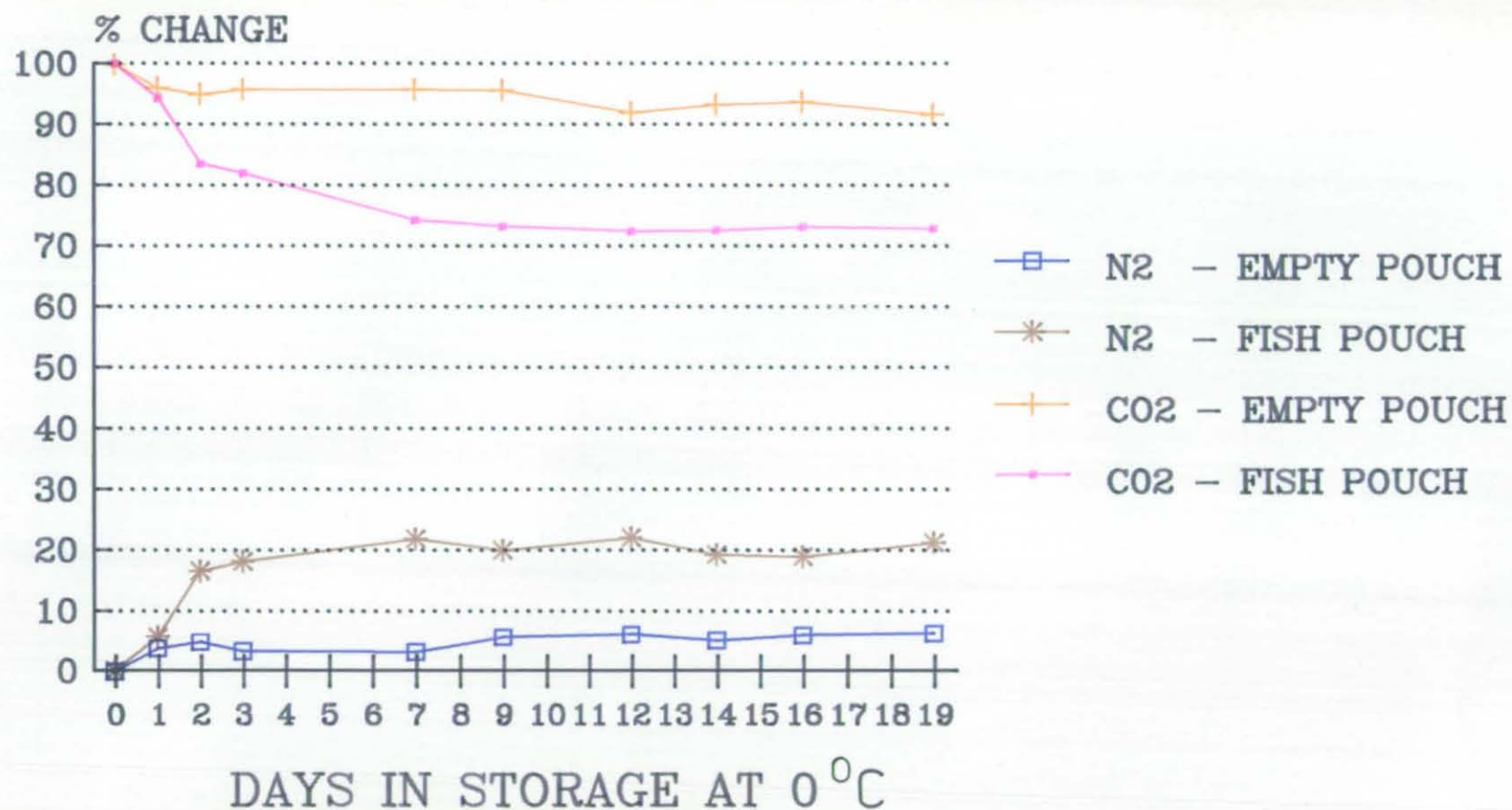
Mitsuda *et al.*, (1975) reported that CO₂ interaction with protein molecules by adsorbing on the Σ - amino group of lysine, the guanidinium group of arginine, and the α - amino group of oligopeptides and amino acids, thus contributing to the observed decrease of CO₂ concentration in the pouch with fish.

It is difficult to maintain consistent pouches for analysis of gas composition for the following reasons: - (1) the fish were not all of the same size, the ones with larger surface area in contact with the atmosphere inside the pouch would dissolve more CO₂ than the ones with smaller surface areas and hence give a different atmospheric change during analysis: (2) some fish could have retained more of the rinsing water than others, giving results as in (1): (3) the machine used for the packaging of the fish could not precisely deliver equal volumes of gas into each package, therefore some pouches could have received more than others. This would increase inaccuracies highlighted in 1 and 2 above.

The level of N₂ gas in the MAP fish pouches during storage was greater than the control

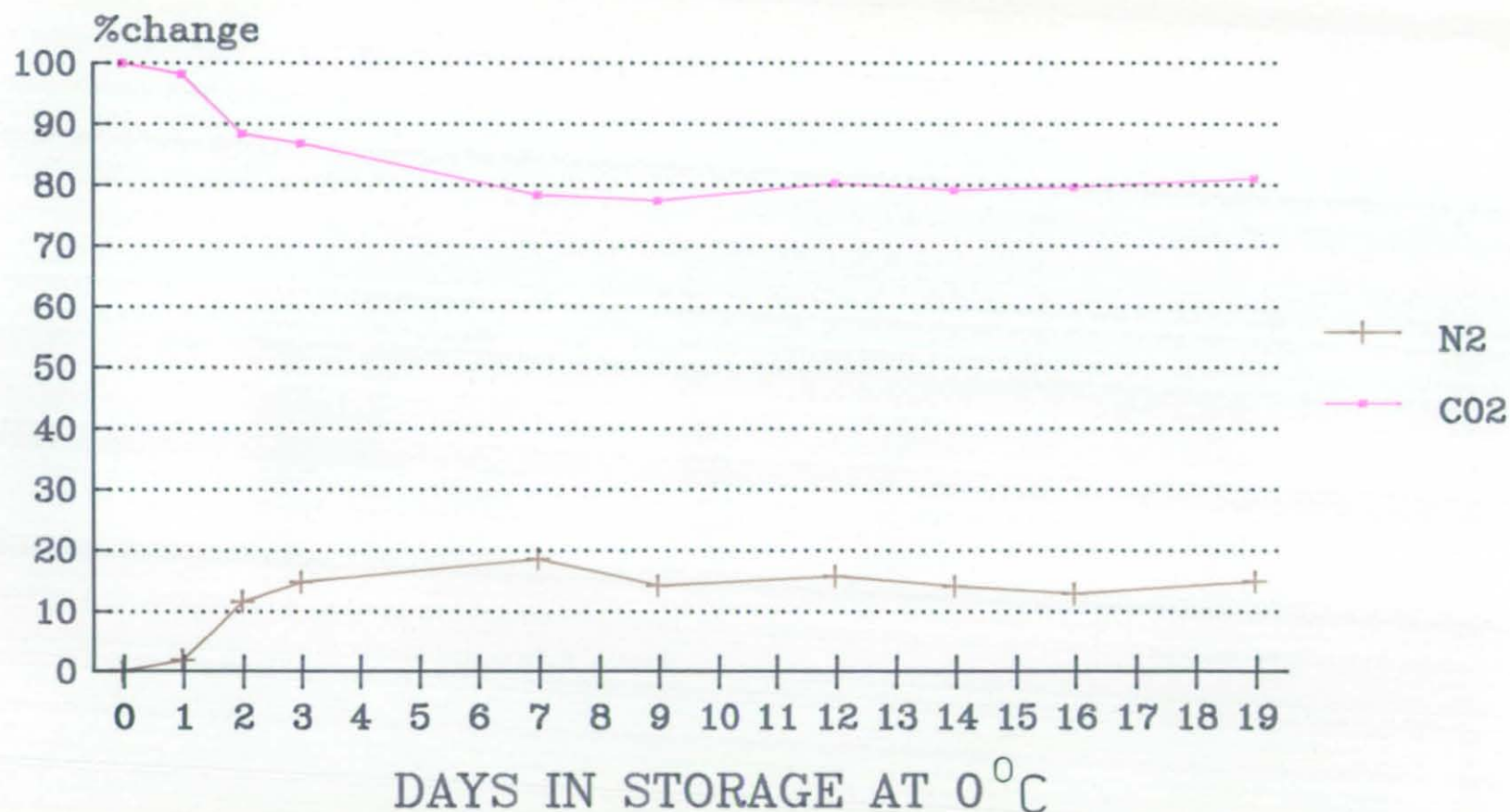
pouches with no fish. It is thought that this occurrence was due to CO_2 level decreasing because of adsorption into the fish (so reducing the total volume of gas in the headspace) thus increasing the relative proportion of Nitrogen in comparison to CO_2 left within the pack.

FIG. 17 MAP GAS - 100% CO₂
CHANGES IN CO₂ AND N₂ LEVELS WITHIN
THE POUCHES WITH AND WITHOUT FISH



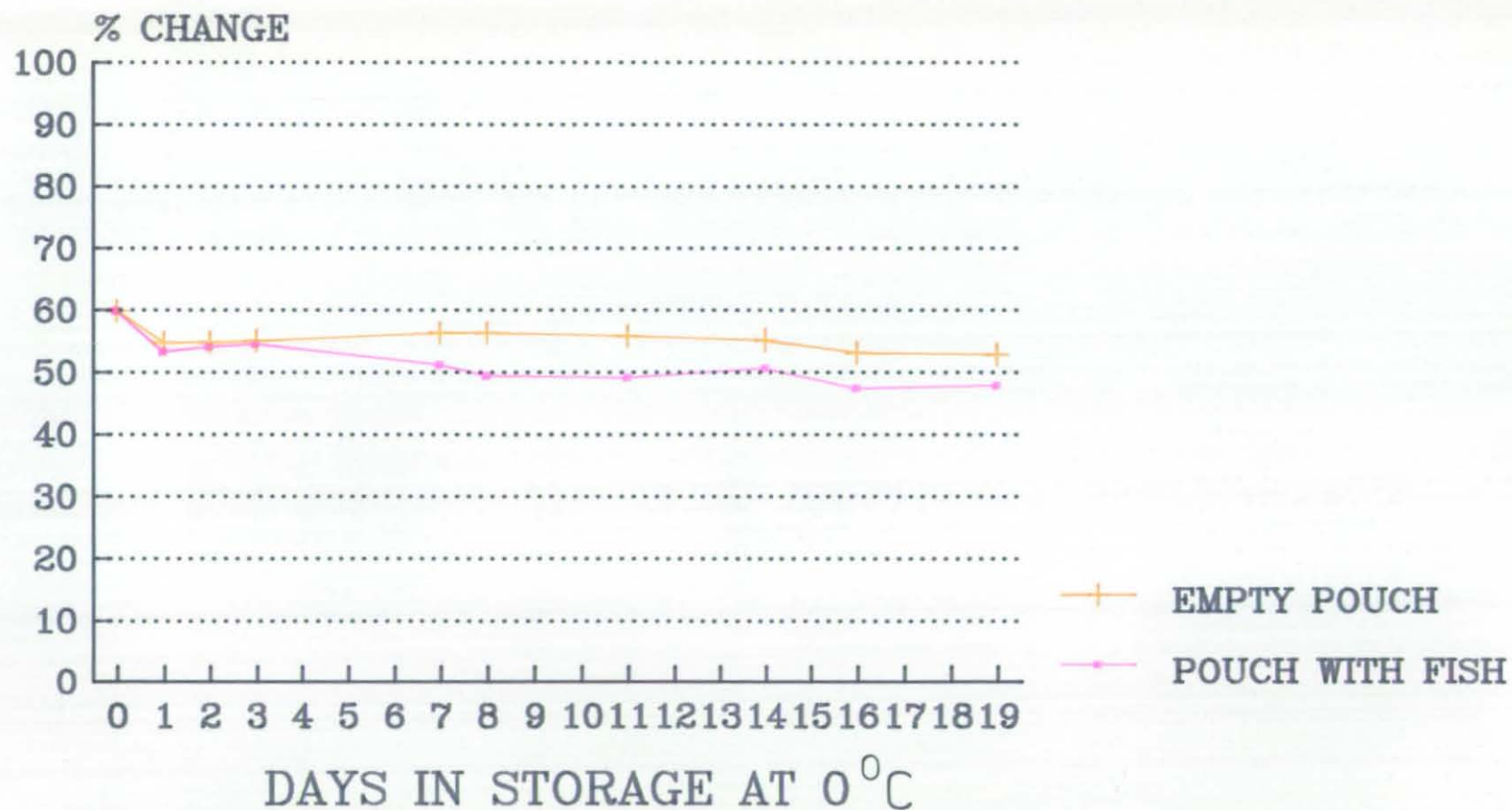
Mean of two experiments, each of three samples.

FIG.18 MAP GAS - 100%
CHANGES IN CO₂ AND N₂ LEVELS WITHIN
THE POUCH DUE TO ABSORPTION BY FISH



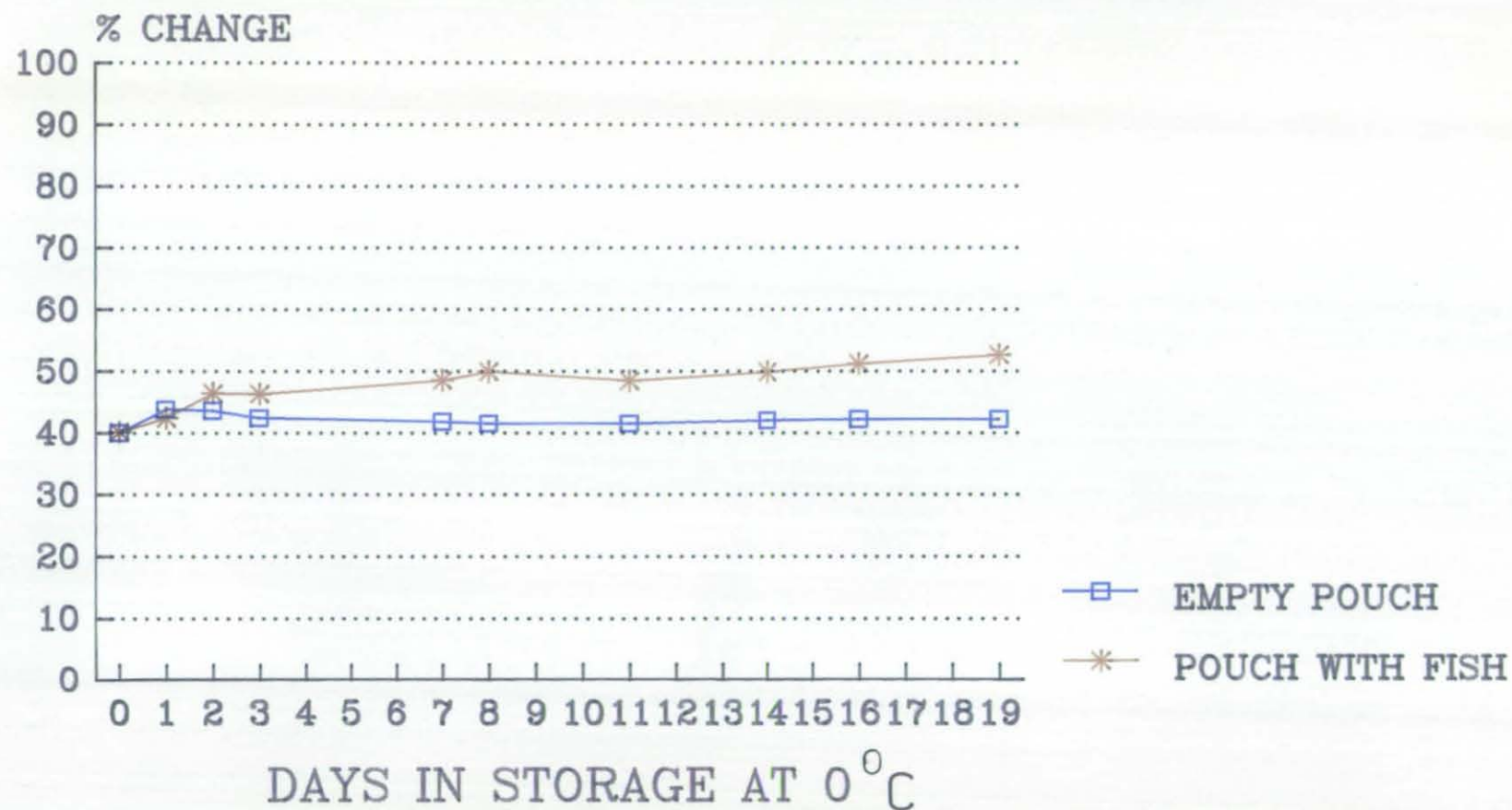
Mean of two experiments, each of two samples.

FIG.19 MAP GAS – 60/40%CO₂/N₂
CHANGES IN CO₂ LEVEL WITHIN THE
POUCHES WITH AND WITHOUT FISH



Mean of two experiments each of three samples.

FIG.20 MAP GAS - 60/40%CO₂/N₂
CHANGES IN NITROGEN LEVEL WITHIN THE
POUCHES WITH AND WITHOUT FISH.



Mean of two experiments, each of three samples.

FIG. 21 MAP GAS - 60/40%CO₂/N₂
CHANGES IN CO₂ AND N₂ LEVELS WITHIN
THE POUH DUE TO ABSORPTION BY FISH



Mean of two experiments, each of three samples.

5 CONCLUSIONS

The following conclusions can be drawn from this investigation:

- 1) Shelflife of iced, gutted rainbow trout (*Salmo gairdneri*) held in a chill store set at +2°C is approximately 14 days. The deterioration is accompanied by a rise in bacterial numbers. It therefore appears that the principal spoilage agent is bacterial growth.
- 2) In iced gutted fish the dominant microflora when shelflife expires consists of gram negative rods of the genus *Pseudomonas* group II.
- 3) The point of unacceptability as determined organoleptically by a score of 16 on the TFRU scheme was found to correspond to a Torrymeter reading of 6.
- 4) With the exception of the 50/50% CO₂/O₂ MAP fish, there is good correlation between the TFRU organoleptic score and Torrymeter reading for MAP treatments and iced fish control.
- 5) The point of unacceptability determined by means of the TFRU scheme and by use of the Torrymeter under the conditions investigated is as follows:

100% CO ₂	10 days
60/40% CO ₂	14 days
50/50 CO ₂ /H ₂	10 days
50/50% CO ₂ /O ₂	7 days
Control (iced)	14 days

- 6) Shelflife of the 50/50% CO₂/O₂ MAP fish as determined by TFRU scheme is 7 days. This corresponds to a Torrymeter reading of 7. This difference appears to be due to the development of rancidity which affects the TFRU scheme more than the Torrymeter.
- 7) The number of micro-organisms at the point of incipient spoilage of fish, normally considered to be 10⁶/cm² of skin surface, is not reached within the experimental period of 21

days in any of the MAP treatments. There is an initial fall in bacterial number during the first 24 hours, followed by a slight increase during the following 20 days. Within the limitations of the treatments used, it appears that the higher the CO₂ level, the greater the bacterial inhibition.

8) The deterioration in MAP fish is not accompanied by a significant rise in bacterial numbers. As microbial spoilage is normally associated with levels of bacteria in excess of 10⁶/cm², it would appear that bacteria are not the principal spoilage agent.

9) If the TFRU scheme is separated into elements concerned with appearance, and odour, then the demerit score which corresponds to the point of unacceptability is determined primarily by the appearance, the major factor being flabbiness of the flesh. The odour is quite acceptable in fish rejected by the scheme.

10) Lactic acid bacteria appear to play no role in the spoilage of the MAP fish.

11) The microflora in the MAP fish at the end of shelflife is not dominated by any particular group but contains a greater proportion of gram positive organisms than the iced (control) fish.

12) Drip loss increased with increased levels of CO₂ in the gas mix used for the MAP.

13) During storage, CO₂ is absorbed by the fish when stored in the gas mixtures used.

14) The pH of the spoilage iced control fish rises from a post mortem pH of 6.1 to a final level of pH of 6.9 after 21 days. In contrast, the pH of the MAP fish does not rise above pH 6.4. This is thought to be associated with the absorption of CO₂ and the lack of bacterial activity.

15) It would appear that MAP fish in the atmospheres used in this study do not spoil by bacterial action. The method is therefore a useful mechanism of preservation, provided that the appearance is not the sole criterion on which acceptability is judged.

Such MAP fish would not obtain such a high price as fresh iced fish because of its appearance, but would be free from off odours associated with spoiling fish. In catering situations, it may not be possible to distinguish MAP from fresh iced fish. Modified atmosphere packs may be most appropriate for bulk transport of fish over long distances in chill containers held at 0°. On reaching their destinations such fish could be unpacked and sold.

6. RECOMMENDATIONS FOR FURTHER WORK

Based on the results obtained in this study, the following further work is recommended.

- (1) Investigation of the acceptability of the current product to the Kenyan population in terms of use of MAP as a method of preservation and the quality of the product itself.
- (2) The use of a taste panel to provide an objective assessment of the cooked product.
- (3) Determination of the level of drip loss acceptable to the consumer and a mechanism for limiting drip loss eg. by the use of polyphosphate dips.
- (4) Evaluation of the potential shelflife attainable after storage in the MAP.
- (5) Scaling up of the packaging process to give a more convenient method for bulk transportation.
- (6) Determination of the causes of belly burst in ungutted fish stored in MAP and development of measures that may inhibit this.
- (7) Measures to inhibit the deterioration in appearance which appears to be the principle reason for consumer rejection eg. the use of skinned fillets.
- (8) Expansion of the work to include studies of other freshwater species.

APPENDIX I

1. SENSORY ASSESMENT SCORE SHEET

FISH IDENT.

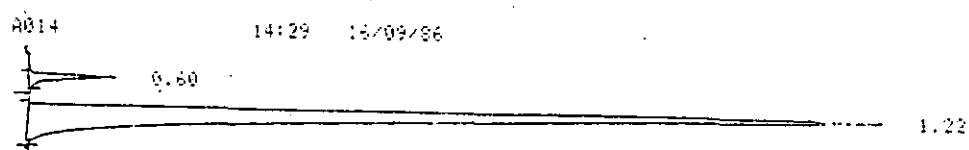
APPEARANCE		(v. Bright/Bright/Sl.Dull/Dull)
		0 1 2 3
SKIN		(Firm/Soft)
		0 1
SCALES		(Firm/Sl.Loose/Loose)
		0 1 2
SLIME		(Absent/Sl.Slimy/Slimy/V.Slimy)
		0 1 2 3
STIFFNESS		(Pre-Rigor/Rigor/Post-Rigor)
		0 1 2
EYES	Clarity	(Clear/Sl.Cloudy/Cloudy)
		0 1 2
	Shape	(Normal/SL.Sunken/Sunken)
		0 1 2
	Iris	(Visible/Not Visible)
		0 1
	Blood	(No Blood/Sl.Bloody/V.Bloody)
		0 1 2
GILLS	Colour	Characteristic (Sl.Dark) (V.Dark)
		(Sl.Faded) (V.Faded)
		0 1 2
	Mucus	(Absent/Moderate/Excessive)
		0 1 2
	Smell	(Fresh Oily) Fishy/Stale/Spoilt
		(Metallic,Seaweed) 1 2 3
BELLY	Discolouration	(Absent/Detectable/Moderate/Excessive)
		0 1 2 3
	Firmness	(Firm/Soft/Burst)
		0 1 2
VENT	Condition	Normal (Sl. Break) (Excessive)
		(Exudes) (Opening)
		0 1 2
	Smell	(Fresh/Neutral/Fishy/Spoilt)
		0 1 2 3
BELLY CAVITY	Stains	(Opalescent/Greyish/Yellow-Brown)
		0 1 2
	Blood	(Red/Dark Red/Brown)
		0 1 2

APPENDIX II

RANGE OF QUALITY SCORES FOR RAINBOW TROUT (*Salmo gairdneri*)

<i>Demerit Points</i>	<i>Quality of Fish</i>
< 4	Prime
4 - 13	Good
13 - 16	Fair
>16	Poor

APPENDIX III An example of separated peaks of CO₂ and N₂ from 100% atmosphere treatment by TRIO



A015 14:32 16/09/86
Method GAS/ANAL
Method Text EUNICE

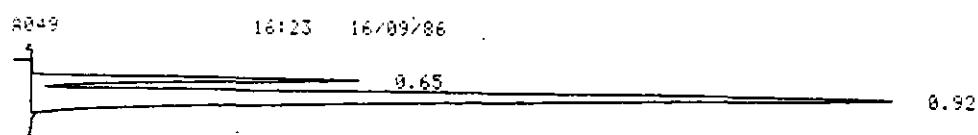
Sample Weight 1.00 Volume 1.00

Report Retention Time	Alieks Peak Start	Peak End	Peak Height	Peak Area	%Area	Peak Code
0.60	0.45	0.78	7013.00	40868	3.45	
1.13	0.76	2.05	72722.60	1143795	96.55	
			79735.60	1184663	100.00	

A017 14:38 16/09/86
Method GAS/ANAL
Method Text EUNICE

Sample Weight 1.00 Volume 1.00

Report Retention Time	Alieks Peak Start	Peak End	Peak Height	Peak Area	%Area	Peak Code
0.60	0.48	0.95	8195.50	49777	4.03	
1.13	0.95	1.95	75132.27	1185149	95.97	
			83327.77	1234926	100.00	



A049 16:23 16/09/86
Method GAS/ANAL
Method Text EUNICE

Sample Weight 1.00 Volume 1.00

Report Retention Time	Alieks Peak Start	Peak End	Peak Height	Peak Area	%Area	Peak Code
0.65	0.32	0.80	20075.25	132034	16.13	
0.92	0.80	1.32	52795.87	686599	83.87	
			72871.10	818633	100.00	

A049 16:20 16/09/86
Method GAS/ANAL
Method Text EUNICE

Sample Weight 1.00 Volume 1.00

Report Retention Time	Alieks Peak Start	Peak End	Peak Height	Peak Area	%Area	Peak Code
0.68	0.60	0.85	19072.78	119520	16.16	
0.97	0.85	1.33	50291.07	619960	83.84	
			69363.86	739500	100.00	

REFERENCES

- Balakrishnan N. R., Tharamani P.K., and Lahiry, N.L. (1971)*
Studies on Chilled Storage of Freshwater Fish. 1. Changes Occuring During Ice Storage. **Journal of Food Science and Technology**, Volume 8, 53-56
- Banks, H., Nickelson, R. and Finne, G. (1980).*
Shelf-Life Studies on Carbon Dioxide Packaged FinFish from the Gulf of Mexico. **Journal of Food Science**, 45 157 - 162.
- Banwart, G. J. (1979).*
Basic Food Microbiology, The AVI Publishing Company, INC. Westport, Conneticut.
- Barnett, H.J., Stone, F.E., Glenn C. R., Hunter, P.J., Nelson, R.W., and Kwok, J. (1982).*
A Study in the Use of a High Concentration of CO_2 in a Modified Atmosphere to Preserve Fresh Salmon. **Marine Fisheries Review**, 44 (3) 7 - 11.
- Blickstad, E., Emfors, S.-O., and Molin, G. (1981).*
Effect of Hyperbaric Carbon Dioxide Pressure on the Microbial Flora of Pork Stored at 4 or 14°C. **Journal of Applied Bacteriology**, 50 493 - 504,
- Blickstad, E. and Molin, G. (1983).*
Carbon Dioxide as a Controller of the Spoilage Flora of Pork, with Special Reference to Temperature and Sodium Chloride. **Journal of Food Protection** 46 (9) 756 - 763.
- Blickstad, E. and Molin, G. (1983).*
The Microbial Flora of Smoked Pork Loin and Frankfurter Sausage Stored in Different Gas Atmospheres at 4°C. **Journal of Applied Bacteriology**, 54 45-56.
- Bligh, E. G. (1971).*
Specific Problems in the Quality Assessment of Freshwater Fish. **Fish Inspection and Quality Control**, edited by R. Kreuzer. Fishing News (Books) Ltd. pp 81 - 85.
- Bramsnaes, F. (1965).*
Handling of Fresh Fish, G. Borgstrom, ed. **Fish as Food** Volume IV pages 1 - 56.
- Bramstedt, F. and Auerbach, M. (1961).*
The Spoilage of Fresh-Water Fish, G. Borgstrom, ed. **Fish as Food** Volume I pages 613 - 634.
- Branch, A.C. and Vail, A.M.A. (1985)*
Bringing Fish Inspection into the Computer Age. **Food Technology in Australia**, Vol 37 (8).
- Bremner, A. H. (1985).*
A Convenient, Easy to Use System for Estimating the Quality of Chilled Seafoods. **Fish Processing Bulletin** 7.

- Brown, D.W., Albright M., Watts, A., Heyer, B., Spruce, B., and Price, R.J. (1980).*
Modified Atmosphere Storage of Rockfish (*Sebastes minictus*) and Silver Salmon (*Oncorhynchus kisutch*). *Journal of Food Science*, **45** 93 - 96.
- Burgess, G. H. O. (1965)*
Fish Spoilage, *Fish Handling and Processing* pp. 346 - 357.
- Cann, D.C. and Taylor L.Y. (1979)*
The Control of the Botulism Hazard in Hot -smoked Trout and Mackerel. *Journal of Food Technology*, **14** 123 - 129.
- Cann, D.C., Smith, G.L. and Houston, N.C. (1983).*
Further studies on Marine Fish Stored Under Modified Atmosphere Packaging.
Aberdeen: Ministry of Agriculture, Fisheries Food.
- Cann, D.C., Houston, N.C., Taylor L.Y., Smith, G.L., Thomson, A.B. and Craig, A., (1984)*
Studies of Salmonids Packed and Stored Under a Modified Atmosphere, Torry Research Station (unpublished).
- Cann, D.C. (1985).*
Packing Fish in a Modified Atmosphere, *Torry Advisory Note*, 88
- Connell, J.J. (1980).*
Control of Fish Quality, 2nd Edition. Fishing News Books Ltd., Farnham, Surrey, England.
- Coyne, F.P. (1932).*
The Effect of Carbon Dioxide on Bacterial Growth with Special Reference to the Preservation of Fish. *Journal of Society of Chemical Industry*, **51** (1) 119T - 21T.
- Coyne, F. P. (1933).*
The Effect on Carbon Dioxide on Bacteria Growth with Special reference to the Preservation of Fish. Part II. Gas Storage of Fresh Fish. *Journal of Society of Chemical Industry*, **52** (11) 19T - 24T.
- Cutting, C.L. (1953).*
Changes in the pH and Buffering Capacity of Fish During Spoilage. *Journal of Science Food Agriculture*, **4** 597 - 603.
- Daniels, J A ., Krishnamurthi , R .and Rizui , S .H ., (1985).*
A review of Effects of Carbon Dioxide on Microbial Growth and Food Quality. *Journal of Food Protection*, **48** (6) 532 - 537.
- Disney, J.G., Cole, R.C. and Jones, N. R. (1974).*
Consideration in the use of Tropical Fish Species. *Fishery Products*, edited by Rudolf Kreuzer. Fishing News (Books) Ltd. pp 329 - 333.

- Ehrenberg, A.S.C. and Shewan, J. M. (1953).
The Objective Approach to Sensory Tests of Food. **Journal of Science and Food Agriculture**, 4 482 - 490.
- Eklund, M.W. (1982).
Significance of *Clostridium botulinum* in Fishery Products Preserved Short of Sterilization. **Food Technology** 107 - 112.
- Enfors, S.O., Molin, G., and Sternstom, A., (1979).
Effect on Packaging Under Carbon Dioxide, Nitrogen or Air on the Microbial Flora of Pork Stored at 4°C. **Journal of Applied Bacteriology**, 47 197 - 208
- Enfors, S.O. and Molin, G. (1980).
Effect of High Concentrations of Carbon Dioxide on Growth Rate of *Pseudomonas fragi*, *Bacillus cereus* and *Streptococcus cremoris*. **Journal of Applied Bacteriology**, 48 409 - 416.
- Farber, L. (1965).
Freshness Tests, G. Borgstrom, ed. **Fish as Food** volume IV Pages 65 - 99.
- Fey, M.S. (1980).
Extending the Shelflife of Fresh Fish by Potassium Sorbate and Modified Atmospheres at 0 - 1°C Celsius. Ph.D. thesis, Cornell University.
- Fey, M.S. and Regestein J.M. (1982).
Extending the Shelflife of Fresh Wet Red Hake and Salmon Using CO₂ - O₂. Modified Atmosphere and Potassium Sorbate ice at 1°C. **Journal of Food Science**, 47 1048 - 1054
- Food Agriculture Organization (1978).
1977 Yearbook of Fishery Statistics, vol. 44 Rome: F A O of the United Nations.
- Genigeorgis, C.A. (1985).
Microbial and Safety Implications of the Use of Modified Atmospheres to Extend the Storage Life of Fresh Meat and Fish. **International Journal of Food Microbiology**, 1 237 - 251.
- Georgala, D.L. (1957)
Changes in the Skin Flora of Cod after Washing and Icing. **Journal of Applied Bacteriology**, 20 (1), 23 - 29.
- Gill, C.O. and Tan, K.H. (1979).
Effect on Carbon Dioxide on Growth of *Pseudomonas fluorescens*. **Journal of Applied and Environmental Microbiology**, 237 - 240.
- Gray, R.J.H., Hoover, D.G., and Muir, A.M. (1983).
Attenuation of Microbial Growth on Modified Atmosphere - Packaged Fish. **Journal of Food Protection**, 46 610 - 613.

- Haines, R. B. (1933).*
The Influence of Carbon Dioxide on the Rate of Multiplication of Certain Bacteria as Judged by Viable Counts. *Journal of Society of Chemistry Industry*, 52 13T - 17
- Hansen, P. (1972).*
Storage Life of Prepacked Wet Fish at 0°C. *Journal of Food Technology*, 7 21 - 26.
- Harrigan, W.F. and Margaret, E. M. (1976).*
Laboratory Methods in Food and Dairy Microbiology. Academic Press, A subsidiary of Harcourt Brace Jovanovich, publishers.
- Hobbs, G. (1982).*
Changes in Fish after Catching. *Fish Handling and Processing*, second edition pages 20 - 27.
- Howgate, P.F. (1982).*
Quality assessment and quality control. *Fish Handling and Processing*, Second Edition pages 177 - 186.
- Jones, M.R., Murray, J., Livingston, E.J., and Murray, C.K. (1964).*
Rapid Estimations of Hypoxanthine. Concentrations as Indices of the Freshness of Chill Stored Fish. *Journal of the Science of Food and Agriculture*, 15. 763 - 774.
- Killefer, D.H. (1930).*
Carbon Preservation of Meat and Fish. *Industrial and Engineering Chemistry*, 22 140 - 143.
- Larmond, E. (1971).*
Food Quality Evaluation: a Review of Sensory Methods. *Fish Inspection and Quality Control*, edited by Rudolf Kreuzer. Fishing News (Books) pp172 - 175.
- Lees, A. and Smith, G.L., (1980).*
Assessment of the freshness of fillets by G R Torrymeter. In *Advances in Fish Science and Technology* 400 - 403. Fishing News Books Ltd. Eamham, Surrey, England.
- Leeson, R. H. (1984).*
The Use of Controlled Gaseous Mixtures. *Conference Notes (UK)*.
- Lima Dos Santos, C.A.M., D. James, and F. Teutscher, (1982).*
Guidelines for chilled fish storage experiments. *FAO Fish Technology. Paragraph; (210):* 22 p.
- Lima dos Santos, C.A.M.*
The Bacteriology of Fresh and Spoiling Freshwater Fish. Unpublished.

- Lindsay, R. (1982).*
Controlled Atmosphere Packaging. **Proceedings of the 4th Annual International Seafood Conference.** Munich, Washington DC 80 -89.
- Malcolm, L. R. (1980).*
Biological Factors Affecting Processing and Utilization. **Advances in Fish Science and Technology.** 130 - 137. Fishing News Books Ltd, Farnham, Surrey, England.
- Martin, R.E., Gray, R.J.H., and Pierson, M.D. (1978).*
Quality Assessment of Fresh Fish and the Role of Naturally Occuring Microflora. **In Food Technology, 32** 188 - 198.
- Matches, J.R. and Layrisse, M.E.(1985).*
Controlled Atmosphere Storage of Spotted Shrimp (*Pandalus platyceros*) **Journal of Food Protection, 48** (8) 709 - 711.
- Mead, G.C. (1983).*
Effect of Packaging and Gaseous Environment on The Microbiology and Shelflife of Processed Poultry Products. **Food Microbiology, 203** - 216.
- Mieclzielski, Z and Krala, C. (1981).*
Effect of the Composition of Gas Mixtures on the Prolongated Storage of Beef. **Acta Alimentaria Polonica, 7** (1/2) 35 - 49.
- Mitruka, B.M. (1976).*
Methods of deterioration and Identification of Bacteria. CRC Press.
- Mitsuda, H., Kamia, F. Yamamoto, A. and Makajima, K. (1975).*
Carbon Dioxide - Protein Interaction on a Gas-Solid Phase. **Journal of Nutritional Science and Vitaminology, 21** 151 - 62.
- Molin, G. and Stenstrom , I. M. (1984).*
Effect of Temperature on the Microbial Flora of Herring Fillets Stored in Air or Carbon Dioxide. **Journal of Applied Bacteriology, 56.** 275 - 282.
- Parkins, K.L. and Brown, W.D. (1982).*
Preservation of Seafood with Modified Atmospheres. **Chemistry and Biochemistry of Marine Food Products, 453** - 465.
- Parkin K.L., Wells , M.J. and Brown, D.W. (1981).*
Modified Atmosphere Storage of Rockfish Fillets. **Journal of Food Science, 47** 181 - 184.
- Partmann ,W. and Frank ,H.K. (1973).*
Observations on the Storage of Meat on Controlled Gaseous Atmospheres. **Proceeding of The International Congress on Refrigeration. 18th Washington. 3** 17 - 24.
- Reay, G.A. and Shewan, J.M. (1949).*
Spoilage of Fish and Its Preservation by Chilling. **Advances in Food Research. Vol.II.** New York: Academic Press.

- Regenstein, J.M. and Regenstein, C.E. (1981).*
The Shelflife Extension of Fish. **Refrigeration Science and Technology**, 4
357 - 364.
- Richter, E.R. and Banwart, G.J. (1983).*
Microbiological and Sensory Evaluation of Fresh Fish Packaged in Carbon Dioxide
for Retail Outlets in the Midwest. **Journal of Food Food Protection**, 46 (3)
245 - 247.
- Sander, E.H., and H.M. Soo (1978).*
Increasing shelflife by carbon dioxide on treatment and low temperature storage of
bulk pack fresh chickens packaged in nylon/surlyn film. **Journal of Food
Science**, 43 1519 - 1527.
- Shewan, J.M. , Hobbs, G. and Hodgkiss, W. (1960a)*
A Determinative Scheme for the Identification of Certain Gram Negative Bacteria with
Special Reference to Pseudomonadaceae. **Journal of Applied Bacteriology**, 23
379 - 390.
- Shewan, J.M. (1977).*
The Bacteriology of Fresh and Spoiling Fish and the Biochemical Changes Induced
By Bacterial Action. In **Proc. Conference. Handling, Processing and
Marketing of Tropical Fish**, pp. 51 - 66. Tropical Product Institute. London.
- Stansby , M. E.and Griffiths , F.P. (1935).*
Carbon Dioxide in Handling Fresh Fish - Haddock. **Industrial and Engineering
Chemistry**, 27 1452 - 1458.
- Statham, J.A. (1984).*
Modified Atmosphere Storage of Fisheries Products: The State of the Art.
Journal of Food Technology in Australia, 36 (S) 233 - 239.
- Stenstrom, I.M. (1985).*
Microbial flora of Cod Fillets (*Gadus morhua*) Stored at 2°C in Different Mixtures of
Carbon Dioxide and Nitrogen/Oxygen. **Journal of Food Protection** 48 (7) 585 -
589.
- Sutherland, J.P., Patterson, J.T., Gibbs, P.G., and Murray, J.G. (1977).*
The effect of several gaseous atmospheres on the multiplication of organisms isolated
from Vacuum - packaged beef. **Journal of Food Technology**, 12 249 - 255.
- Tiffney, P. and Mills, A. (1982).*
Storage Trials of Controlled Atmosphere Packaged Fish Products. **Technical
Report**, No. 191.
- Wheaton, F.W. (1985)*
Quality Changes in Aquatic Food Products. **Processing Aquatic Food
Products**. Publisher - John Wiley.
- Wilhelm , K.A. (1982).*
Extended Fresh Storage of Fishery Products with Modified Atmospheres: A Survey.
Marine Fisheries Review, 44 (2) 17 - 20.

