This is to certify that the

thesis entitled

EFFECT OF MODIFIED ATMOSPHERE
ON QUALITY RETENTION DURING REFRIGERATED STORAGE
OF RAINBOW TROUT (Onchorhynchus mykiss)

presented by

SUTIKNO

has been accepted towards fulfillment
of the requirements for

Masters degree in Food Science

Theodor Wiadnyana
Major professor

Date May 16, 1991

EFFECT OF MODIFIED ATMOSPHERE ON QUALITY RETENTION DURING REFRIGERATED STORAGE OF RAINBOW TROUT (*Onchorhynchus mykiss*)

by

SUTIKNO

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirement

for the degree of

MASTER OF SCIENCE

Department of Food Science

and Human Nutrition

1991

ABSTRACT

EFFECT OF MODIFIED ATMOSPHERE
ON QUALITY RETENTION DURING REFRIGERATED STORAGE
OF RAINBOW TROUT (Onchorhynchus mykiss)

By

Sutikno

The effects of temperatures (0 C and 5 C) and atmospheres (modified atmosphere packaging or MAP and air atmosphere packaging or AAP) and combinations of the temperatures and atmospheres on shelf life of fish were evaluated. Fish spoilage was monitored weekly during 4 week storage.

The shelf life of fish stored at 0 C was significantly higher than that of fish stored at 5 C. The shelf life of fish at 0 C was more than 2.6 weeks.

The shelf life of fish under MAP was significantly higher than that of fish under AAP. The MAP extended shelf life of fish by a factor of more than 1.5.

Combination of 0 C and MAP was the most effective way to extend shelf life of fish. The shelf life of fish under MAP at 0 C was 4+ weeks.

After removing from MAP and storing under air packaging at 5 C for 3 days, fish were still acceptable.

To my wife, Yuli, and my daughter, Wulan

for their patience, kindness and understanding

ACKNOWLEDGMENTS

The author is appreciative of his major advisor, Dr. Theodore Wishnetsky, for continuous guidance given and enthusiasm shown throughout these studies, and for his assistance in the preparation of the thesis manuscript.

The author also wishes to thank Drs. James F. Price Bruce Harte, and Donald L. Garling for their assistance and guidance during the course of the research being reported here. Dr. Garling also provided invaluable help in procurement and maintenance of the rainbow trout colony used in this research.

Special acknowledgment goes to his wife, Siti Zulaikah Sutikno, and his daughter, Titiek Wulandari Sutikno, for their patience, understanding, and help during the course of his graduate program.

His Indonesian friends and many others at Michigan State University gave freely of their time with various types of assistance, for which the author wishes to express his appreciation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

There is an ever increasing demand for fresh fish, especially in the developing countries, many of which rely on fish to great extent for their animal protein requirement (James, 1986). With such increasing demand, an expanded distribution to non-coastal areas may be necessary.

The traditional method of distribution and marketing of fresh fish in ice presents limitations : (1) the extension of shelf life provided by ice is limited; (2) freight cost of bulk transportation of fish packed in ice is expensive; (3) microbial cross-contamination and other forms of abuse cannot be avoided since the fish are not surface protected by overwrapping or other packaging; (4) drip from melting ice during transportation can result in additional spread of bacteria.

One method of packaging that could potentially reduce or eliminate the deficiencies associated with bulk holding of fish in ice is modified atmosphere packaging (MAP). This method, which is currently being used to extend the shelf life of pork, poultry and beef, utilizes a carbon dioxide-rich atmosphere to inhibit growth of bacteria. Commercial MAP systems normally involve evacuation of air from an oxygen-impermeable container, and its replacement with the desired gas mixture, followed by sealing of the container.

MAP systems for fresh saltwater fish have been studied with promising results. However, little is known regarding

the spoilage characteristics of freshwater fish packed in such systems. The structure and composition of fish vary widely. Data available for saltwater fish packed in MAP systems can thus not readily be transferred to freshwater fish.

The objective of this research was to determine the effects of modified atmosphere on quality retention during refrigerated storage of rainbow trout.

LITERATURE REVIEW

Post Harvest Fish

Post mortem changes in fish

Quality of fish is determined by several factors prior to harvest : (1) sexual maturation, (2) the type and amount of feed consumed and (3) environmental conditions such as water temperature and the presence of organic and inorganic pollutants in the water (Castell, 1971)

At the time of death, a series of complex changes begins to occur caused by both natural physiological processes and by the bacteria found on the fish (Regenstein, 1983). The stage of post-mortem change in fish is distinguished by : (1) Secretion of mucus; (2) rigor mortis; (3) autolysis; and (4) bacterial decomposition (Novikov, 1983).

Secretion of Mucus

Secretion of mucus is a process in which the mucus glands located under the skin release mucus onto the surface of the body. When the dead fish are stored, the mucus forms a thick coat around the body which constitutes 2 to 2.5 % of the weight of the fish (Novikov, 1983). Mucus is a very good medium for the development of putrefactive bacteria, which eventually cause turbidity of the skin and give off an unpleasant odor. However, by carefully washing in fresh water the slime can be removed, the odor disappears and the fish may be of normal quality (Novikov, 1983).

Rigor Mortis

Rigor mortis (death stiffening) of fish takes place when the adenosine triphosphate (ATP) level falls below a certain critical level (Pedrosa-Menabrito and Regenstein, 1988). As described by Amalacher (1961), rigor mortis in muscle tissue retards the post mortem autolytic and bacterial decomposition of the flesh and its protein. Rigor mortis in fish generally is of shorter duration than in mammals. It starts 1 to 7 hours after death. Its peak in slaughtered fish, kept in ice, lies between 5 and 22 hours after death. The total duration of rigor mortis is 30 - 120 hr. A prolongation of the rigor mortis period, consequently, is of great economic importance.

Hobbs (1982) explained the biochemical processes which lead to rigor mortis. When a fish dies, some of the enzymes in the muscle still perform their functions, including those that maintain muscle in a state of readiness to contract. The energy for contraction comes from the glycogen stored in the muscle. When glycogen is metabolized either aerobically or anaerobically, ATP is split rapidly to adenosine diphosphate (ADP) and a phosphorous group (Pi) by the Mg-activated actomyosin ATP-ase. The free energy derived from the splitting of ATP in the tissue is used for contraction. The contraction occurs through the sliding of the actin and myosin filaments past one another. In living fish the ATP is restored by biochemical reaction, but it does not occur after death. In the absence of ATP, permanent actomyosin

crosslinks are formed. This action results in the stiffening of fish associated with rigor mortis. It remains until enzymatic activity, probably proteolysis, releases the tension. The release of tension is referred to as the resolution of rigor (Bendall. 1969)

The lack of oxygen as a result of termination of the heart's activities and of the brain causes glycogen to be metabolized anaerobically (glycolysis) to lactic acid (Novikov, 1983). If present in sufficient quantity, lactic acid lowers the pH of the muscle tissue, suppressing the development of microorganisms (Hobbs, 1982). Depending upon fish species, the pH immediately after rigor mortis has been resolved is usually between 6.2 to 6.5 (Amalacher, 1961).

Autolysis

Glycolysis occurs until the glycogen reserve is exhausted or the enzymes are inactivated. Thereafter, autolytic breakdown of protein and some other autolytic changes start taking place (Amalacher, 1961). The activities of the endoenzymes of fish muscle such as proteases, cathepsins, and peptidases play important roles in the degradation of peptides and proteins. The degradation establishes an optimal medium for growth and reproduction of spoilage microorganisms. If, in addition, fish tissue is mechanically damaged, enzymes stored in the lysosomes will also be released (Hobbs, 1982).

Microbial Contamination and Spoilage

Even though autolytic activity and natural chemical changes start the degradation process in fish, the spoilage of fish held in ice is mainly a bacteriological phenomenon . The chemical changes that take place are mainly due to bacterial enzymes (Liston, 1982).

Microflora of Fresh Fish

Fish are prone to rapid microbial spoilage. The high perishability of the commodity is attributed to intrinsic factors which favor rapid microbial growth, namely, low collagen and lipid contents and comparatively high levels of soluble nitrogen compounds in muscle (Venugopal, 1990). The higher ultimate pH of fish flesh as a result of the lack of formation of lactic acid in fish muscle by struggling before death facilitates the decreased lag time and the more rapid growth and reproduction of bacteria, even with refrigeration (Menabrito and Regenstein, 1988).

The flesh and body fluids of newly-caught, healthy fish are generally considered to be sterile (Shewan, 1949) although a few researchers have reported the presence of bacteria in the muscle (Gee, 1930; Bisset, 1949). On the other hand, the slime, gills, and in 'feedy' fish, the intestines, usually carry heavy bacterial loads. Bacterial numbers of 100 to 100,000,000 and 1,000 to 1,000,000 per milliliter of intestinal fluid and per gram of gill tissue,

respectively, have been reported by Shewan (1949) and Georgala (1958).

After the death of fish, the regulatory mechanisms preventing invasion of the tissue by bacteria cease to function and, in short time, microorganisms can be detected in some tissues (Shewan, 1961). It is generally believed that the main route of attack is from the gills and kidney into the flesh via the vascular system and directly through the skin and peritoneal lining (Shewan, 1971). However, it is not known with certainty how long it takes for bacteria to penetrate the skin. Furthermore, species differences may exist. Three to five days is believed to be a reasonable estimate (Martin et al., 1978).

The initial microflora of the newly-caught fish is directly related to its aquatic environment (Shewan, 1971). The bacteria most frequently isolated from fish living in temperate waters belong to the genera Pseudomonas, Acinetobacter-Moraxella, and Vibrio. Flavobacterium, Corynebacterium and Micrococcus also seem to occur consistently but at much lower levels (Liston et al., 1963). In warm water like the Gulf of Mexico, however, only Moraxella-like, Moraxella, Coryneform and Micrococcus species have been reported (Neil, 1977); Pseudomonas sp. have not been isolated. Liston (1980) found that the microflora of many different cold water fish species at the time of spoilage were dominated by Gram-negative bacteria usually, identified as Achromobacter, Flavobacterium,

Pseudomonas, or less frequently Vibrio or Enterobacterium genera. There are a few reports of large numbers of Gram-positive bacteria (e.g. bacilli and micrococci) being the dominant microflora of warm water species.

Lerke et al. (1965) reported that some species of Pseudomonas and Achromobacter groups were involved in the spoilage of raw fresh fish stored at low temperatures. The Aeromonas and Vibrio sp. that they encountered were also active spoilers. The common spoilage odors were rapidly produced by Pseudomonas, Proteus, Achromobacter, and Serratia species, while Micrococcus and Flavobacterium sp. produced mild forms of spoilage and much more slowly (Castell and Anderson, 1948). Flavobacterum sp. are, according to Castell and Mapplebeck (1952), among the organisms most frequently encountered on fish. However, even though 50 % of the Flavobacterium isolated were proteolytic and 16 % reduced trimethylamine oxide (TMAO), they found little conclusive evidence as to their role during spoilage.

Bacterial Activity and Spoilage

The metabolic processes of the microflora contribute partly to the gradual decrease of fish quality and lead ultimately to spoilage due to partial proteolysis and accumulation of unpleasant metabolites.

The microflora of fresh fish initially use many of the low molecular weight substances in the tissue

(carbohydrates, free amino acids, small peptides, and lactic acid) as sources of energy for further growth (Gill and Newton, 1977). Liston (1982) reported that the Pseudomonas and Achromobacter groups rapidly metabolize most amino acids, dipeptides, and tripeptides found in the nonprotein nitrogen (NPN) fraction of muscle. Oxidative deamination of amino acids seems to be the primary pathway leading to ammonia and volatile fatty acid accumulation. Proteolysis does not seem to be significant in the early stages of spoilage because the high concentration of NPN (free amino acid) apparently inhibits the proteinases (Chung, 1968).

The bacterial spoilage reaction in chilled fish has been elucidated by Liston (1982). According to him, the contaminant organisms initially utilize lactic acid and NPN compounds, particularly trimethylamine oxide (TMAO) with the liberation of trimethylamine (TMA), dimethylamine (DMA), ammonia, and other volatile compounds. During 4 to 7 days of storage on ice, a sharp reduction in the content of free amino acid was noticed while no significant proteolysis was evident. Thereafter, the bacterial count increased to above 1,000,00 CFU per square centimeter skin surface or per g of tissue, causing breakdown of protein accompanied by increase in amino acid and volatile sulfur compounds. Sulfur compounds, such as hydrogen sulfide (H2S), dimethyl sulfide ((CH3)2S) and methanethiol (CH3SH) are produced mainly from the sulphur amino acids: H2S from cysteine and ((CH3)2S) and (CH3SH) from methionine (Herbert and Shewan, 1976). This

stage was also marked by severe changes in organoleptic quality of the fish. The number of H2S producing bacteria could, thus, be taken as a general index of the extent of proteolysis and hence spoilage (Herbert, 1979; Gram and Huss, 1987).

TMA is associated with the odor of fish spoilage and is clearly a part of the spoilage pattern of many species (Herbard et al., 1982). According to these authors, when TMA reacts with fat in the muscle of fish, the characteristic fishy odor of low quality fish is produced. Odors appear when TMA level of 2.9 to 4.3 millimol (4 - 6 mg N/100mL) of muscle extract. At a level of 7.2 millimol (10 mg N/100 mL) of muscle extract, there are definite off odors. A number of experiments have shown positive correlations between TMA levels and organoleptic scores.

TMAO is found in a large number of marine fish and shellfish. Generally the largest amounts are in the elasmobranch fishes. Negligible amounts are found in freshwater fish (Dryer, 1952; Groninger, 1959; Ruiter, 1971 as cited by Herbard et al., 1982). Regenstein et al. (1982) stated that TMAO is believed to be involved in osmotic regulation in marine fish, as well as being part of the body's buffer system. TMAO is a waste product in fish, a detoxified form of TMA derived from choline, betaine, methionine etc. (Yamada, 1967). Ogilvie and Warrent (1957) suggested that the presence of TMAO in fish was the result of both exogenous and endogenous processes. Saltwater fish

have varying amounts of TMAO because of differences in TMAO content of their food, but mostly because they must lower their TMAO excretion to the level that counteracts the osmotic pressure of sea water.

TMAO is degraded by the bacterial enzyme triamineoxidase. The enzyme apparently activates the substrate (TMAO) so that a bacterial dehydrogenase can reduce it to TMA (Regenstein et al., 1982). Among the many potential hydrogen donors, the most important is probably lactic acid (one of the final products of glycolysis) and pyruvic acid. Psychrotrophic bacteria, particularly Achromobacter are capable of reducing TMAO to TMA (Lee et al. 1967; Laycock and Regier, 1970). However, significant amounts of TMA are not produced until after the bacterial lag phase, which extends from the onset of rigor to its resolution, due to a postulated bacteriostatic effect of rigor mortis. After rigor mortis, the amount of TMAO reduced to TMA is significant when bacterial counts are sufficiently high.

Temperature and Microbial Growth

The initial loss of the attributes of prime freshness in fish is mainly due to the activity of endogenous enzymes, desiccation, as well as oxidation of lipids and pigments. Further undesirable quality changes and the final spoilage are caused by bacterial putrefaction (Herbert

et al., 1971). The rate of bacterial spoilage is controlled mainly by temperature (Sikorski, 1990).

The relationship between temperature and growth rate of bacterial cultures has been elucidated by several researchers. Plots of microbial growth rate as a function of temperature over a limited temperature range fit the Arrhenius equation, which is commonly used to describe chemical reaction rates as a function of temperature (Ingraham, 1958) :

$$k = A \exp(-Ea/RT)$$

where, A is collision factor or the Arrhenius constant, k is the reaction rate constant, Ea is the energy of activation, R is the universal gas constant, and T is the absolute temperature.

In microbiology, it has been recognized that temperature is also an important factor controlling the rate of development of microbial populations. Microbiologists have simply substituted a growth rate constant r, which is determined assuming an exponential growth model and which is also the reciprocal of the generation time, for the rate constant k in the Arrhenius equation and have replaced Ea by a quantity "u" which they called the temperature characteristic (Ratkowsky at al, 1982). Thus, the new equation is :

$$r = A \exp(-u/RT) \quad \text{or} \quad \ln r = \ln A - (u/RT)$$

Ingraham (1958) found that three psychrophilic pseudomonads had much lower temperature characteristics (u) than two mesophiles, Escherichia coli and Pseudomonas aeruginosa.

Plots of ln r vs 1/T yield a straight line. The line illustrates the temperature optimum and, if extended, would indicate the temperature maximum and minimum.

The u in the equation above is supposed to be constant. However, Baig and Hopton (1969) found that the value of u is a decreasing function of temperature. The consequence of this is that when ln r is plotted against reciprocal temperature, 1/T, to produce what is commonly known as an Arrhenius plot, a curve is obtained instead of a straight line. It indicates that the Arrhenius Law does not adequately describe the effect of temperature on the growth of bacteria.

Based on empirical data, Ratkowsky et al. (1982) found an alternative linear growth relationship for bacterial culture growing between the minimum and optimum growth temperature. Their equation is :

$$Vr = b(T - Ti)$$

where Vr is square root of bacterial growth rate, b is the slope regression line, T is temperature, and Ti is a conceptual temperature of no metabolic significance. The authors suggested that T and Ti are in degree Kelvin to avoid the occurrence of negative temperature.

Owen and Nesbitt (1984) and Storey and Owen (1985) have produced a version of the Ratkowsky et al. equation for

chilled fish and propose that the spoilage rate at 5 and 10 C be 2.25 and 4.0 times the rate at 0 C, respectively. It is reasonable to use the equation since it is by now generally accepted that, at chilled temperatures, fish spoilage and consequent inedibility is due to bacterial growth (Herbert et al., 1971).

Pathogenic Bacteria in Fish

Typically, only two species of pathogenic bacteria seem to occur naturally on fish, *Clostridium botulinum* type E and *Vibrio parahemolyticus* (Liston, 1980).

Cl. botulinum has been described by Eklund (1982) as a spore forming anaerobic bacterial species that exists in either the spore or vegetative state. The spores are widespread in nature and frequently contaminate food products. With appropriate environmental conditions the spores will germinate and develop into vegetative cells which then grow and produce their lethal toxin. The vegetative cells will form spores during the later stage of their growth cycle. The spores are very resistant to heat, drying, salting, freezing , and other physical and chemical treatments and can remain dormant for many years in the soil and in places such as food processing plants. The spores are very difficult to destroy when they contaminate food.

The primary type of *Cl. botulinum* in marine environments in the northern area is type E; however, other types are occasionally found. Bottom sediments of marshes,

lakes, and costal ocean waters contain Cl. botulinum. This type is unusual in being able to grow and produce toxin at low temperature (down to 3.3 C) in suitable media (Hobbs, 1979). However, it does not seem to grow and produce toxin in living fish but is carried passively. From a practical standpoint, therefore, it only becomes a hazard in mishandled processed products.

On the basis of antigenic specificity of neurotoxins produced by Cl. botulinum cells, Eklund (1982) divided the strains of Cl. botulinum into types A, B, C, D, E, F, and G. Type G is weakly proteolytic and type C, D , E, and some F are nonproteolytic.

Types A, B, E, and F have caused the majority of human botulism outbreaks. These botulinum types can be further divided into two groups based upon their biochemical and physiological characteristics (Eklund, 1982). Group 1 consists of the proteolytic organisms of type A, B, and F; and group 2 consists of nonproteolytic organisms of type A, B, F, and E.

Group 1 species are more heat resistant. Some spores withstand boiling water for 6 to 8 hours. The minimum temperature at which they will grow is 10 C (50 F). They attack complex proteins, and their growth is often accompanied by off odor.

Type B, E, and F of group 2 are more sensitive to heat than group 1 types, being rapidly killed in buffer solution at 212 F. These three types of Cl. botulinum have

the important characteristic of growing and producing toxin at temperature as low as 38 F (3.3 C). They do not attack complex proteins and their growth in food cannot be detected by off odor and off flavor.

Cl. botulinum toxins are the most potent poisons known. Under optimum conditions, the growth of Cl. botulinum is accompanied by the release of this potent neurotoxin into the food. When the food is consumed, the toxin enters the circulatory system through the small intestines. The toxin causes paralysis by acting on the nervous system. If sufficient toxin is present in the blood, the diaphragm and chest muscles are paralyzed and death may occur because of asphyxiation. Usually symptoms develop between 8 and 72 hours after eating the toxic food (Eklund, 1982).

Other potentially pathogenic bacteria occasionally associated with fish and shellfish are Cl. perfringens, Staphylococcus, Edwardsiella, Salmonella, E. coli, Shigella, Erysipelothrix, Fransiscella, Vibrio parahemolyticus, Vibrio cholerae and other Vibrios. All of these organisms are probably derived by contamination from terrestrial sources (Shewan, 1971) through contaminated waters.

Lipid Changes in Fish

Lipids in fish contain a high proportion of unsaturated fatty acids. After death, the lipids are subject to two

major changes, namely, lipolysis and autoxidation (Liston, 1980). With the exception of one or two fish products (Van Veen, 1965) the effects of these changes are considered undesirable and are often the major cause of spoilage (Fukuda. 1955; Toyama, 1956).

Of the two processes, autoxidation is the more important deteriorative reaction, causing flavor, color, and possibly textural changes associated with rancidity (Hobbs, 1982). Although oxidative color changes are more significant in frozen fish (Jones, 1962), the oxidation of myoglobin (purple red) to metmyoglobin (brown) has been reported as a major cause of discoloration of both fresh and frozen fish (Benedict et al., 1975). Fat and myoglobin oxidation can be avoided or at least retarded by lowering the oxygen level by vacuum packaging or by modified atmosphere packaging (MAP) with different concentrations of nitrogen and carbon dioxide in the absence of oxygen.

One of the intermediate products of oxidative rancidity in products with highly unsaturated fatty acids is malonaldehyde (Dugan, 1976). Malonaldehyde forms when trienoic and tetraenoic acids are present, and reacts readily with thiobarbituric acid (TBA) to form a characteristic pink color. Vynche (1975) successfully used the TBA procedure to determine rancidity in mackerel.

Modified Atmosphere Packaging

Definition and scope

Modified atmosphere is "the initial alteration of gaseous environment in the immediate vicinity of the product, permitting the packaged product interactions to naturally vary their immediate gaseous environment" (Brody, 1989). MAP is the process of removing the normal air enveloping a product and replacing it with a less reactive or possibly inert gas to eliminate or retard the deteriorative reactions betwen the product and reactive gases (oxygen) in the air. This is done prior to the package being sealed. After the package is sealed, the interior environment is dependant upon the effectiveness of the barrier created by the package and reactions occurring inside the package. MAP differs from controlled atmosphere packaging (CAP). In CAP, there is an effort to monitor and maintain a certain gas mix to compensate for changes occurring inside the package. The gases usually used to replace the air enveloping the product are carbon dioxide and nitrogen.

The first observation regarding the effect of carbon dioxide on retarding bacterial growth were made about 100 year ago (Frankel. 1889; Kolbe, 1882). Since then, the potential for retarding spoilage through application of carbon dioxide has been examined in relation to a number of commodities. A partial list includes : fruits (Kadar, 1980; Smith, 1963), vegetables (Singh et al., 1972), bakery

products (Seiler 1985), carbonated beverages (Insalata, 1952), pork (Huffman, 1974), poultry (Ogilvy, 1951), beef (Taylor and McDougal, 1973), lamb (Smith et al, 1983), fish (Coyne, 1932; Coyne, 1933; Brown et al, 1980; Woyewoda et al., 1984). Through these and other studies, carbon dioxide has been shown to be effective for foods whose spoilage microflora are dominated by gram-negative, aerobic, psychrotrophic bacteria.

Mechanisms of Carbon Dioxide Action

There are several explanations of mechanisms of carbon dioxide action. These are displacement of oxygen, influence on pH and cellular penetration (Daniels et al., 1980).

Displacement of Oxygen

One of the first explanations for the action of carbon dioxide was that it displaced some or all of the oxygen available for bacterial metabolism, thus slowing growth by a proportional amount. This possibility was discounted early in the study of such system by experiments which showed that anaerobic bacteria were also inhibited by carbon dioxide atmosphere (Frankel, 1889). Callow (1932) confirmed these results by replacing the bacterial growth atmosphere with 100 % nitrogen. He did not observe a degree of inhibition equal to that obtained when carbon dioxide was present. Although reducing available oxygen may have some effect on

bacterial growth, it does not appear to be the most limiting factor.

Influence on pH

Most research on carbon dioxide atmosphere and bacterial growth makes the observation that pH of the medium is decreased (King and Nagel, 1967; Parkin and Brown, 1983; Villemure et al., 1986). Several investigators have suggested that when gaseous carbon dioxide is applied to a biological tissue, it is first dissolved into the liquid phase of the tissue, then absorbed as carbonic acid in the undissociated form (Sears and Eisenberg, 1961); Barnett et al, 1979; Mitsuda et al.,1980). This mechanism for movement of carbon dioxide into a cell would help to explain the observation by many researchers that application of carbon dioxide atmosphere also causes a rapid pH drop in the tissue (King and Nagel, 1967). After rejection of oxygen exlusion as a major mechanism for the action of carbon dioxide, many early researchers suggested internal acidification as the cause of its bacteriostatic effect. Once again, however, a few relatively straightforward experiments demonstrated that the observed effects were not due to acidification alone. Coyne (1933) adjusted the pH of bacterial growth media to standard levels (approximately pH 5.8), then grew pure cultures of Achromobacter, Pseudomonas and bacillus under either air or carbon dioxide atmosphere. In all trials, the carbon dioxide treatment produced a far greater degree of

inhibition, as measured by culture growth. In another investigation, Becker (1933) studied other acids that produced equal acidification in the cell, but found that they were not able to inhibit growth to levels achieved through application of carbon dioxide.

The observations by several researchers that the effect of carbon dioxide is increased at low temperature tends to support the theory that carbon dioxide acts first by dissolving in liquid phase. Barnett et al. (1971) showed this to occur to a minimum temperature of 1 C, below which no additional bactericidal effect was achieved. Sears and Eisenberg (1961) reported that increased inhibition at low temperature has been correlated to increased solubility of the gas in the water phase. This was confirmed by Wolfe (1980) who found an increased effect at low temperature and attributed it to increased solubility of carbon dioxide. Recent investigation by Daniels et al. (1985) showed that direct application of carbonic acid to fresh fish fillet was effective in reducing surface microbial growth during refrigerated storage.

Cellular Penetration

An alternative theory of the mechanism of carbon dioxide action suggests that carbon dioxide and bicarbonate ions may alter contact between the cell and its external environment by affecting the structure of cell membrane. Sears and Eisenberg (1961), using a model system, observed

that the concentration of bicarbonate ions influenced the molecular arrangement at the interface between lipid droplets and water. They further indicated that higher concentrations caused a decrease in the interfacial tensions and increased hydration of the 'membrane'. They concluded that bicarbonate would cause an increase in a membrane's permeability to ionic species and could alter the balance between the internal and external metabolic processes. If carbon dioxide is first dissolved in the form of carbonic acid, then bicarbonate ions would be present as dissociation products, and thus be available to produce these changes in cell permeability.

Many researchers alluded to the interference of carbon dioxide with the various metabolic chemical reactions once it is in the cell. Elsden (1938) first reported that Bacillus showed an increased rate of succinate formation in the presence of carbon dioxide, and that removal of carbon dioxide caused a decrease in succinate concentration. The specific inhibition of fumaric acid formation from glucose in Rhizopus nigricans occurs when grown anaerobically under high concentration of carbon dioxide (Foster and Daves, 1949). In addition, the researchers reported that oxaloacetate decarboxylase was inhibited by the presence of carbon dioxide. Fanestil et al. (1963) showed that carbon dioxide stimulated mitochondrial ATP-ase activity, and that such action would have a coupling effect on oxidative phosphorylation, resulting in a decreased level of energy

available to the organism in the form of ATP for metabolism and growth. King and Nagel (1967) studied different growth rates for Pseudomonas grown on various subtrates, and postulated that carbon dioxide may interfere with formation of coenzymes that break down the substrate before absorption by bacteria. The investigators further tried to determine whether carbon dioxide has a general effect on all enzymes, or a specific effect on selected enzymes. They found that there was no inhibition by carbon dioxide of the enzymatic reaction rates of oxaloacetate decarboxylase, fumarase, succinate dehydrogenase, or cytochrome C oxidase, but carbon dioxide at concentrations above 50 % inhibits the activity of isocitrate dehydrogenase and malate dehydrogenase. From these results the investigators concluded that carbon dioxide has a specific effect on particular enzymes, and inhibits certain decarboxylation enzymes through a mass action effect. Mitsuda et al. (1980) reported that on the basis of work with a model system, carbon dioxide interacts with enzymes to cause a transient inactivation, particularly to many hydrolases that cause autolysis after death. Wolfe (1980) related disruption of normal intracellular activity to pH changes related to absorption of carbon dioxide.

Although there seems to be ample evidence that carbon dioxide inhibition is related to enzymatic interference, Gill and Tan (1979) stated that the basis of such inhibition

is not known, although the specific inhibition of certain enzymes may be involved.

In regard to inhibition by carbon dioxide of bacterial growth, Daniels et al. (1980) wrote : "(1) The exclusion of oxygen by replacement with carbon dioxide may contribute slightly to the overall effect, by slowing the growth rate of aerobic bacteria; (2) The ease with which carbon dioxide penetrates the cells may facilitate its chemical effects on the internal metabolic processes; (3) Carbon dioxide is able to produce a rapid acidification of the internal cells with possible ramification relating to metabolic activities; (4) Carbon dioxide appears to exert an effect on certain enzyme systems. Such effects do not appear to be similar among species, and may well be affected by different growth conditions among members of the same species."

Application of MAP to Fishery Products

Extension of Shelf Life

Many researchers found that the shelf-life of fish increases when stored in MAP as compared to the same material stored in air at the same temperature. Kiffeler (1933), using pure carbon dioxide, showed that the shelf-life of both meat and fish could be extended 2 to 3 times, as compared to the same material stored in air. Coyne (1933) stated that the shelf-life of fish stored in MAP at 0 C is at least twice as long as in air at the same

temperature. Haddock kept twice as long in carbon dioxide atmosphere compared to air (Stansby and Griffiths, 1935). Bank et al. (1980) indicated that an extension of the shelf-life of fresh fish can be obtained by packaging and storing the fish in a carbon dioxide atmosphere. Parkin et al. (1982) showed that fish stored under refrigeration in carbon dioxide-enriched atmosphere have a longer shelf-life as compared to similar samples stored in refrigerated air. Villemure et al. (1986) studied the storage life of bulk stored cod under carbon dioxide and air atmospheres, and found the shelf-life of cod under carbon dioxide atmosphere to be nearly twice as long as in air. The shelf-life of fish under carbon dioxide atmosphere storage can be extended by a factor of 1.5 to 2.0 (Veranth and Robe, 1979; Brown et al, 1980; Woyewoda et al., 1984).

Factors Affecting Effectiveness of MAP

Three factors affecting use of MAP need to be addressed in regard to extension of the shelf-life of fresh fish. The first is the type of fish; the second is the concentration of carbon dioxide needed to produce optimal inhibition of bacterial growth; and the other is the potential for growth of pathogenic bacteria under modified atmosphere conditions.

Jacquat (1961) classified fish into three categories based on fat content. These are fatty, semi fatty and lean fish. Based on its meats, there are two group of fish, namely, 'white' fish and 'dark' fish (Love, 1989). Finne

(1980) stated that for fishery products the shelf-life and other benefits obtained by MAP may well be species dependent.

With regard to optimal concentration, there is considerable variation among results reported by various investigators, as well as differences in methodology for approaching the question. Coyne (1933), in one of the original studies on using carbon dioxide atmosphere to extend shelf-life of fresh fish, recommended concentrations between 40 and 60 %, and suggested that, above the upper concentration, no additional benefits could be derived. Shewan (1949) recommended concentrations between 30 and 40 % for improving the quality of white fish. Tarr (1954) suggested a minimum of 40 to 50 % to derive maximum benefit in the storage of fresh fish. Brown et al. (1980) studied storage of rockfish filets and salmon steaks in both 20 and 40 % carbon dioxide atmospheres and found superior quality at the higher concentration. Cann (1985) performed research with the aim of determining a suitable gas mixture for MAP of fish and the extent of benefits to be gained. He concluded that, for a gas : fish ratio of 3 : 1, a gas mixture of CO2/O2/N2 of 40/30/30 is suitable for white fish and 60/0/40 for fatty fish such as trout.

There is evidence that MAP may not introduce a significant risk from fish if proper sanitation and temperature controls are employed. However, fish is likely to be contaminated with spores of Cl. botulinum since they

exist in the fish environment (Daniels et al. , 1985). Johannsen (1965) stated that those bacteria which predominate in carbon dioxide atmosphere, such as the Lactobacilli, form peroxide and acid that may inhibit growth of Clostridia. Schimdt et al. (1961) and Hobbs (1978) reported that Cl. botulinum type E does not grow and produce toxin below 3.3 C, thus the potential for toxin formation could be minimized by maintaining temperature below this level. Licciardello et al. (1967) reminded us that, even if the toxin is formed , normal cooking operation would inactivate the toxin. However, Wilhelm (1982) cautioned that until the safety from botulism can be demonstrated, use of MAP cannot be recommended for retail use. Lyndsay (1984) suggested that modified-atmosphere master-package systems with careful, low-temperature controls could transport traditionally tray-packed fish in oxygen-permeable films and provide the benefits of MAP during distribution to the retail level. The sale of individual packages from opened master packages would effectively remove the potential of botulinum toxin formation since aerobic conditions would prevail within the package during retailing and afterwards, a period when low-temperature control cannot be assured.

Effect of Temperature on MAP

There is little descriptive information (Coyne, 1932; 1933) and no quantitative data concerning temperature effect

on MAP for shelf-life extension of fish products. However, there are limited data dealing with temperature effects of MAP on microorganisms growing on meat, chicken, or artificial media.

Ogrydziak and Brown (1982) stated that : "(1) at temperatures below the optimum for growth of a microorganism, the lower the temperature the lower the growth rate ; and (2) in a given medium there is a temperature below which the microorganism does not grow." Harder and Veldham (1971) reported that the growth rates of facultative psychrophilic bacteria in fish decrease steadily from 26 to 5 C, then decrease more sharply from 5 C to - 2 C; while the growth rate of obligate psychrophiles decreases steadily from 16 C to - 2 C.

Enfords and Molins (1981) showed the influence of temperature on the growth- inhibitory effect of carbon dioxide on Pseudomonas fragi and Bacillus cereus. The following discussion will concentrate on their results with Pseudomonas fragi, a psychrotrophic food-spoilage species since Bacillus cereus is a mesophile which does not grow at refrigerated storage. The Pseudomonas fragi was grown in a complex liquid medium in a fermenter. The pH was automatically controlled, and the culture was continuously flushed with either air or 50 % carbon dioxide in air.

The growth rate of Pseudomonas fragi in both atmospheres was determined at 5 degree increments between 5 and 35 C at both pH 5.7 and 6.7. The effect of carbon

dioxide was expressed as a 'relative inhibitory effect'(RI). RI equals to ((rC -rCO2)/rC) x 100; where rC and rCO2 are the growth rate of the control culture and the carbon dioxide-inhibited culture, respectively.

The results indicated that the relative inhibitory effect decreased as the temperature increased. Since carbon dioxide solubility decreases when the temperature is increased, this suggested that the relative inhibitory effect might be a function of carbon dioxide solubility. The authors concluded that, taking the solubility of carbon dioxide into account, the carbon dioxide inhibitory effect on Pseudomonas fragi was independent of temperature.

The data on the effect of temperature on MAP of seafood are qualitative. Coyne (1933) compared cod stored at 0 C and 10 C in air and in various concentrations of carbon dioxide. The shelf life of fish stored in carbon dioxide atmosphere at lower temperature was longer than that at higher temperature.

Extension of shelf life is a consequence of the effects of carbon dioxide on both growth rate and lag time (Ogrydziak and Brown, 1982). According to these authors, estimation of increase in shelf life based only on the relative inhibitory effect will be an underestimate.

Enford and Molins (1981) reported that lag phase was prolonged as temperature decreased, but lag phase was not significantly influenced by the presence or absence of carbon dioxide. Gill and Tan (1979) showed that an increase

in lag time at 30 C only occurred in minimal medium at the highest carbon dioxide concentration studied (about 60 % carbon dioxide). Ogilvy and Ayres (1951) found that there was a tendency for lag time to increase when carbon dioxide concentration was increased. However, the authors stated that their results were not consistent in this respect. At 5 C, lag time increased as the carbon dioxide concentration increased (Haine, 1933; Clarck and Lentz, 1972).

Rainbow Trout

In terms of zoological classification, the rainbow trout (Onchorhynchus mykiss) (Smith and Stearley, 1989) belongs to sub family salmonini of the family salmonidae (Lair and Needham, 1988).

Willers (1981) described the rainbow trout as having, in its typical coloration, blocks of blue to olive green which fade to a silver tone below. Along the lateral line there is a pink band, and back, sides, head, and fins are generally covered with a profusion of small black spots. As a rule, rainbow trout darken somewhat with age, and the pink lateral band deepens in color, but color variations are seen, many apparently due to local environmental conditions.

The distribution of this fish, whose original scientific name, Salmo gairdnerii, honors Dr. Meredith Gairdner, a naturalist employed by Hudson's Bay Company, extends from Mexico north to the Bering Sea in Alaska. Almost all naturally-occurring populations are found west of the Continental Divide (Laird and Needham, 1988).

Introductions outside of their native range occurred in 1874 when fish from the Mc Cloud River, California were transferred to Caledonia River, New York State. Since then, rainbow trout, which have 'anadromous' forms, have been transplanted all over the world (Willers, 1981; Laird and Needham, 1988).

Fish that are normally resident in saltwater, but which run to freshwater to spawn are said to be "anadromous". Spawning is in freshwater and the young grow in it. Some species of rainbow trout never migrate to the sea but most of them reach a stage known as the smolt stage when the fish is capable of surviving in the sea (Laird and Needham, 1988).

The blood of fish, including rainbow trout, in either fresh or saltwater, must undergo continuous changes in order to maintain a balance between the salts in solution in the body fluids and the surrounding water (Sedgwick, 1982; Stevenson, 1987; Laird and Needham, 1988). The process by which this is carried out is known as osmosis. When solutions of different concentration are separated by a semi-permeable membrane, water will pass through the membrane from the dilute to the more concentrated solution until the concentration on both sides of the membrane is the same. The body fluid of fish in freshwater is more saline than the environment. The gills, the gut and, to a lesser extent, the skin are semi-permeable membranes (Sedgwick, 1982; Stevenson, 1987). Water enters the bloodstream

through these membranes and must be constantly discharged through the kidney to maintain the correct saline balance in the body fluids.

In the marine environment, the sea water is a more concentrated salt solution and water passes out through the semi-permeable membranes and is lost to the body. The fish must drink seawater to compensate for this loss and, in doing so, takes in water that already contains more salt than their own body fluids.

Most rainbow trout produced on fish farms have been marketed at what is known as 'portion size' (Sedgwick, 1978). This is a graded size suitable for an individual portion of whole trout to be served to one person in a restaurant or in the home. Preferred weights (portion size) of rainbow trout in most European countries and in the USA are between 160 grams (.35 lb) and 250 grams (.55lbs) (Sedgwick, 1978).

However, fish size can be measured in a number of ways. The description of fish size are cited from Laird and Needham (1988). The two most common measurements are length and weight. In salmonids, such as rainbow trout, the usual measure of length is the fork length. This is defined as the distance between the tip of snout and the fork of the tail (Figure 1). The total length is subject to error since the size of fish with damaged tails would be underestimated. The standard length (to the base of tail) overcomes this problem. However, in salmonids the fork length is easy to

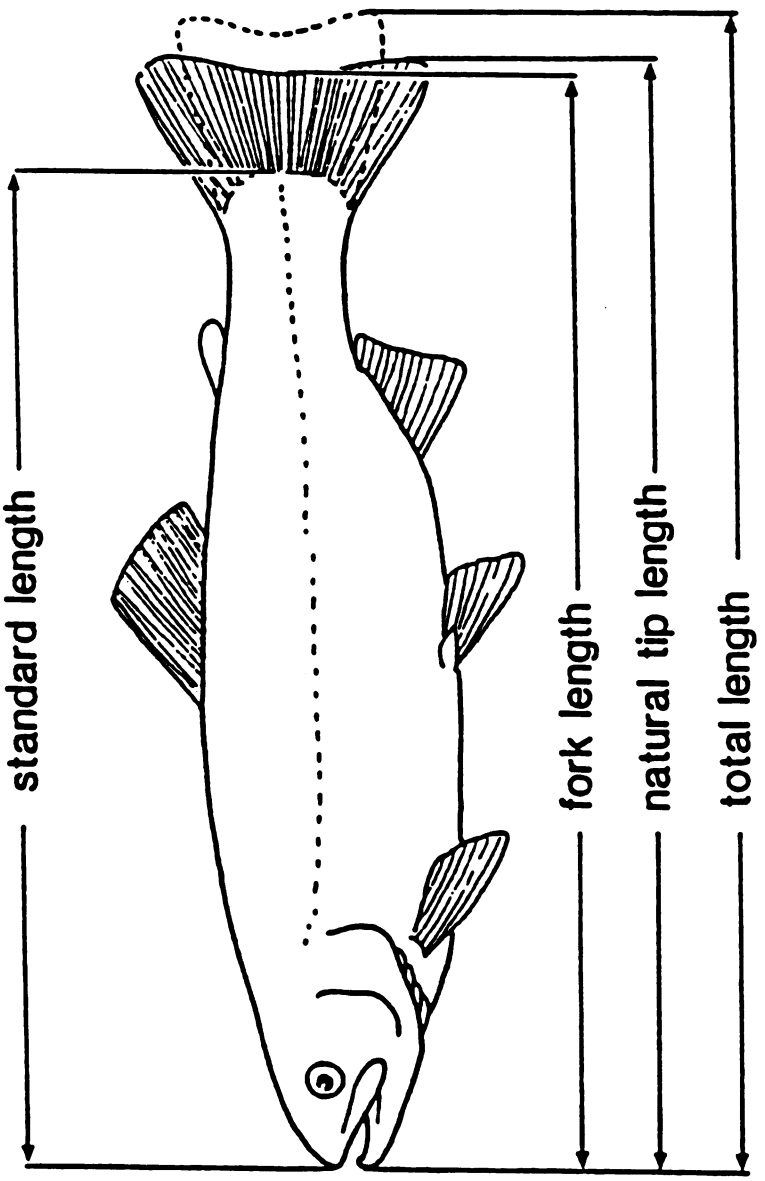


Fig. 1. — Different methods of measuring fish length. Unless otherwise stated fork length is normally used.

see when the fish is lying on a measuring board because the tail start of this fish is not always clear. Length should be measured in millimeters and centimeters.

Weight is usually taken of the whole fish with excess water removed from the body surface. This is described as the wet body weight. This is the normal weighing that can be measured on the farm of live sedated fish or freshly slaughtered fish. It includes a variable weight of viscera and recent meals. For some commercial purposes, the gutted wet weight is of more interest, with viscera removed.

The chemical composition of aquatic animals is extremely varied. The factors affecting composition are numerous, being either of intrinsic nature bearing upon genetics, morphology, and physiology, or of an environmental nature, relating to the living conditions, particularly the diet (Jacquat, 1961). Based on lipid content, fish are classified into three categories (Jacquat, 1961). These are fatty, semi fatty, and lean. Trout is classified as a fatty fish. Jacquat (1961) reported that the average composition of fatty fish is 68.6 % water, 20.0 % protein, 10.0 % lipid , and 1.4 % ash. One should not, however, draw too strict a line between fat and lean fish species because of the range within species and of the existence of individual fish-to-fish variations. For instance, salmon contain between 0.35 and 14.00 % of lipid, depending on when they are caught (Lovern and Wood, 1947). This fish is sometimes

lean, sometimes fat. Poulter and Nicolaides (1985) reported that the composition of freshwater rainbow trout was 78.50 % water, 20.52 % protein, 3.51 % fat, and 1.06 % ash.

Shelf-life Determination

Total time from freshly-caught fish to fish spoilage and unacceptability due to odor, flavor, color, texture or taste is called shelf life of fresh fish or keeping time of fresh fish. Cann et al. (1983) "defined shelf life as the time since packaging for the fish to reach a score of cooked flavor = 6.0 units of Score Sheet in Appendix B". Gibson (1985) stated that shelf life of fish was the total time since packaging for the fish to reach a score of cooked flavor equal to 5.5 units of Score Sheet in Appendix B. During that time, deterioration occurs and the quality of fresh fish decreases.

Factors affecting shelf-life of fresh fish are : (1) Factors prior to the harvest, such as, sexual maturation, type and amount of food consumed, and environmental conditions (Castell, 1976); (2) Season when the fish were caught (Love, 1989); (3) Species of the fish (Martin et al., 1978); (4) Methods of catch (Shewan, 1949); Onboard handling, such as, bleeding, gutting, gilling, icing or keeping fish at low temperature (Shewan, 1961; FAO, 1973; Samuels et al., 1984; Ravesi et al., 1985); (6) Fishing vessel sanitation (Huss et al, 1974; Samuels, 1984). (7)

Preservatives used such as sorbic acid and its potassium salt (Fey and Regenstein, 1982; Satham et al., 1985); and (8) Packaging including modified atmosphere packaging (Lannelongue et al., 1982 a, b; Gray et al., 1983; Parkin et al., 1983).

The tests used for the measurement of fish quality may be divided into two categories : one encompasses the sensory test; the other, the objective ones (Louit and Ransivalli, 1988). Burt et al. (1975) classified the measurement of fish quality into two groups, namely, sensory and non-sensory tests. Further, the authors divided non-sensory into two types, chemical and instrumental methods. Farber (1965) described a lot of methods to measure quality changes of fish. These are : (1) Sensory test; (2) Physical methods, including measurements of textural change, refractive index, electric conductivity, optical test. surface tension, viscosity, and internal friction; (3) Physicochemical methods, such as determination of pH, buffering capacity, and oxidation-reduction potential; (4) Biochemical methods including bacterial count, enzymatic activity, oxygen consumption and dye reduction test; and (5) Chemical methods. The chemical methods include measurements of : (a) Volatile basic nitrogen compounds (e.g. total, ammonia, trimethylamine or TMA); (b) Volatile acids; (c) succinic acid; (d) indole and skatole; (e) Hydrogen sulfide; (f) Carbinol compounds; (g) Steam-volatile oxidizable substances; (h) Reducing substances that are

volatile at room temperature; (i) Histamine and histamine-like compounds; (j) Nucleotides and their derivatives; (k) Fat spoilage including 2-thiobarbituric acid test or TBA test, aldehydetest and free fatty acid content; and (l) Miscellaneous methods.

Among the many methods listed above, only a few were used widely by investigators (e.g., sensory test, TMA test, total volatile base, TBA test and bacterial count) due to accuracy and simplicity. For the same reasons, sensory, TBA, and bacterial tests were utilized in this study for measuring the shelf life of fresh fish under various conditions.

There are no standard conditions to evaluate quality of fish and to determine the end of shelf life of fresh fish (Ward and Baj, 1988). According to these authors, there are differences in parameters and conditions used by most investigators in evaluating shelf life of fish because of differences in species of fish, methodology of evaluation, or both. For example, the end of shelf life of fresh fish has been based upon (a) bacterial count (100,000; 1,000,000 CFU/g sample); (b) Organoleptic (Slightly off odor); (c) Chemical (TMA, total volatile nitrogen, TBA tests); (d) Combinations of a, b, and/or c. Bacterial plate count incubation temperatures have varied (20, 21, 25, 30, 35, or 37 C), as did storage temperature (- 1 or salt ice, ice, 1, 2, 3, or 4 C) (Ward and Baj, 1988). These differences as well as other differences not mentioned here make it

difficult to draw valid conclusions from comparative data in the literature.

Sensory Evaluation

Sensory evaluation is the oldest and still most widespread means of evaluating the acceptability and edibility of fish (Farber, 1965). The evaluation may include evaluating odor, flavor, texture, appearance, and/or taste. The reasons for the preferential use of sensory testing are clear : (1) the evaluation can be performed quickly; (2) No special laboratory equipment is needed; (3) The fish can be evaluated wherever they happen to be; and (4) many samples can be examined in a relatively short time.

However, there are a number of disadvantages inherent in the organoleptic method that significantly detract from its usefulness. Use of the senses is a subjective procedure. The impressions received are the result of the interactions between a number of physiological, psychological, environmental, and even economic factors, including state of health, personal prejudices, preference and interests, sensory acuity, freedom from disturbing and influencing conditions in the examining environment and motives of possible profit or loss (Farber, 1965). Sensory testing is not appropriate when it is applied to distinguish and assess the stage of incipient spoilage, that is when a fish sample to be judged is in the last stage of freshness

or in the first stage of spoilage. This task is well recognized as a difficult one, where even experienced panelists often differ in their evaluations. Castell et al (1956) stated that "in actual practice the grading of fresh fish is, and for some time will remain, an art and not a science".

The disadvantages associated with sensory evaluation can be minimized by use of trained or experienced panelists to judge the samples and elaboration of numerical systems of scoring and recording the sensory judgements. Objective criteria, based on physical, biochemical or chemical tests, can eliminate disadvantages inherent in the sensory, subjective criteria, but have limitations of their own.

In this study, sniff tests were performed to evaluate off-odor intensity of fish samples.

"Odor is the property of a substance or substances that is perceived, in the human and higher vertebrates, by inhalation in the nasal or oral cavity; that makes an impression upon the olfactory area of the body; and that, during and as a result of inhalation, is distinct from seeing, hearing, tasting or feeling, and does not cause or result in choking, irritation, cooling, warmth, drying, wetting or other functions foreign to the olfactory area" (Sagarin, 1954). Odorless would be the verdict when such a sensory stimulus was not received.

Odor has three elements, namely intensity, type, and variety, where variety is the deviation from the main type (Amerine et al., 1965).

Compounds associated with off-flavors and off-odors are produced by spoilage bacteria which attack various substances in the fish tissue. The compounds are reported to be methyl mercaptan, dimethyl sulfide, dimethyl trisulfide, 3-methyl-1-butanal, trimethylamine, and ethyl esters of acetate, butyrate and hexanoate (Miller et al., 1973).

The compounds responsible for the off-flavor or off-odor in freshwater fish are derived from protein, not trimethylamine oxide (Love, 1989). Spoilage bacteria degrade protein into amino acid and sulfur compounds such as, hydrogen sulfide, disulfide and methanethiol (Herbert and Shewan, 1976). These authors stated that the higher the sulfur compounds, the higher the intensity of off-odor.

The end of shelf life of fresh fish has been reported to be the time when the sensory score (raw-fish odor, cooked-fish flavor) is 4 units of an 11 unit scale from 10 (fresh) to 0 (putrid) (Hansen, 1963; 1972; Spencer and Baines, 1964; Huss, 1972; Villarreal and Howgate, 1987). Baines and Shewan (1965) stated that a score of 4.5 units is the end of keeping time. For packed chilled fish Murray et al. (1971) used a higher score, 5.5 units as a level of acceptability. Cann et al. (1983) defined the end of shelf life of packed fish as 6.0 units. A score sheet for

organoleptic studies used by all researchers above are in Appendix C. The score sheet used in this study is in Appendix A.

In this study, it was arbitrarily decided that the end of acceptable shelf life of fish would be equal to a sensory score of 3.0 or "slightly off odor"

Thiobarbituric Acid Test (TBA Test)

Numerous chemical and physical methods have been developed for monitoring oxidation in oils and lipid-containing foods. One of the most commonly used methods for measuring lipid oxidation in muscle food is the 2-thiobarbituric acid (Gray, 1978; Melton, 1983). The principle of this method is the condensation of two molecules of TBA with one molecules of malonaldehyde to produce a red complex which is quantified spectrophotometrically in the region of 530 to 532 n.m. (Sinnhuber et al., 1958).

The malonaldehyde found in oxidized muscle foods is a secondary oxidation product, formed mainly from polyunsaturated fatty acid containing three or more double bonds (Pryor et al., 1976).

There are several ways in which the TBA test can be carried out on muscle foods. One of them is a modified distillation method in which distillates of samples are reacted with TBA without the use of any acid (Tarladgis et al., 1964). This method was used in this study.

The degree of lipid oxidation is expressed in term of a TBA number having units of mg of malonaldehyde per kg of sample. TBA number is obtained by multiplication of the measured absorbancy value by a constant. The constant for the modified distillation method developed by Tarladgis et al. (1964) is 6.2 (Crackel, 1986).

Tarladgis et al. (1960) reported that the range in TBA number at which rancid odors were detected by panelists was between 0.5 and 1.0. The TBA threshold range for "discriminating" panelists is 0.6 to 2.0 (Greene and Cumuze, 1981).

In this study, it was arbitrarily decided that the end of acceptable shelf life of fish would be equal to a TBA number of 0.5.

Bacterial Count Test

One attribute of food quality is a state of freshness as opposed to a state of deterioration. In fish products, quality changes in flavor, texture, appearance, and taste reflect deterioration caused by the multiplication of bacteria (Nickelson and Finne, 1984). The microbial quality of fish is usually expressed in a total number of bacteria present in the product.

Methods which have been used extensively for determining the viable microbial population in foods are colony count methods (Speck, 1984). These methods are based on the assumption that each microbial cell in a sample will

form a visible separate colony when mixed with the agar medium and permitted to grow.

Colony count methods such as, aerobic, psychrotrophic, and anaerobic plate count are easy to carry out, but they have limitations. The methods provide an estimate of the number of viable microflora in the food according to the medium used and the incubation time and temperature applied. Procedures for shaking and dilution of samples are designed to uniformly distribute the clumps of bacteria, but may not completely disrupt them. The mixing of the initial dilution in a mechanical mixer, which may provide a better breakdown of the clumps, does not insure that the microorganism will be separate as single cells. Consequently, each colony that appears in the agar plate can arise from a clump of cells or from a single cell. The number of bacteria in samples using these methods is expressed in colony forming unit (CFU), not total viable cell count.

The end of shelf life of fresh fish has been defined as the time when its aerobic plate count (APC) reaches 1,000,000 CFU/g (Anonymous, 1974; Nickelson and Finne, 1984; Stenstrom, 1985).

In the present study, it was arbitrarily decided that the end of acceptable shelf life of fish would be equal to a log CFU APC of 6.

MATERIALS AND METHODS

Materials

To accomplish the objectives described under "Experimental Methods" (page 49) required materials included fish, modified and air atmosphere containers, gases, reagents and solvents. Container contruction required the use of Plexiglas, sealants, septums, toggle valves, and fish supports. Sealants were used to seal sides, top, and bottom of the containers and to prevent air leakage where Plexiglas connections were mounted in the containers.

Fish

Live rainbow trout were purchased from Old State Fish Hatchery, Baldwin, Michigan. After delivery, they were held in a 500 gal fish tank supplied with 1.5 gpm well water at a constant temperature of 11.5 C in the Fishery Laboratory of the M.S.U. Fisheries and Wildlife Department. Fish were held for 2 - 3 months, and fed with Aquaculture Zeigler Quality Feed (manufactured by Zeigler Bros., Inc.; PO Box 95, Garners, PA, 17324-0095). Their standard length and wet weight at time of slaughter for use in this study were 23 to 25 cm, and 150 to 170 g, respectively.

Plexiglas

Cutting Plexiglas, 0.25 inch thickness (Lucite L Acrylic Sheet, manufactured by Dupont De Nemours & Co. Inc.), was purchased from Plas-Labs, Inc; 917 E Chilson Street, Lansing, MI, 48906.

Sealants

Acrylic Solvent Cement (net weight 2 Fl oz, stock No 1106002, and manufactured by Crafties, Inc.; Chicago, Illinois, 60639) was used as permanent sealant. One hundred percent Silicon Rubber (General purpose sealant, and manufactured by Dow Corning Corporation, Midland, MI, 48640, USA) was used as semi-permanent sealing material. Vacuum Grease (manufactured by Dow Corning Corporation, Midland, MI, 48640, USA, cat. # 14-635-5D) was used as temporary sealing material.

Septums

Septums (100GR-2 Septa, 12.5 mm, manufactured by Supelco, Inc., Supelco Park, Belefonte, Pennsylvania, 16823) were used to cover small holes on the upper side of each container, through which gas samples were withdrawn.

Toggle Valves

Toggle Valves (Parker CPI Toggle, 2F-V4LQ-BP, manufactured by Parker Hannifin Corporation, Instrument Commercial Division, Jacksonville, AL, 36265) were purchased

from Mid-State Fluid Power, Lansing, Michigan and used to construct modified atmosphere containers.

Fish Supports

Undervents 4 in x 16 in (622R 16 x 4 W, Part No. 21209) were purchased from Meijer Store, Okemos, Michigan. The undervents were cut into 30 cm lengths before placement in storage containers.

Oxygen Absorbers

Oxygen absorbers (Ageless G-type, manufactured by Mitsubishi Gas Company, Inc) were donated by Cryovac Division, W.R. Grace & Co., Duncan, SC, 29334.

Tubes

Tygon flexible plastic tubing (I.D. 5/16", O.D. 7/16", manufactured by Norton, Plastic and Synthetics Division; PO BOX 350, Akron, Ohio, 44309) was used to connect gas tanks to gas filter and containers.

Gas Mixer

A Matheson Gas Mixer model No. 7342H (manufactured by Matheson Gas Equipment Technology Group, 166 Keystone Drive, Montgomeryville, PA, 186936) was used in this study to quantitatively mix carbon dioxide and nitrogen.

Gas Filter

An Acro 50 Filter Unit (Cat. No.: F3059-1, Mfr No.: 4251, Pore size 0.2 u, manufactured by Baxter Healthcare Corporation, Scientific Product Division, 1430 Waukegan Road, McGraw Park, IL, 60085-9988) was used to filter gases in order to avoid microbial contamination from nitrogen and carbon dioxide during gas-flushing of modified atmosphere containers.

Gases

Carbon dioxide , 99.5 % purity, Michigan State University, General Store, Catalog # 1605336. Nitrogen, 99.9 % purity, Michigan State University, General Stores, Catalog # 1607176. The gases were used to create modified atmosphere conditions within containers used for MAP-stored fish samples.

Agar

Bacto Plate Count Agar (dehydrated standard method agar, Difco Laboratories, Detroit, MI, USA) was used to perform microbial plate count tests.

Peptone

Bacto Peptone ("Difco certified", Difco Laboratories, Detroit, MI, USA) was used to make agar media..

Petri Dishes

SIP diSPO Petri Dishes (Cat. D1906, size 100 x 15 mm, distributed by Baxter Healthcare Corporation, Scientific Product Division, McGraw Park, IL, 60085-6787, USA) were purchased from General Stores, Michigan State University.

Pipets

Pipets (1 ml, individual pack distributed by Baxter Healthcare Corporation, Scientific Product Division, McGraw Park, IL, 60085-6787, USA) were purchased from General Stores, Michigan State University.

CampyPak plus

CampyPak Plus (BBL cat #71045, Microaerophilic System Envelope, with Palladium catalyst, manufactured by Becton Dickinson, Microbiology Systems, Cockeysville, MD, 21030, USA).

GasPak Disposable Anaerobic Indicators

GasPak disposable anaerobic indicator (manufactured by Becton Dickinson, Microbiology Systems, Cockeysville, MD, 21030, USA).

Stomacher Bags

Stomacher Lab-Blender model 400 (size 7" x 12" in 50's, cat no. BA6041, manufactured by Seward Medical, 131 Great Suffolk Street, London, SE11PP, UK).

Air Permeable Film

Hi-Y-Gold Meat Stretch Film (Hi-Y-G5, 301-17; 65 gauge, manufactured by Filmco, 1450 S Chillicothe Rd, Aurora, Ohio, 44202) was used to cover foam containers in which post-MAP fish samples were stored.

Polystyrene Foam Plates

Polystyrene foam plates (manufactured by Mobil Chemical, Packaging Department/Canandaigua, NY) were used to pack post-MAP fish samples.

Reagents and Solvents

All reagents and solvents utilized in this study were reagent grade.

Experimental Methods

Objectives of experimental menthods were to construct air-tight containers, to evaluate container tightness, to determine carbon dioxide and nitrogen flow rates which yield gas composition of 60 % carbon dioxide - 40 % nitrogen, to create modified atmosphere (60 % carbon dioxide-40 % nitrogen) within MAP containers, and to prepare and store fish samples.

Construction of Containers for Modified Atmosphere and Air Storage

Plexiglas was cut to the desired size so that the outer dimensions of the constructed containers would be 11.0 cm in width, 31.0 cm in length, and 11.5 cm in height. Acrylic Solvent Cement was applied to the contact surfaces on the bottom and sides. Vacuum grease was used on the upper contact surfaces.

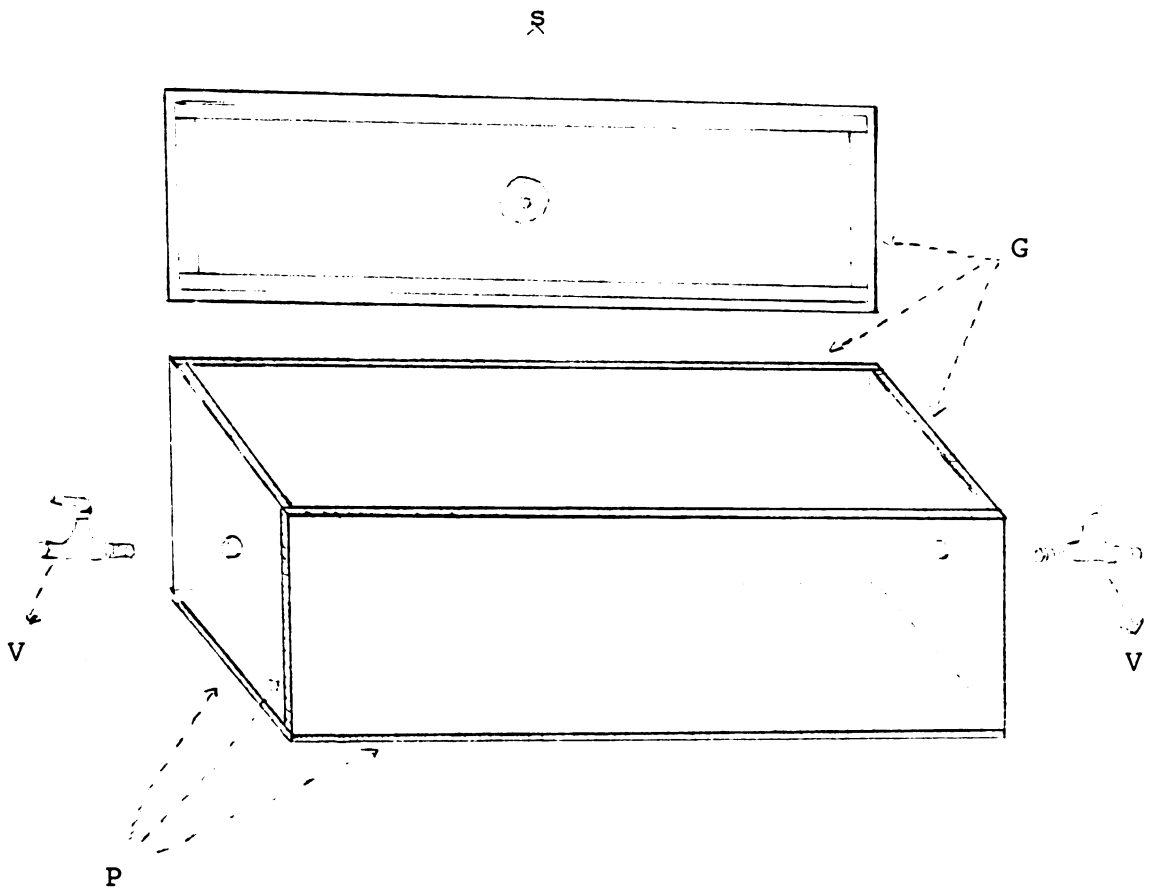
On each of the two end pieces (the 11.0 x 11.5 cm sides), a 5/16"-diameter hole was drilled, in which a toggle valve was planted. On the upper side, a smaller hole (2/16" in diameter) was made. The hole was filled with silicon rubber and then covered with a septum, through which gas samples were to be withdrawn.

Four small lengths (0.5 x 1.5 x 5.0 cm) of Plexiglas were attached vertically to the inner wall of the two larger sides (the 11.0 x 31.0 cm sides) using silicon rubber to serve as 'fish supports' on which the fish samples were placed. A sketch of the modified atmosphere packaging (MAP) container can be seen in Fig. 2.

Identical containers were used for AAP-stored fish, but without the toggle valves or septa.

Flow-Rate Ratio Determination

A study was designed to determine the flow rate ratio between carbon dioxide and nitrogen to achieve a gas mixture of 60 % carbon dioxide - 40 % nitrogen. Carbon dioxide and



P = permanent cement applied

S = septum attached with silicont sealant

G = grease applied

V = a toggle valve screwed into Plexiglas

Fig. 2. A sketch of a MAP container.

nitrogen were mixed with Matheson Gas Mixer in a certain flow rate ratio. The composition of the mixing gas was measured with Analytical Gas Chromatography (GC) model 111 (manufactured by Carle, Care Instrument Inc., A Hach Knollwood Circle, Anaheim, California, 92801). This procedure was repeated with other flow rate ratios until the desired composition was obtained.

It was found that, at flow rates of 50 units on the carbon dioxide scale and 24 units on the nitrogen scale of the mixer, a gas composition in the container of approximately 59.8 % carbon dioxide, 39.7 % nitrogen, and less than 0.5 % oxygen was obtained.

Evaluation of Container Tightness

A preliminary study was performed to evaluate the tightness of the containers. Containers were flushed with 100 % carbon dioxide for 15 minutes, then stored at 0 C. Gas samples were withdrawn and analyzed weekly with GC. If oxygen content increased more then 1.5 % per week, the procedures were repeated after securing and repairing all connections.

To eliminate unavoidable oxygen inside the containers, oxygen absorbers were used. Use of 5 or more packs reduced oxygen content from the initial value of approximately 1.8 % down to a value of less than 1 % after one day of storage at 0 C. Oxygen content remained below 1 % during one month of 0 C storage.

Determination of Gas Composition Inside Containers

Containers were flushed with the mixture of carbon dioxide at 50 unit scale of flow rate and nitrogen at 24 unit scale of flow rate. Every 5 or 10 minutes during flushing, gas samples from the containers were withdrawn and analyzed with GC until gas composition inside the containers was the same as that in the mixer. The toggle valves were closed, then gas samples were taken and analyzed every 5 or 10 minutes to determine how long it took the gases inside the containers to achieve equilibrium. Reflushing was needed if appropriate gas composition was not achieved.

At least 10 minutes of flushing was needed to achieve about 59.8 % carbon dioxide, 39.6 % nitrigen and less than 0.6 % oxygen. A longer flushing time did not change the gas composition within the container.

Preparation and Storage of Samples

Rainbow trout from the Fishery Laboratory were netted, iced, and quickly transferred to the MSU Meat Laboratory to be gutted, gilled and rinsed to remove adhering blood and viscera along the back bone and debris from the mouth. Gutted fish were iced and quickly transferred to room 223A Food Science. Four fish (two fish on each fish support) as well as 8 packs of oxygen absorber were put inside each sanitized container. Sanitization was accomplished by rubbing the container interiors with 80 % ethyl alcohol.

Sixteen containers were prepared, 8 containers for air atmosphere packaging (AAP) treatments and the reminder for modified atmosphere packaging (MAP) treatments. Four containers from each treatment were stored in a cubicle at 0 C (32 F) and the four others were stored at 5 C (41 F). The cubicles were located in 217 Food Science.

MAP-treatment samples were flushed with carbon dioxide and nitrogen for 15 minutes. The flow rates of carbon dioxide and nitrogen were 50 carbon dioxide scale units and 24 nitrogen scale units on the Matheson Gas Mixer. Before and after storage, gas samples were withdrawn and analyzed by GC at the M.S.U. School of Packaging Laboratory.

Fish samples (one complete container of fish) from each treatment were withdrawn for test measurements after storage of 1, 2, 3, and 4 weeks and at the start of storage (0 weeks).

Preparation and Storage of Post-MAP Samples

Fish were packed under MAP conditions as stated above, and, thereafter, stored in their containers at 0 C for 0, 2 and 3 weeks.

Before and after storage, gas samples from inside the closed containers were taken and analyzed with GC at the Packaging Laboratory.

Fish were then removed from their MAP containers and placed directly in a single layer on polystyrene foam plates, then overwrapped with air-permeable film. The

repacked fish were stored at 5 C. Fish samples (one complete container of fish from each treatment) were removed for test measurements on day 0, 1, 2, and 3.

Methods of Analysis

Gas Composition Analysis

Gas Samples (500 uL each sample) from containers were taken with Hamilton Microliter Syringes (Gastight # 1750, Pat No. 3-150801, Capacity 500 uL, manufactured by Hamilton Company, PO BOX 10030, Reno, Nevada, 89520) and then injected into the GC, which was operated under the following conditions : Flow rate of carrier gas (Helium) 84 mL/min and column temperature 50 C.

Sensory Evaluation

Whole fresh fish (control) and whole fish representing other treatments were cut into cubes measuring about 0.5 x 1.5 x 3.0 cm, put into covered cups (one cube per cup), and stored overnight at 0 C. The samples were transferred to a refrigerator at 5 C in the Sensory Laboratory (101 Food Science) for 1 to 3 hours prior to evaluation.

Eighteen semi-trained panelists were asked to evaluate the intensity of off-odor, using a 7-point rating scale, where a rating of 1 represented "no off-odor" and a rating of 7 was "strong off-odor". At each session, all panelists were presented with 10 coded samples representing four

experimental treatments and a control. The first set of five samples (the control was sniffed first, but the panelists were not informed that it was a control) contained four treatments and one control. The second set of five samples was identical to the first set of five, but the control position was randomized. The procedures and the sensory score sheet can be seen in Appendix A.

Thiobarbituric Acid Test (TBA Test)

The TBA distillation method of Tarladgis et al. (1960), as modified by Tarladgis et al. (1964), was used to measure the development of oxidative rancidity in the fish samples.

Microbial Analysis

Samples were tested for aerobic plate count, psychrotrophic plate count and anaerobic plate count. Measurements were carried out according to Speck (1984).

Gutted fish were cut with a sterilized knife. Samples of 10 g were blended with 90 ml of sterile 0.1 % peptone water in model 400 stomacher bags for 5 minutes. Appropriate dilutions were made with 0.1 % peptone water and 1 mL of each dilution was spread on prepoured plate count agar.

Duplicate plates from each dilution were incubated at 35 C for 48 hours (aerobic plate count), at 7 C for 10 days (psychrotrophic plate count), and at 35 C for 48 hours (anaerobic plate count). The anaerobic mesophilic samples

were put in BBL anaerobic jars, into which 6 CampyPak Pluses and 2 GasPak disposable anaerobic indicators were placed.

Bacterial colonies were counted with a Quebec Colony Counter (Model 3327, Buffalo, NY). The results were expressed in log Colony Forming Unit (CFU) per gram sample.

Statistical Analysis

Gas composition, sensory scores, TBA numbers, and microbial plate counts were analyzed by analysis of variance (ANOVA) to evaluate significant differences among treatments (Steel and Torry, 1980). Regression equations showing the relationship of sensory score to aerobic plate count for each of the treatments were calculated, from which correlation coefficients were also determined.

RESULTS AND DISCUSSION

Gas Composition of MAP Before and After Storage

Changes of gas composition inside containers depend on surface area, thickness, and permeability constant of the containers, partial pressure differences, storage temperature, storage time, and reactions between products and gases inside the containers (Karel, 1975).

According to Downes and Giacin (1990), "Permeability of a material is the flux or the rate at which a quantity of permeant gas or vapor passes through a unit surface area in unit time, dependent upon partial pressure, film thickness, surface area, and temperature". Permeant gas or vapor moves from higher partial pressure to lower. The formula of permeability is :

$$Q = (p \times A \times t \times dP)/L$$

where Q is permeability; p is permeability constant; A is total surface area; t is storage time; dP is partial pressure difference between inside and outside of the containers; and L is thickness of packaging materials. The formula shows that the permeability of the containers will be lower when the partial pressure difference decreases.

Containers used in this study were hand made as described in Materials and Methods. In this case, permeation occurs through sealants and greases that were used to prevent or minimize leakage into or out of the storage containers, as well as through the Plexiglas

material that constituted most of the surface area of the containers.

In this study, partial pressure differences between inside and outside of the containers were not the same for carbon dioxide, oxygen, and nitrogen. Thus , the permeabilities of the gases were different from one another. Moreover permeation rate of a permeant gas during the course of a MAP storage study is not constant due to the decrease in partial pressure difference during storage.

Ageless (oxygen absorber) chemically absorbs oxygen completely to eliminate oxygen as a cause of food deterioration (Nakamura and Hoskino, undated). The authors classified Ageless into 5 types, namely , Ageless "Z, S, FX, E, and G". Each type is suitable for specific products. Z-type is good for dry food; S-type is suitable for moist food; E-type, which has ability to absorb carbon dioxide as well as oxygen, is used for roasted coffee only; and G-type, which generates the same volume of carbon dioxide from absorbed oxygen, is suitable for modified atmosphere packaging.

Each type is manufactured in several sizes (e.g. size 20, 30 , 50, 100, 200, 500, 1000, 2000). The size numbers indicate total oxygen in CC which can be absorbed by one pack of Ageless. Thus, Ageless G-200, for example, can absorb 200 CC oxygen and release 200 CC carbon dioxide per pack.

Deoxygenation time is the time required to absorb oxygen from the food package. It varies with types of Ageless. Deoxygenation times at room temperature are : 1 to 4 days for Z-type, 0.5 to 2 days for S-type, and 0.5 to 1 days for FX-type (Nakamura and Hoskino), but no data is availabe for G-type. From preliminary study, it was found that use of 4 packs of Ageless G-200 inside a gas-flushed MAP container produced an oxygen content of less than 1 % after the container was stored at 0 C for 1 day. Longer storage did not change the oxygen content. This indicated that the Ageless G-200 needed one day to absorb unavoidable oxygen inside MAP containers (less than 2 %) and kept the oxygen content less than 1 % during 4 weeks of storage at 0 C. Thus, initially-higher oxygen content (1.72 %) was not considered to significantly affect the quality changes of fish within the MAP containers.

In this study, 8 packs of Ageless G-200 were used in each MAP to eliminate unavoidable oxygen during storage although data from a preliminary study indicated that the use of 4 packs was enough to maintain oxygen content of less than 1 % for one month`s storage at 0 C.

Permeating gases both enter and exit the containers, and it is therefore difficult to quantify because neither the permeability constant of the containers nor the actual oxygen absorber capacity were measured. However, the final gas composition in the containers was measured by an Analytical Gas Chromatograph. Based on the initial and

final gas compositions in the containers, the gas changes during storage were explained qualitatively, not quantitatively.

Tables 1 and 2 show that the gas composition for all treatments was different before and after storage. Carbon dioxide content decreased during storage. Content of carbon dioxide inside the containers before storage was higher (56.64 %) than outside the containers (about 0.03 %). Therefore, carbon dioxide permeated from inside to outside. At the same time, Ageless inside the containers produced carbon dioxide. The decrease in carbon dioxide content within containers during storage indicated that the rate at which carbon dioxide permeated through the containers was higher than that of carbon dioxide release by the Ageless material.

During storage, oxygen permeated from outside to inside of the containers due to the partial pressure difference. Outside concentration was 20.95 % while inside it was 1.72 %. Cocomitantly, the Ageless inside the containers absorbed oxygen. Since the oxygen content was lower after storage, the rates at which the Ageless material absorbed oxygen during storage appears to have been higher than the rate of entry of oxygen into the containers.

Partial pressure of nitrogen in air was higher than inside the containers before storage, 78.09 % compared to 41.64 % or 42.95 % (initial nitrogen content averages shown in Tables 1 and 2); thus, nitrogen permeated into the

Table 1. Initial and Final Gas Composition* of MAP Containers.

Treatment	Before Storage (%) CO2	O2	N2	After Storage (%) CO2	O2	N2
LW1	57.42	1.89	42.67	50.99	0.84	48.17
LW2	56.55	1.73	41.72	51.19	0.24	48.57
LW3	57.46	1.58	40.96	52.16	0.91	46.93
LW4	56.13	1.76	42.11	52.17	0.49	47.34
HW1	55.32	1.63	43.05	52.76	0.49	46.75
HW2	55.81	1.82	42.37	52.95	0.22	46.83
HW3	57.47	1.66	40.87	50.60	0.75	48.65
HW4	56.92	1.71	41.37	50.30	0.96	48.74
Average	56.64	1.72	41.64	51.64	0.61	47.75

Table 2. Initial and Final Gas Composition* of MAP Containers from Which Fish Were Removed for Post-MAP Storage in Air.

Treatment	Before Storage (%) CO2	O2	N2	After Storage (%) CO2	O2	N2
LM2D0	54.87	1.79	43.34	50.97	0.78	48.35
LM2D1	55.06	1.82	43.12	51.36	0.80	47.84
LM2D2	55.22	1.82	42.96	51.38	0.78	47.84
LM2D3	55.16	1.89	42.95	51.01	0.74	48.25
LM3D0	55.07	1.70	43.23	50.51	0.54	48.95
LM3D1	55.60	1.66	42.74	50.97	0.79	48.24
LM3D2	55.17	1.81	43.02	51.06	0.79	48.15
LM3D3	56.00	1.76	42.24	50.37	0.66	48.97
Average	55.27	1.78	42.95	50.95	0.64	48.41

*) One container for each value shown in the Table, but analyses were performed in duplicate.
L is low temperature storage (0 C).
H is higher temperature storage (5 C).
W followed by 1, 2, 3, or 4 is time storage in weeks.
M followed by 2 or 4 is weeks in MAP prior to post MAP storage in air.
D followed by 0, 1, 2, or 3 is day in air package at 5 C following MAP storage.
a, values in the same column bearing the same letter do not differ significantly at $p < 0.05$.

containers, resulting in higher nitrogen content after storage (47.75 % and 48.41 % for Tables 1 and 2, respectively).

Analyses of variance (ANOVAs) were performed to determine significant differences among treatments with respect to carbon dioxide content and oxygen content. The results of ANOVAs are presented in Tables 17 - 24 in Appendix C. These data show that, before storage, neither carbon dioxide nor oxygen content for all treatments differed significantly at a level of $P < 0.05$. Neither were there significant differences in the data after storage. These results mean that, statistically, all MAP samples were enveloped by the same gas composition. Thus, measured spoilage-related changes were not influenced by container-to-container variations in gas composition.

Effect of Temperature and Atmosphere on Shelf Life of Fish

Two temperatures (0 and 5 C) and two atmospheres (air atmosphere packaging (AAP) and modified atmosphere packaging (MAP) were applied in this current study. Thus, there were four combinations of temperature and atmosphere treatments. They were 0 C-MAP, 0 C-AAP, 5 C-MAP, and 5 C-AAP treatments. Average of initial gas composition in MAP containers was 56.64 % carbon dioxide, 1.72 % oxygen, and 41.64 % nitrogen (Table 1). After storage, average composition of the gas was 51.64 % carbon dioxide, 0.61 % oxygen, and 47.75 % nitrogen (Table 1).

Sensory evaluations, TBA tests, and microbial plate counts were performed on samples from each treatment weekly during 4 weeks of storage.

Having collected data from each analysis in each treatment, ANOVAs were carried out to determine if significant differences existed : (a) within temperature treatments, (b) within atmosphere treatments, (c) within storage times, and (d) within combinations among temperature, atmosphere and storage time treatments with respect to shelf life of the fish. Linear regression equations showing the relationship of sensory score to aerobic plate count for each of the treatments were calculated, from which correlation coefficients were also determined.

Sensory Evaluation

Sensory evaluation (sniff test) was performed to assess fish quality and to determine shelf life of fish stored under different conditions. In this study, it was arbitrarily decided that the end of acceptable shelf life of fish would be equal to a score of 3.0 or "slightly off odor". The result of ANOVA is presented in Table 3. Sensory scores for samples within temperature treatments (C), atmosphere treatments (A), storage time (W), and within combinations of A, C, and W treatments were all significantly different at a level of $p < 0.01$.

Table 3. ANOVA for sensory score.

Source of Variation	df	SS	MS	F	
Temperature (C)	1	34.753	34.753	720.7717	**
Atmosphere (A)	1	17.657	17.657	366.1545	**
C x A	1	5.080	5.080	105.3480	**
Storage Time (W)	3	13.604	4.535	94.0378	**
C x W	3	2.866	0.955	19.8135	**
A x W	3	4.588	1.529	31.7141	**
C x A x W	3	3.032	1.011	20.9581	**
Error	16	0.772	0.048		
Total	31	82.355			

Coefficient of variation = 6.53 %

** is significantly different at a level of $P < 0.01$

df is degree of freedom.

SS is sum of squares

MS is mean square.

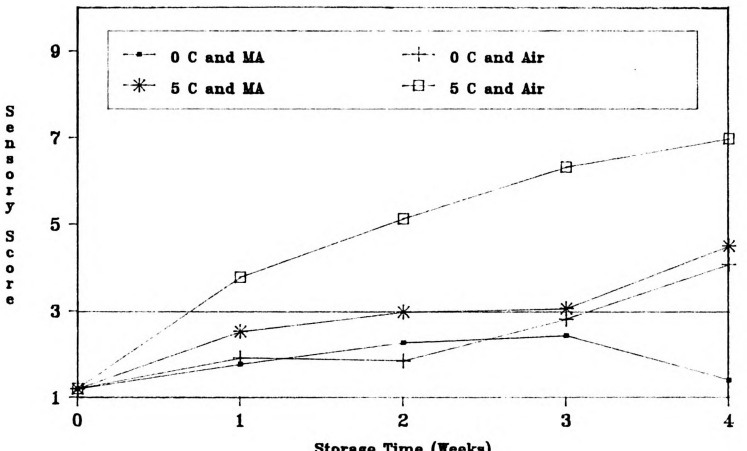
F is calculated F value.

Mean scores for all samples from different treatments are tabulated in Table 14 in Appendix C and shown graphically in Fig. 3.

Figure 3 indicates that the shelf life of rainbow trout stored at 0 C was longer than that of fish samples stored at 5 C under the same atmosphere. The difference in sensory scores was due to different spoilage rates at 0 and 5 C. As described in the Literature Review, the spoilage rate at 5 C is approximately 2.25 times that at 0 C (Owen and Nesbitt, 1984; Storey and Owen, 1985).

Shelf life for fish stored under AAP at 0 C in this study was more than 3 weeks (Figure 3). These data disagree with findings of other researchers which reported shelf life of fish stored in ice (0 C) of about 2 weeks (Shewan et al., 1953; Burt et al., 1975; Dawood et al., 1986). These authors used 18 to 30 fish per sample and stored them in ice without packaging, while the present 0 C study used 4 fish per container as a sample and stored the containers in mechanically refrigerated cubicles at 0 C. The higher shelf life at 0 C obtained in the current study may have resulted from the use of containers ,which reduced cross contamination. Differences between data from the present study and cited literature data may also have been caused by different criteria for the end of shelf life and different test conditions (e.g. gas composition, storage temperature) , as well as differences in the fish themselves.

Fig. 3. Effect of Temp. and Atmosphere On Sensory Score



Score 1 = No off odor
Score 3 = The end of shelf life
Score 7 = Strong off odor

Figure 3 shows that the shelf life of fish stored under MAP was longer than that of fish stored under AAP at the same temperature. Since compounds associated with off flavor and off odor are produced by spoilage bacteria (Miller et al., 1973), the spoilage rate, which correlates linearly with growth rate of the bacteria, was lower under MAP than under AAP. It indicates that MAP, at its measured 56.64 % carbon dioxide content, inhibited microbial growth. A range of 40 to 60 % carbon dioxide was previously found to be the most effective concentration range to inhibit spoilage (Coyne, 1933). As stated in the Literature Review, Ogrydziak and Brown (1980) found that carbon dioxide retards spoilage rate by increasing the lag phase and decreasing the growth rate of the spoilage bacteria. However, Enfors and Molin (1981) reported that carbon dioxide did not significantly increase the lag phase of the bacteria growth cycle.

The present study found, as shown in Fig. 3, that the shelf life of rainbow trout stored under 0 C-MAP, 0 C-AAP, 5 C-MAP, and 5 C-AAP treatments was approximately 4+, 3.1, 2.2, and 0.7 weeks, respectively. It means that the shelf life of fish under MAP was about 4+/3.1 or about 1.5 times longer than fish under AAP if stored at 0 C, and about 2.2/0.7 or3.1 times longer when stored at 5 C. Results from the present study support other reported results with respect to carbon dioxide-enriched atmosphere providing a longer shelf life than comparable samples stored in

refrigerated air (Bank et al., 1980; Parkin et al., 1982; Villemure et al., 1986). However, data from this study disagree with Coyne's (1933) finding that the shelf life of fish stored in MAP at 0 C is at least twice as long as in air at the same temperature. Differences between data from the current study and cited literature data, may be caused by different criteria of acceptable shelf life and different test conditions (e.g. storage temperature, storage time. gas composition, fish species). Therefore, direct comparison with literature values may be inappropriate.

The most effective treatment to extend shelf life of fresh fish in the current study was 0 C-MAP treatment. Since bacteria are frequently responsible for spoilage in chilled fish (Herbert et al., 1971), it seems that 56.64 % carbon dioxide inhibits growth of spoilage bacteria more effectively at 0 C than at 5 C. The mechanisms of carbon dioxide action as well as the effect of low temperature on inhibition of bacterial growth has been clearly described in the Literature Review.

Thiobarbituric Acid Test (TBA Test)

The objective of this portion of the study was to use a chemical method (the TBA test) to assess the shelf life of fresh fish stored under different conditions.

For the current study, it was arbitrarily decided that the end of shelf life of the fish would be equal to a TBA number of 0.5.

The results of ANOVA for TBA number are shown in Table 4. TBA numbers of samples within temperature treatments (C), within atmosphere treatments (A), within storage time (W) and within combinations of A, C, and W treatments were all significantly different at a level of $p < 0.01$.

Mean TBA numbers for all samples representing different treatments are tabulated in Table 14 in Appendix C and shown graphically in Fig. 4.

From Figure 4, TBA values seem to indicate that shelf life of rainbow trout stored at 0 C was shorter than that of fish samples stored at 5 C under the same atmosphere. It means that oxidation rates at 0 C were higher than at 5 C. These findings are in disagreement with those reported by Hardy (1980), who found that the oxidation rate in fatty fish decreases with decreasing temperature, usually by a factor of 2 to 3 for every 10 C decrease.

A possible explanation for the TBA differences shown in Figure 4 is that the rate of malonaldehyde formation at 5 C is much lower than the rate of malonaldehyde destruction. As stated in the Literature Review, the TBA test has this drawback if applied to fish tissue (Smith et al., 1972). In fish tissue, the formation of malonaldehyde does not correlate linearly with autoxidation rate. McGill (1976) reported that, in oxidized cod and haddock tissue, malonaldehyde production or retention in the tissue was not detected.

Table 4. ANOVA for TBA number

Source of Variation	df	SS	MS	F
Temperature (C)	1	2.114	2.114	92.4912 **
Atmosphere (A)	1	1.993	1.993	87.1771 **
C x A	1	1.858	1.858	81.2723 **
Storage Time (W)	3	2.786	0.929	40.6264 **
C x W	3	2.322	0.774	33.8573 **
A x W	3	1.452	0.484	21.1653 **
C x A x W	3	1.472	0.491	21.4658 **
Error	16	0.366	0.023	
Total	31	14.363		

coefficient of variation = 28.15 %

** is significantly different at a level of P < 0.01

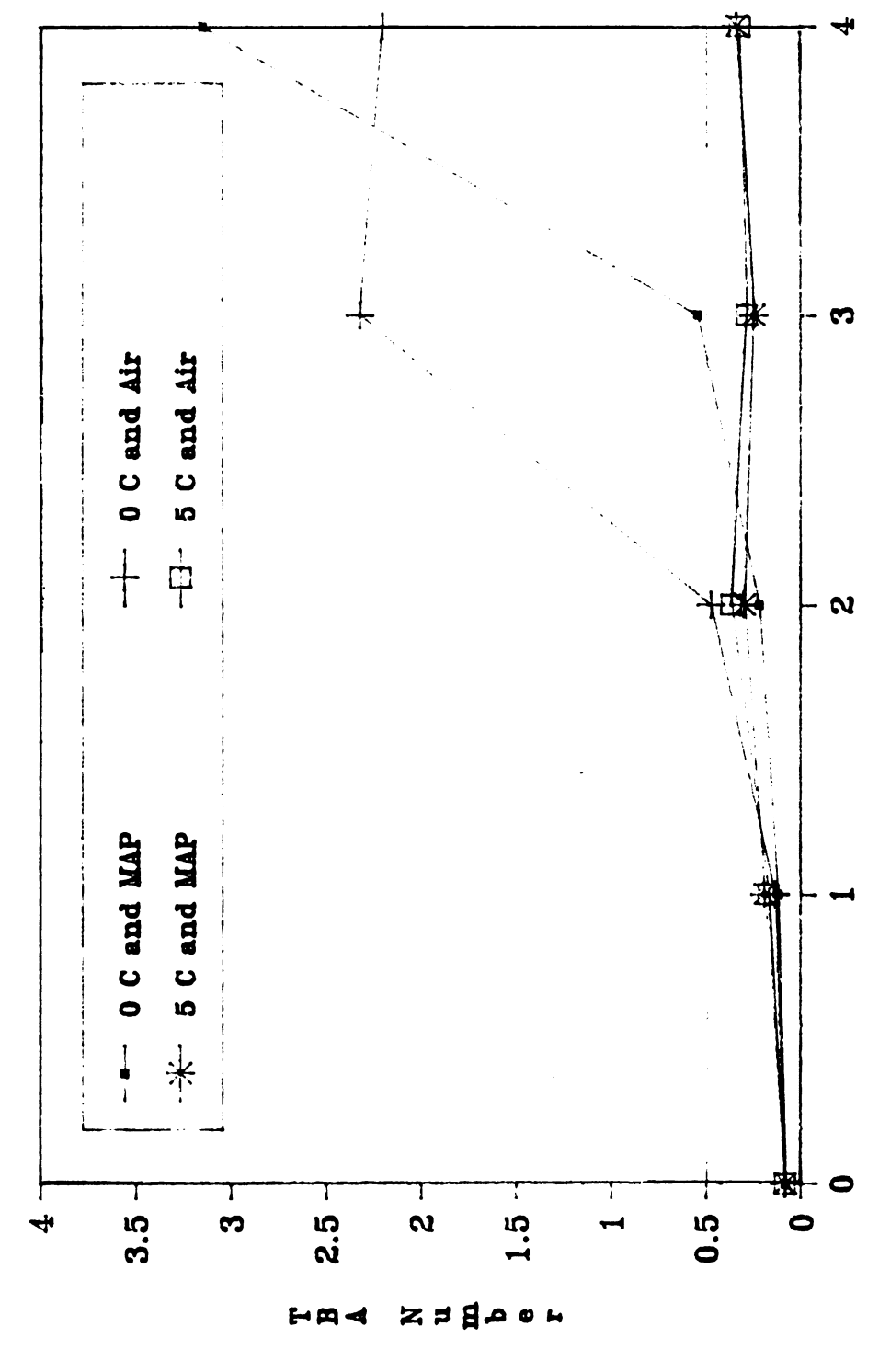
df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

Fig. 4. Effect of Temp. and Atmosphere On TBA Number



TBA number = 0.5 is the end of shelf lie

Use of TBA test for measurement of oxidation in fish products has a drawback (Smith et al, 1976). TBA is said to react with carbonyl components, especially malonaldehyde, which are hydroperoxide decomposition products. In fish oil oxidation, such reactions follow fairly consistent pathways and thus, production of TBA reacting substances correlate linearly with autoxidation (Hardy, 1980)

However, in fish tissue this is less likely and good correlations are not obtained. In the oxidation of cod and haddock, for instance, malonaldehyde production or retention in the tissue was not detected (McGill, 1976).

TBA values plotted in Figure 4 suggest that the shelf life of rainbow trout stored under MAP is higher than that of samples stored under AAP if the temperature is 0 C, but approximately the same if the temperature is 5 C. The 0 C data agree with those of Hardy (1980), who found that exclusion of oxygen retards the propagation stage of autoxidation mechanisms, thus retarding oxidation rate.

Figure 4 indicates that the end of acceptable shelf life, based on the time to reach a TBA number of 0.5, is approximately 2.8, 2, 4+, 4+ weeks if stored under 0 C-MAP, 0 C-AAP, 5 C-MAP, and 5 C-AAP treatments, respectively. 5 C-MAP and 5 C-AAP treatments were more effective in extending the shelf life of fish than 0 C-MAP or 0 C-AAP treatment,based on TBA results.

Data from the present study do not conform to the pattern reported by Hardy (1980), who found that a

combination of low temperature and exclusion of oxygen was the most effective way to prevent autoxidation. At low temperature, he reported that for every 10 C reduction, the oxidation rate fell by a factor 2 to 3.

The current study indicates that the TBA test is an inappropriate method for evaluation of shelf life of rainbow trout stored under the conditions of this study.

Bacterial Plate Counts

Three kinds of microbial count tests were carried out in this study, namely, aerobic plate count (APC), psychrotrophic plate count, and anaerobic plate count. The objective of APC was to relate APC values to the shelf life of fish and to determine the nature and degree of correlation between APC and sensory evaluation. For psychrotrophic and anaerobic plate counts, the objective was to evaluate the effect of different treatments applied in this study on the growth of microbes tolerant to cold or lack of oxygen.

In this study, it was decided that the shelf life of fish would be equal to the time required for the samples to reach a value of 6 log CFU APC /g sample. The decision agrees with criteria of other reseachers, who defined the end of shelf life of fresh fish as the time when its APC reached 1,000,000 CFU/g (Anonymous, 1974; Nickelson and Finne, 1984; Stenstrom, 1985).

Psychrotrophic Plate Count

The results of ANOVA for mean log CFU psychrotrophic bacterial count are shown in Table 5. Differences of psychrotrophic count within temperature treatments (C), within atmosphere treatments (A), within storage time (W) and within combinations of A, C, and W treatments were all significant at a level of $p < 0.01$.

Mean log CFU for psychrotrophic plate counts from each treatment at each sampling period are numerically tabulated in Table 15 in Appendix C, and graphically presented in Fig. 5.

From Fig. 5, it can be seen that the growth rate, which is indicated by the size of angle between the x-axis and the plotted graph, of psychrotrophic bacteria at 0 C was lower than that at 5 C under the same atmosphere, especially during 2 weeks of storage. The data support the conclusion of other researchers that, at temperature below the optimum for growth of a microorganism, the lower the temperature the lower the growth rate (Ogrydziak and Brown, 1982). The optimum temperatures for growth range between 15 C (59 F) and 25 C (77 F) for psychrophiles, and is at 37 C (98.6 F) for mesophiles (Gorge and Ronsivalli, 1988).

Figure 5 indicates that the lag phase for psychrotrophs lasted for 2 weeks in the case of samples stored at 0 C, whether under MAP or AAP, 1 week for 5 C-MAP treatment, and less than 1 week for 5 C-AAP treatment. It indicates that

Table 5. ANOVA for psychrotrophic plate count.

Source of Variation	df	SS	MS	F	
Temperature (C)	1	82.099	82.099	1271.7569	**
Atmosphere (A)	1	82.292	82.292	1274.7360	**
C x A	1	0.234	0.234	3.6237	ns
Storage Time (W)	3	72.757	24.252	375.6815	**
C x W	3	7.945	2.648	41.0246	**
A x W	3	0.682	0.227	3.5227	*
C x A x W	3	32.361	10.787	167.0948	**
Error	16	1.033	0.065		
Total	31	279.403			

coefficient of variation = 4.63 %

ns is not significantly different at a level of $P < 0.05$

* is significantly different at a level of $P < 0.05$

** is significantly different at a level of $P < 0.01$

df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

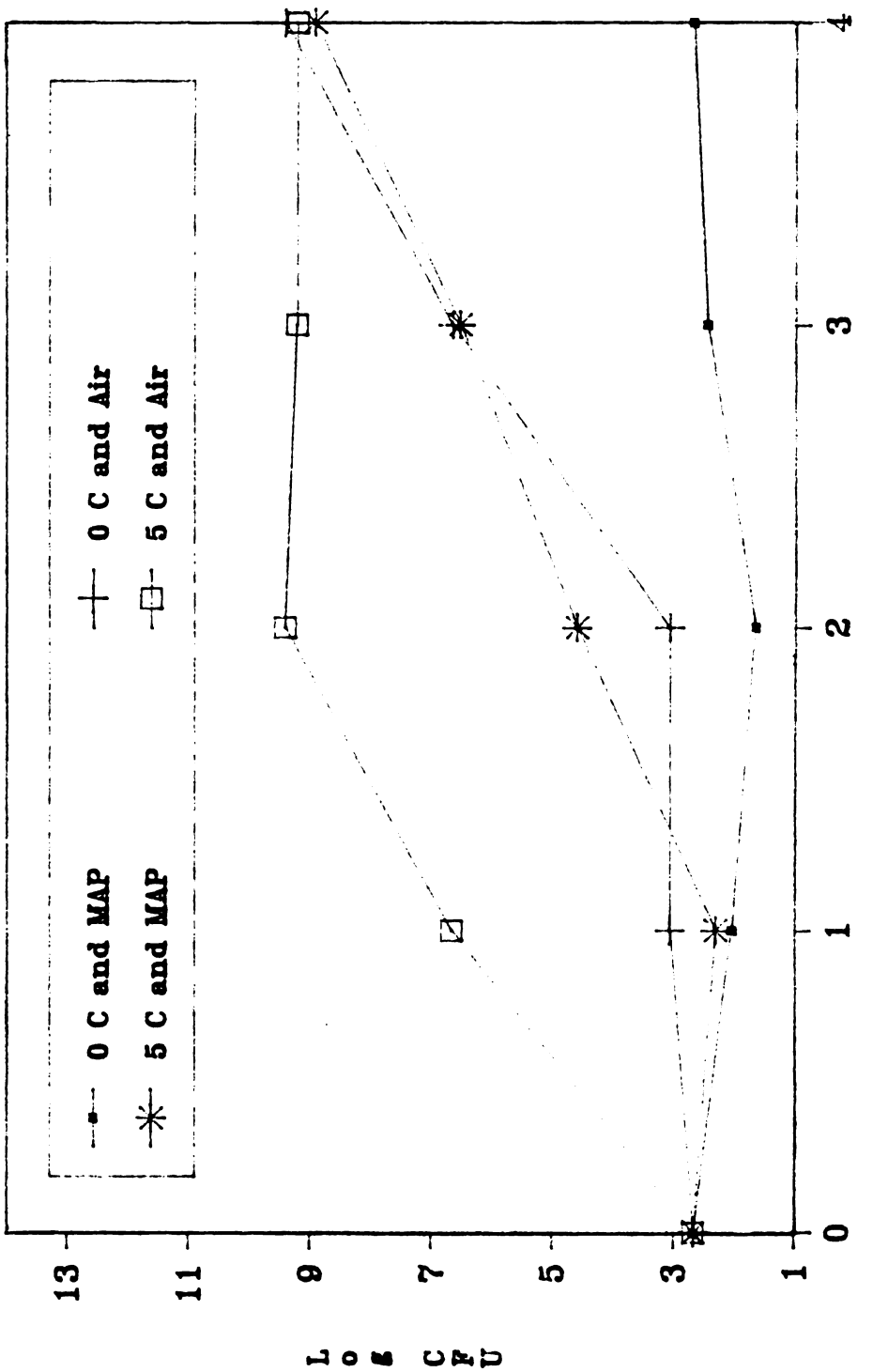


Fig. 5. Effect of Temp. and Atmosphere On Psychrotrophic Plate Count

temperature has bigger effects on the lag phase than MAP (carbon dioxide).

The growth rate following the lag phase, which is indicated by the size of the angle between the x-axis and the plotted graph of each treatment, was lowest for the 0 C-MAP treatment, followed by 5 C-MAP, by 0 C-AAP, and finally by 5 C-AAP treatment. It seems that under low temperature carbon dioxide effectively inhibits the growth rate of microorganisms.

The phenomena agree with cited literature data. As stated in the Literature Review, carbon dioxide inhibits spoilage bacteria by increasing the lag phase and decreasing the growth rate (Ogrydziak and Brown, 1982). Enfors and Molin (1981) reported that lag phase was prolonged as temperature decreased, but lag phase was not significantly influenced by the presence or absence of carbon dioxide. However,lag phase increase with increase in carbon dioxide concentration was reported by Coyne (1933). Sears and Eisenberg (1961) and Wolf (1980) found that growth inhibition by carbon dioxide increases at low temperature.

At the end of 4 weeks, log CFU/g for psychrotrophic bacteria stored at 0 C-MAP, 0 C-AAP, 5 C-MAP, and 5 C-AAP treatments (Table 15 in Appendix C) were 2.203, 5.581, 5.577, and 8.613, respectively. These data support the conclusion of Gilliland et al. (1984) that a few cells of psychrotrophs capable of growing at low temperature may result in a large population (1,000,000 to 100,000,000 CFU/g

or ml) in days or weeks, particularly if the food is held at marginal refrigeration temperatures.

The enumeration of psychrophiles in foods that are to be stored at refrigeration temperatures (0 to 10 C) is important. Large number of these bacteria in food serve to indicate that there is contamination at some points prior to or during storage of the food, and also as a warning that there is a high potential for spoilage during extended storage. Whether or not flavor or physical defects appear depends largely on the number and biochemical characteristics of the microbial species. Even if no defects appear, the microbial count may exceed legal limits (Gilliland et al, 1984).

Anaerobic Plate Count

The results of ANOVA for log CFU anaerobic count are shown in Table 6. Differences of anaerobic count within temperature treatments (C), within atmosphere treatments (A), within storage time (W) and within combinations of A, C, and W treatments were all significant at a level of $p < 0.01$.

Mean log CFU/g for anaerobic counts from each treatment at each sampling period are numerically tabulated in Table 15 in Appendix C, and graphically presented in Fig. 6.

From Figure 6, it can be seen that the growth pattern for anaerobic bacteria in each treatment was relatively

Table 6. ANOVA for anaerobic plate count

Source of Variation	df	SS	MS	F	
Temperature (C)	1	70.707	70.797	266.9742	**
Atmosphere (A)	1	36.536	36.536	137.9533	**
C x A	1	5.559	5.559	20.9881	**
Storage Time (W)	3	42.824	14.275	53.8977	**
C x W	3	1.269	0.423	1.5967	ns
A x W	3	3.065	1.002	3.8579	*
C x A x W	3	29.709	9.903	37.3915	**
Error	16	4.238	0.265		
Total	31	193.906			

coefficient of variation = 10.81 %

ns is not significantly different at a level of P < 0.05

* is significantly different at a level of P < 0.05

** is significantly different at a level of P < 0.01

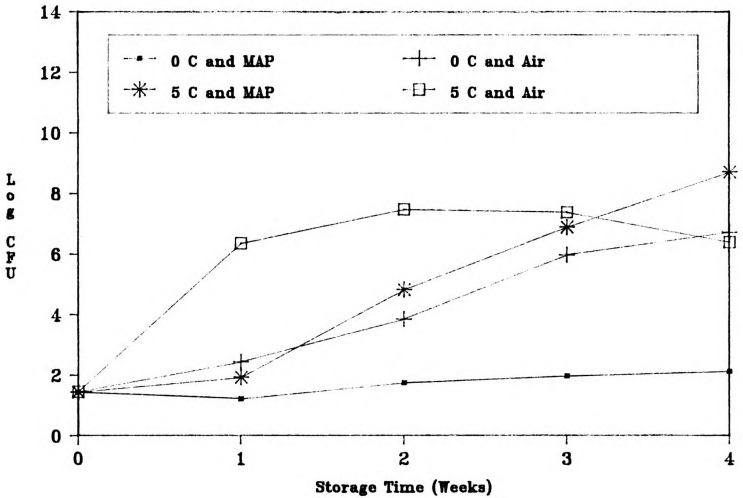
df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

Fig. 6. Effect of Temp. and Atmosphere On Anaerobic Plate Count



close to that of psychrotrophic bacteria described above.

These data indicate that carbon dioxide inhibits aerobic bacteria as well as anaerobic bacteria, by extending lag time and decreasing growth rate. Thus, it was not true that the growth rate of anaerobes is increased by anaerobic environment in the presence of carbon dioxide. Data from this study agree with the conclusion of other researchers who found that anaerobic bacteria were inhibited by carbon dioxide atmosphere (Frankel. 1889; Collow, 1932). The mechanism of carbon dioxide action has been described in the Literature Review.

Aerobic Plate Count (APC)

The results of ANOVA for log CFU APC are shown in Table 7. Differences of aerobic count within temperature treatments (C), within atmosphere treatments (A), within storage time (W) and within combinations of A, C, and W treatments were all significant at a level of $p < 0.01$.

Mean log CFU/g for aerobic counts from each treatment at each sampling period are numerically tabulated in Table 15 in Appendix C, and graphically presented in Fig. 7.

From the Figure, it can be seen that the end of shelf life, as indicated by a log CFU APC value equal to 6, was approximately 4+, 2.5, 2.6, and 1.6 weeks for 0 C-MAP, 0 C-AAP, 5 C-MAP, and 5 C-AAP treatments, respectively.

The shelf life of fish stored at 0 C is longer than that of samples stored at 5 C under the same atmosphere. It

Table 7. ANOVA for aerobic plate count

Source of Variation	df	SS	MS	F	
Temperature (C)	1	50.577	50.577	358.8062	**
Atmosphere (A)	1	52.968	52.968	375.7695	**
C x A	1	7.323	7.323	51.9513	**
Storage Time (W)	3	43.476	14.492	102.8109	**
C x W	3	1.918	0.639	4.5353	*
A x W	3	3.110	1.037	7.3550	**
C x A x W	3	30.015	10.005	70.9797	**
Error	16	2.255	0.141		
Total	31	191.642			

coefficient of variation = 7.04 %

* is significantly different at a level of P < 0.05

** is significantly different at a level of P < 0.01

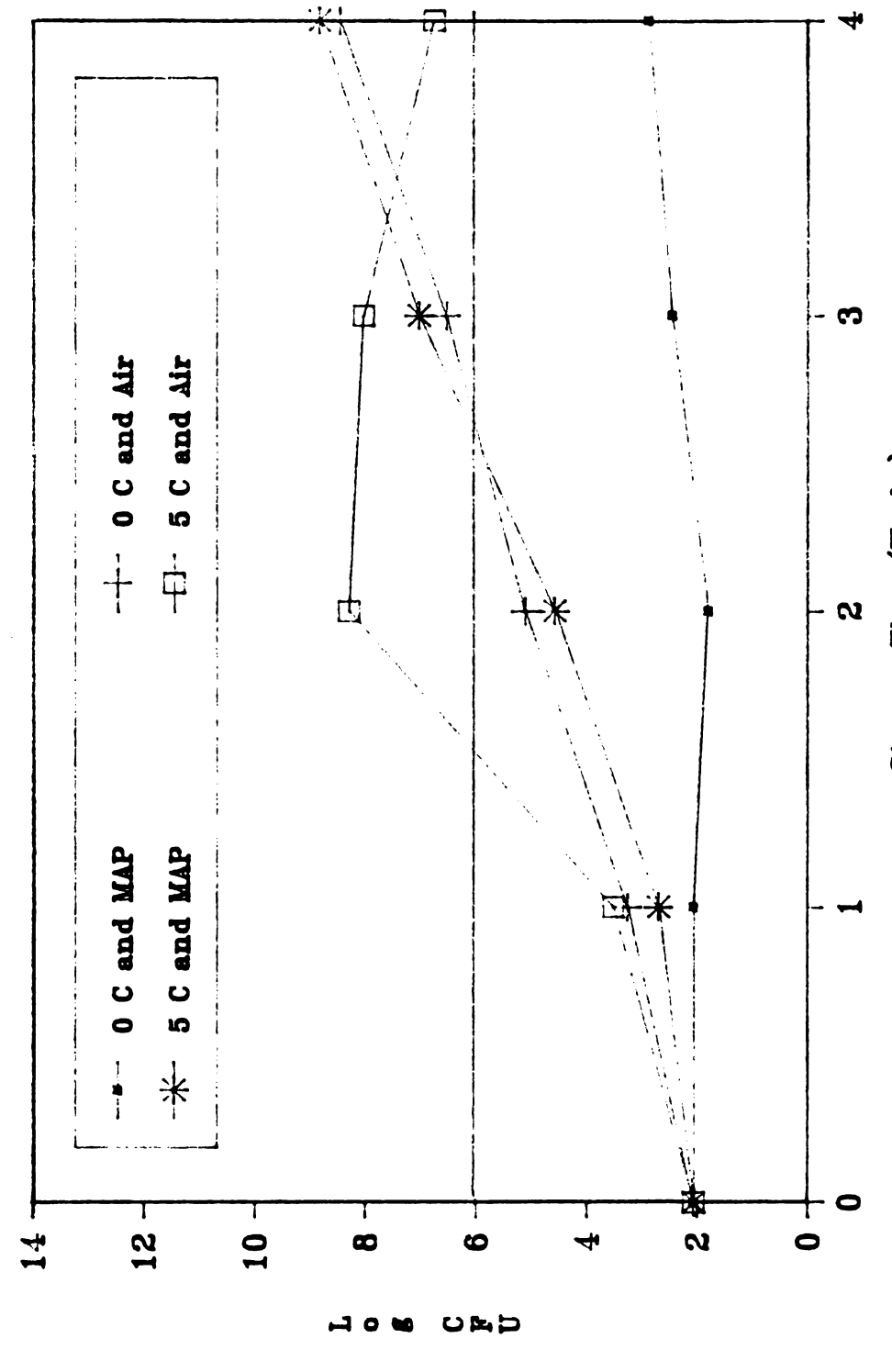
df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

Fig. 7. Effect of Temp. and Atmosphere On Aerobic Plate Count



Log CFU = 6 is The end of shelf life

indicates that spoilage rate at 5 C was higher (approximately 4+/2.6 or 1.5+ times if under MAP and 2.5/1.6 or 1.6 times under AAP) than that at 0 C. Data from this study disagree with the conclusion of Owen and Nesbitt (1984) and Storey and Owen (1985) who found that the spoilage rate at 5 C is approximately 2.25 times that at 0 C.

The end of shelf life for fish under MAP was later than that for fish under AAP at the same temperature. The shelf life of fish under MAP was approximately 4+ weeks at 0 C and 2.5 weeks at 5 C, and under AAP was approximately 2.6 weeks at 0 C and 1.6 weeks at 5 C. These results suggest that MAP, under the conditions of this experiment, can extend shelf life of fish by factor 4+/2.6 or 1.5+ if stored at 0 C, or 2.5/1.6 or 1.6 if at 5 C. Data from the current study support the conclusions of other investigators , as described in the Literature Review, which indicate that the shelf life of fish under carbon dioxide atmosphere storage can be extended by a factor of 1.5 to 2.0 (Veranth and Robe, 1979; Brown et al., 1980; Woyewoda et al., 1984). However, the present data differ from several cited literature data, which found that the shelf life of fish could be at least doubled in carbon dioxide atmosphere as compared to the same material stored in air (Coyne, 1933; Kiffeler, 1933; Standby and Griffiths, 1935). Differences between the gains reported in the present study and the literature data mentioned above

may be attributable to variations in the parameters used to determine the end of shelf life and to the experimental conditions used including gas composition, temperature of storage, and fish species.

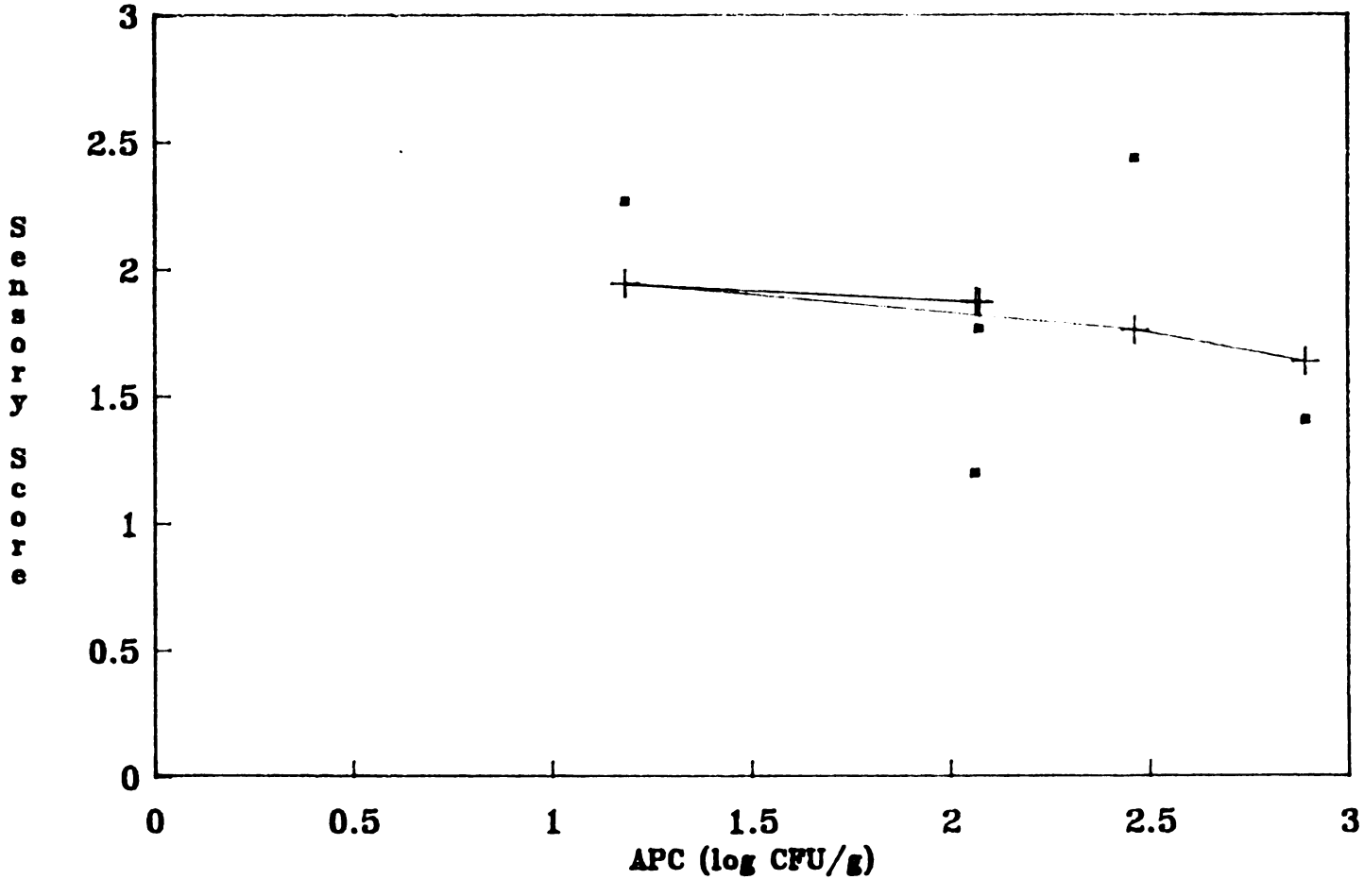
Based on the APC criterion, the present study found that 0 C-MAP treatment was the most effective way to extent shelf life of fresh rainbow trout. Since bacteria are frequently responsible for spoilage in chilled fish (Herbert et al., 1971), and the solubility of carbon dioxide is higer at lower temperature, inhibition of spoilage bacteria is more effective at 0 C than at 5 C. The mechanisms of carbon dioxide action as well as the effect of low temperature on inhibition of bacterial growth has been described in the Literature Review.

Linear Regression of Sensory Score vs. APC

Parameters of linear regression of sensory score vs. APC, including correlation coefficients are shown in Table 8. Such regression equations can be useful in utilization of APC to predict sensory score. The highest correlation ($r = 0.948$) between sensory score and APC was given by samples held under AAP at 0 C. A good correlation ($r = 0.904$) between sensory score and APC was also obtained with the samples held under MAP at 5 C. Correlation coefficient for the two treatments, 5 C-AAP and 0 C-MAP, were 0.838 and 0.225, respectively. The scatter diagrams

and regression lines for the four treatments are graphically presented in Figs. 8, 9, 10, and 11

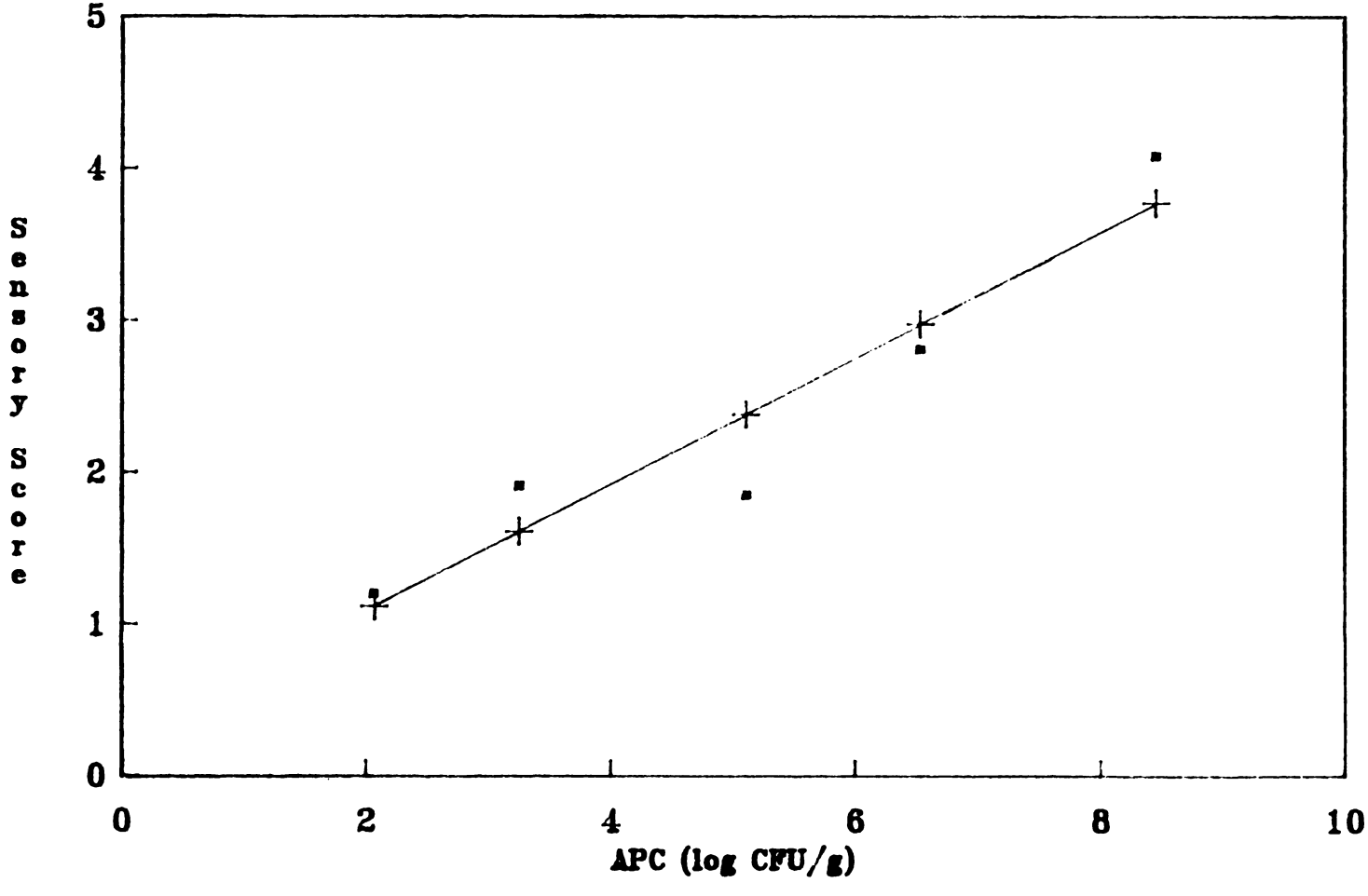
Fig. 8. Linear Regression of Sensory Score vs. APC Under MAP at 0 C.



Correlation coefficient = 0.225

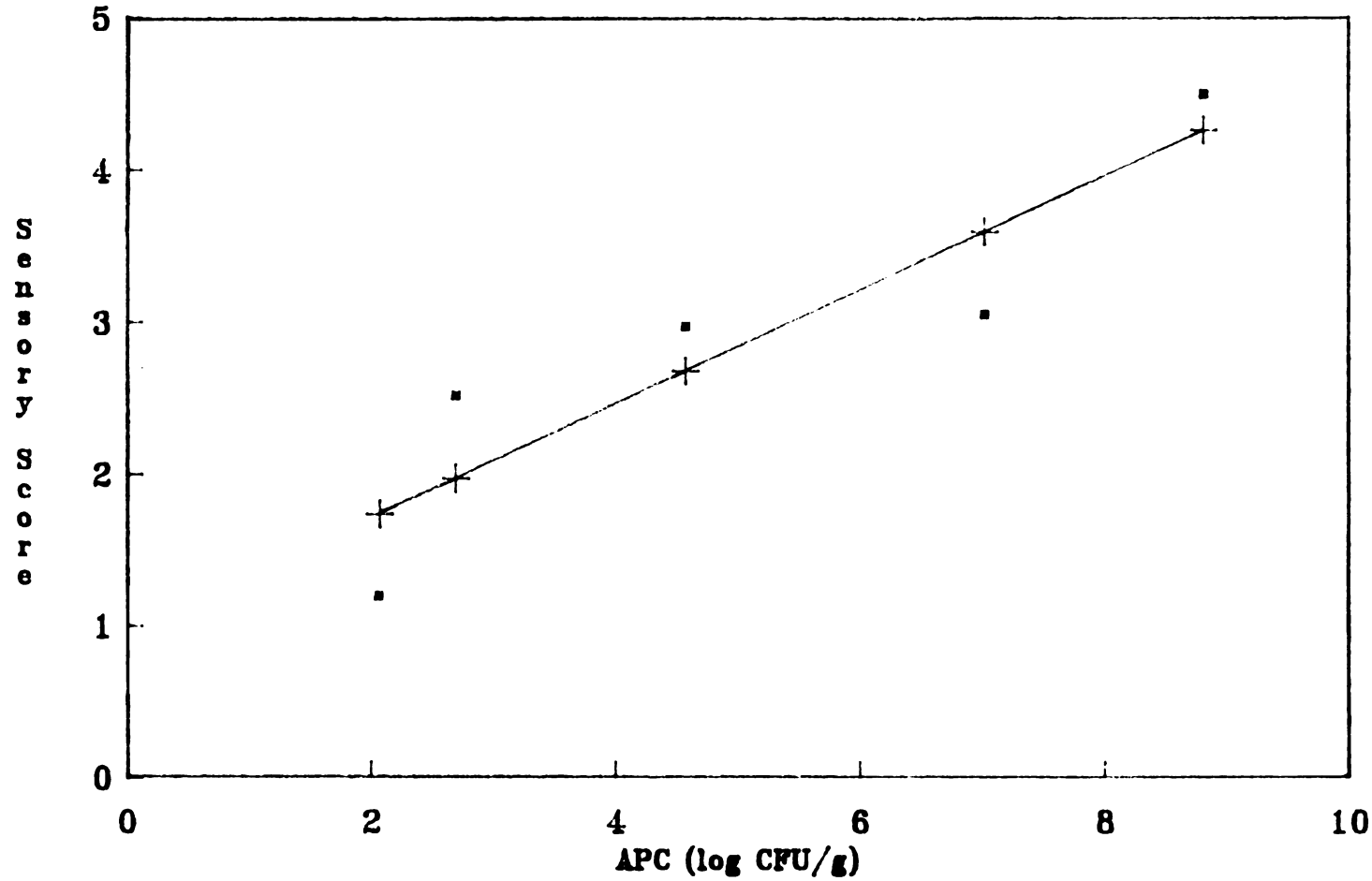
Y = 2.460 X − 0.0284

Fig. 9. Linear Regression of Sensory Score vs. APC Under AAP at 0 C.



Correlation coefficient = 0.948
Y = 0.062 X + 0.415

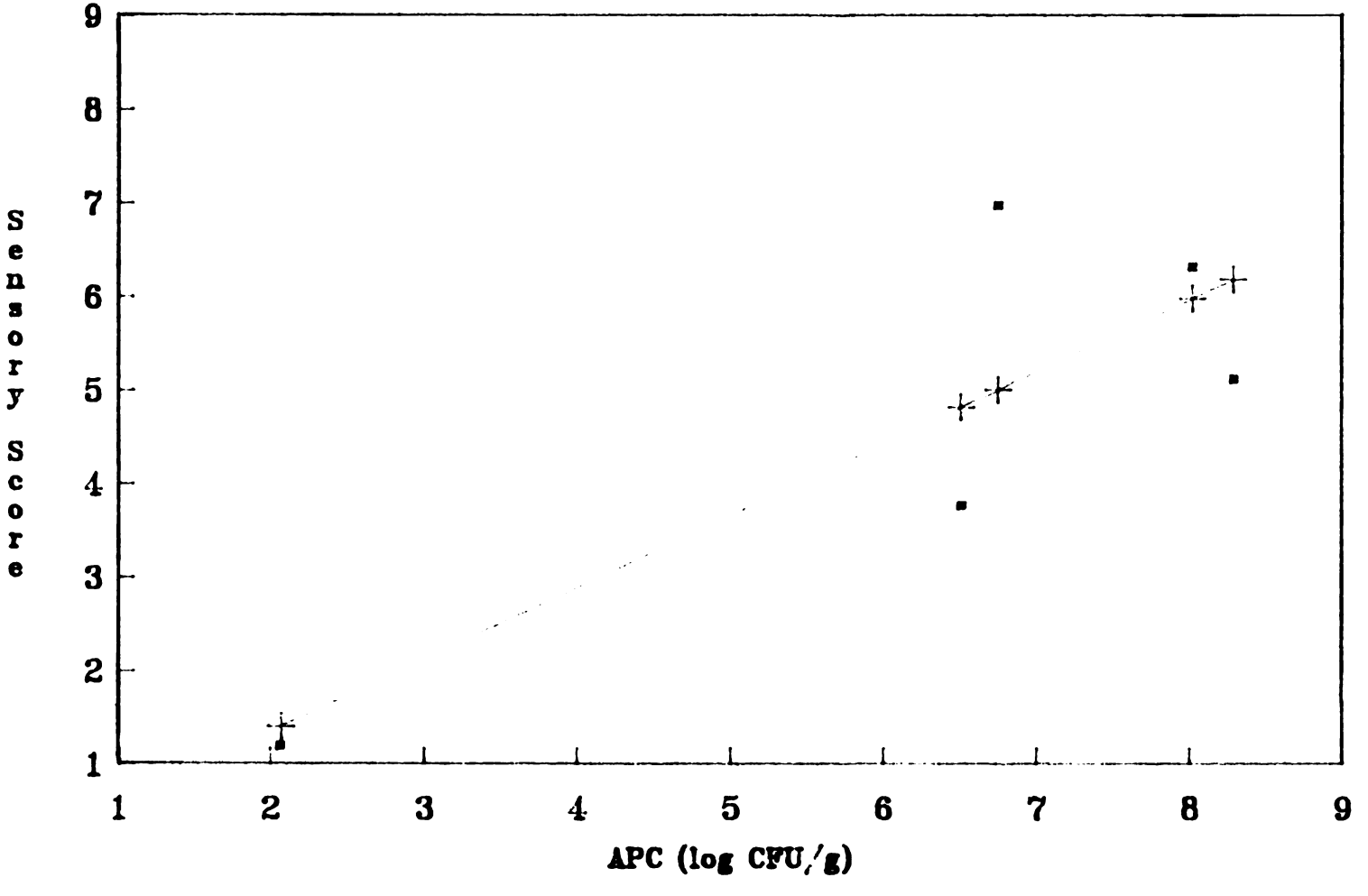
Fig. 10. Linear Regression of Sensory Score vs. APC Under MAP at 5 C.



Correlation coefficient = 0.904

Y = 0.967 X + 0.374

Fig. 11. Linear Regression of Sensory Score vs. APC Under AAP at 5 C.



correlation coefficient = 0.838

Y = −0.176 X + 0.767

Effect of MAP-Storage Time on Fish Shelf Life During Post-MAP Storage

The objective of this portion of the experimental plan was to evaluate the acceptability of fish after removal from MAP storage, followed by placement in air-atmosphere packaging for storage at 5 C. This was intended to simulate typical refrigerated retail storage.

The results reported earlier in this thesis showed that rainbow trout were still acceptable after storage at 0 C for either 2 or 3 weeks under MAP. Based on these findings, treatments to be used in the post-MAP study were determined. The treatments that were decided upon were freshly-killed fish as the control treatment, namely MAP-0 C for 0 weeks (M0); MAP-0 C for 2 weeks (M2); and MAP-0 C for 3 weeks (M3). Samples from each treatment were withdrawn and tested on days 0, 1, 2, and 3.

Since the TBA test was found to be inappropriate in the earlier-reported studies for evaluation of shelf life of chilled rainbow trout, fish acceptability was evaluated only by sensory test and bacterial plate counts.

ANOVAs were carried out to determine if significant differences existed : (a) within MAP storage times (M), (b) within post-MAP storage times (P), and (c) within combinations of M and P. Linear regression equations showing the relationship of sensory score to aerobic plate count for each of the treatments were calculated, from which correlation coefficients were also determined.

Sensory Evaluation

The results of ANOVA for sensory scores are shown in Table 9. Significant difference at a level of $P < 0.01$ existed within MAP Storage Time treatments (M), within Post-MAP storage Times (P), and within combinations of P and M treatments.

Means of sensory scores for each treatment at each sampling period are numerically presented in Table 16 in Appendic C, and graphically shown in Fig. 12. From this Figure, it can be seen that all treatments were still acceptable (sensory score being less than 3) after 3 days of 5 C, AAP storage. The M3 treatment exhibited the highest sensory score during post-MAP storage time. It seems that the longer storage time in MAP results in shorter shelf life during post-MAP storage.

Bacterial Plate Counts

Psychrotrophic Plate Count

The results of ANOVA for psychrotrophic plate counts during post-MAP storage are shown in Table 10. Differences of psychrotropic plate count within MAP Storage Time treatments (M) and within Post-MAP storage Times (P) were significant at a level of $P < 0.01$, and within combinations of P and M treatments was at a level of $P < 0.05$.

Mean log CFU for psychrotrophic plate counts are graphically presented in Fig. 13 and numerically tabulated in Table 16 in Appendix C. From Figure 13, it can be seen

Table 9. ANOVA for post-MAP sensory score.

Source of Variation	df	SS	MS	F
Storage Time in MAP (M)	2	3.941	1.971	84.1967 **
Post-MAP Storage Time (P)	3	0.883	0.294	12.5720 **
M x P	6	0.728	0.121	5.1809 **
Error	12	0.281	0.023	
Total	23	5.823		

Coefficient of variation = 10.18 %

** is significantly different at a level of $P < 0.01$

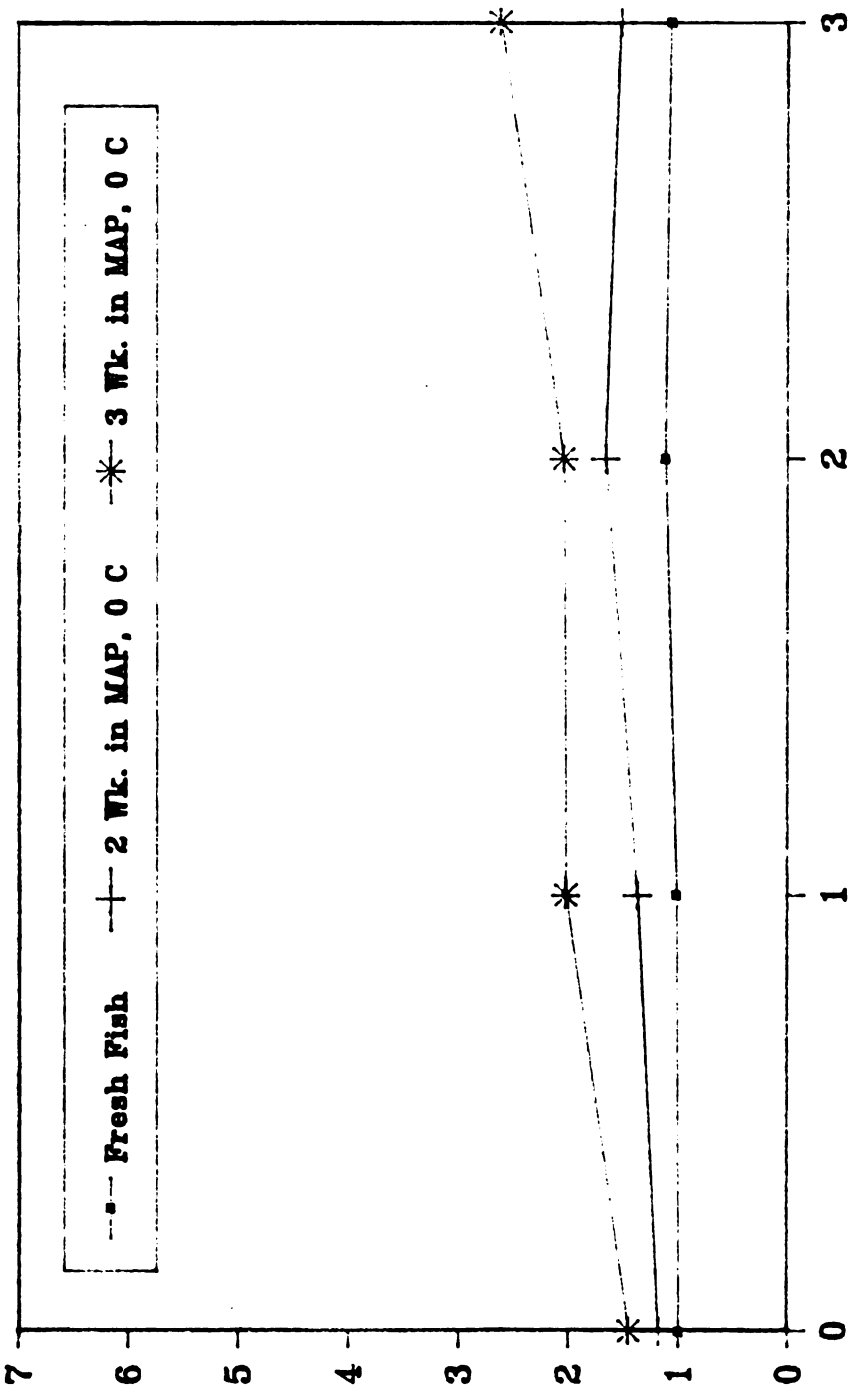
df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

Fig. 12. Effect of Storage Time in MAP On Post-MAP Sensory Score



Score 1 = No off odor
Score 3 = The end of shelf life
Score 7 = Strong off odor

Table 10. ANOVA for post-MAP psychrotrophic plate count

Source of Variation	df	SS	MS	F	
Storage Time in MAP (M)	2	8.728	4.393	19.1150	**
Post-MAP Storage Time (P)	3	58.291	19.430	84.5467	**
M x P	6	4.125	0.687	2.9911	*
Error	12	2.758	0.230		
Total	23	73.960			

Coefficient of variation = 13.05 %

* is significantly different at a level of P < 0.05

** is significantly different at a level of P < 0.01

df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

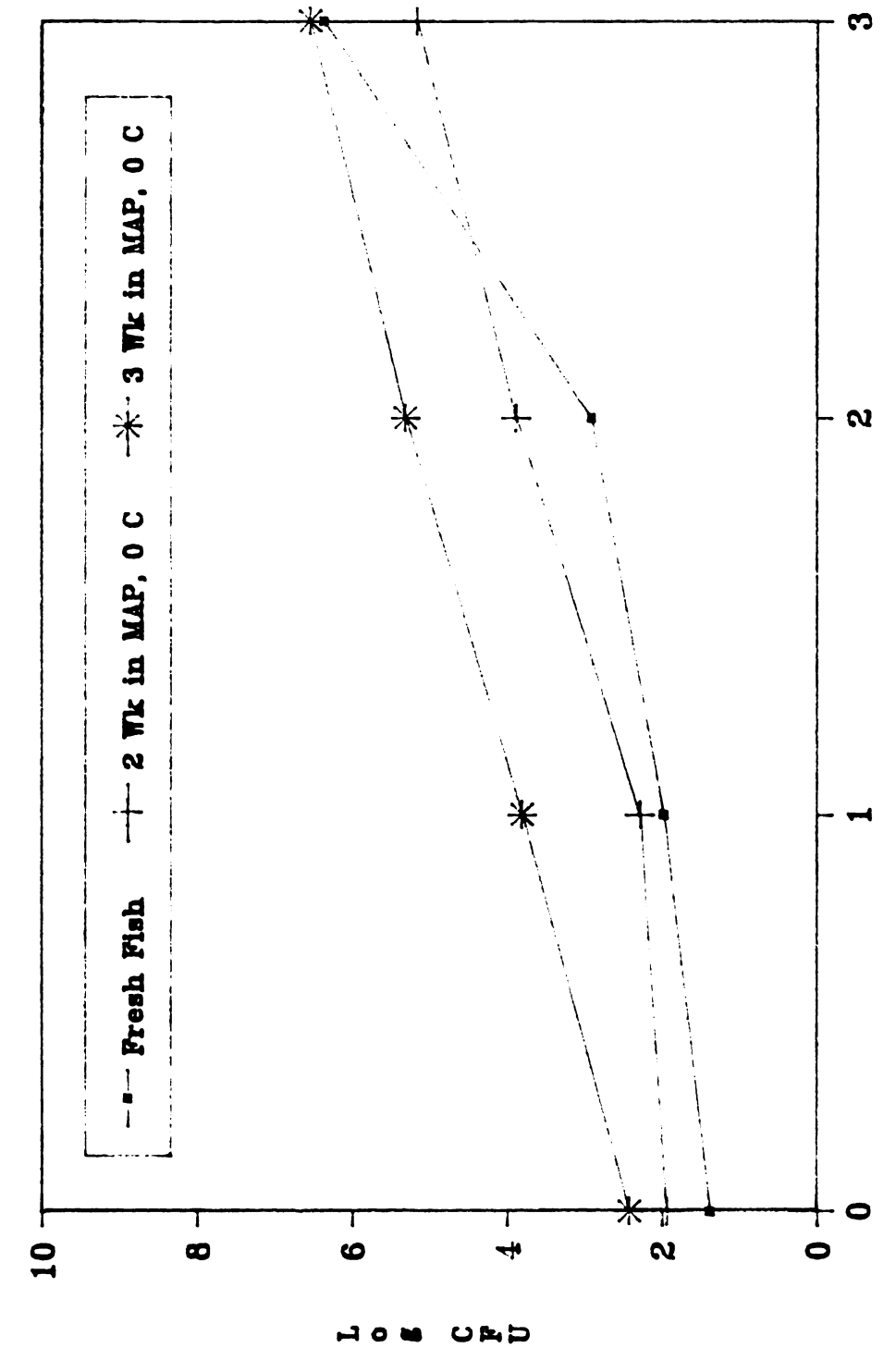


Fig. 13. Effect of Storage Time in MAP On Post-MAP Psychrotrophic Plate Count

that log CFU for samples stored under M0, M2, and M3 treatments at the end of day 3 in post-MAP storage were relatively close. This appears to indicate that there were no carbon dioxide residues from prior MAP storage to inhibit bacterial growth in fish tissue of M2 and M3 samples.

Figure 13 shows that bacterial growth rate for the 3-day period, which relates to the size of the angle between x-axis and the plotted graph for each treatment, was essentially the same in each of the 3 treatments. This also suggests the probable absence of carbon dioxide residue after removal from MAP storage.

Anaerobic Plate Count

The results of ANOVA for anaerobic plate counts during post-MAP storage are shown in Table 11. Differences of anaerobic plate count within MAP Storage Time treatments (M), within Post-MAP storage Times (P), and within combinations of P and M treatments were all significant at a level of 0.01.

Mean log CFU for anaerobes from each treatments at each sampling period are numerically presented in Table 15 in Appendic C and graphically presented in Fig. 14. From the Figure, it can be seen that growth of anaerobes under M0 treatment has a lag phase of about 2 days, but that under M2 and M3 treatments apparently have no lag phase during post-MAP storage.

Table 11. ANOVA for post-MAP anaerobic plate count.

Source of Variation	df	SS	MS	F
Storage Time in MAP (M)	2	9.630	4.815	32.1871 **
Post-MAP Storage Time (P)	3	22.774	7.591	50.7448 **
M x P	6	4.735	0.789	5.2758 **
Error	12	1.795	0.150	
Total	23	38.934		

Coefficient of variation = 13.01 %

** is significantly different at a level of P < 0.01

df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

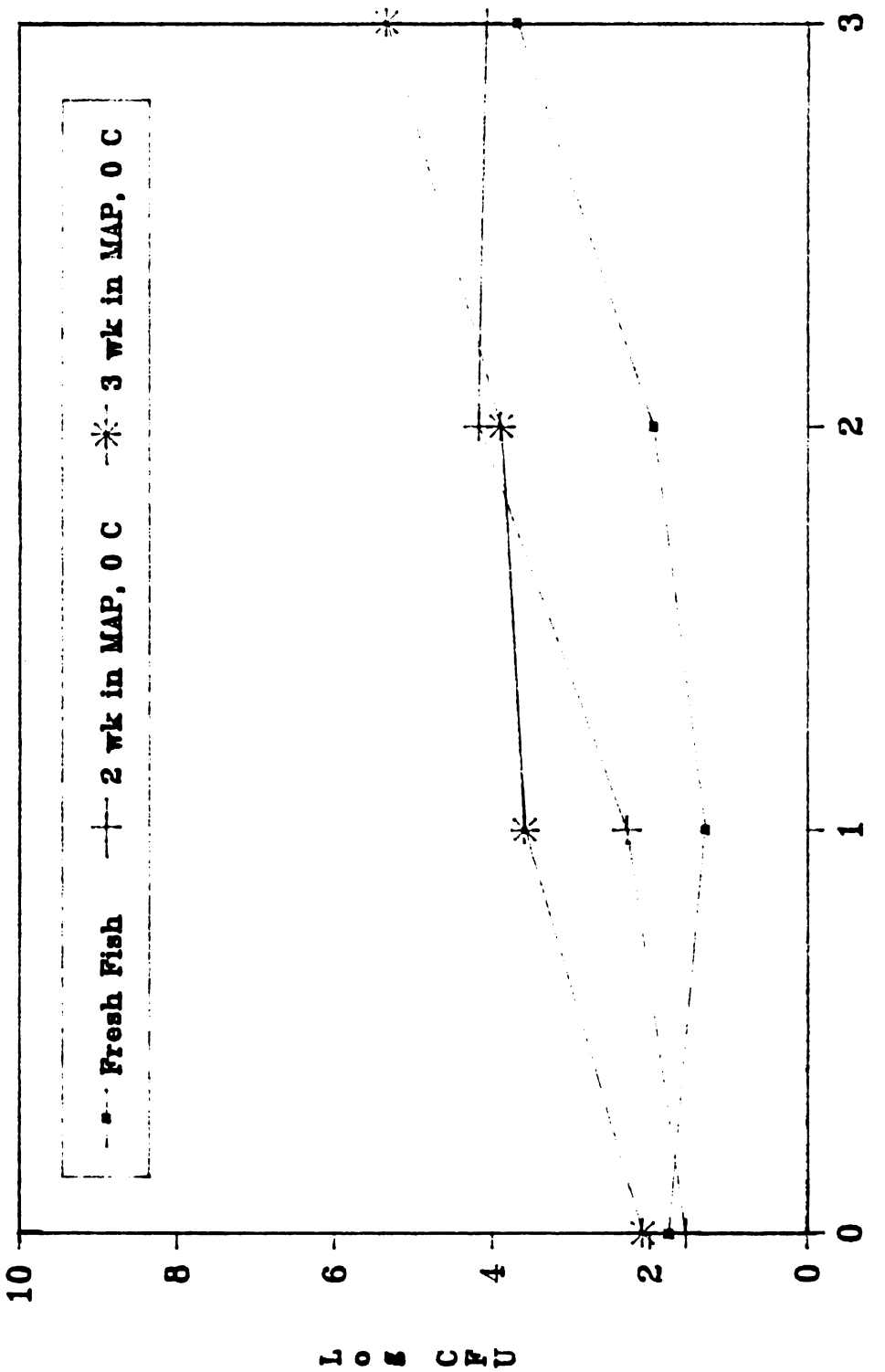


Fig. 14. Effect of Storage Time in MAP
On Post-MAP Anaerobic Plate Count

The resuls of ANOVA for APC during post-MAP storage are shown in Table 12. Differences of APC within MAP Storage Time treatments (M), and within Post-MAP storage Times (P) treatments were significant at a level of 0.01, and within combinations of P and M treatment was at a level of 0.05.

Mean log CFU aerobic plate counts for each treatment at each sampling period are numerically shown in Table 16 in Appendic C and graphically presented in Fig. 15. From the Figure, it can be seen that all samples under all treatments mentioned above were still acceptable (log APC less than 6) at the end of day 3 in post-MAP storage. Lag time for aerobic bacteria under M0 treatment was about 2 days, with no apparent lag phase for the other treatments. These data suggest that there were no carbon dioxide residues in fish stored in MAP for 2 to 3 weeks prior to post-MAP storage. Data from this study agree with data of Bank et al. (1980) who found that the growth rate of aerobic bacteria after storage under carbon dioxide parallels the growth rate of the bacteria in fish stored without carbon dioxide.

Linear Correlation of Post-MAP Sensory Score vs. APC

Parameters of linear correlation of post-MAP sensory score vs. APC for each treatment are shown in Table 13. The scatter diagrams and regression line for each treatment are graphically shown in Figs. 16, 17 and 18. From Table 13, it can be seen that the highest correlation coefficient

(r = 0.879) was for the samples stored under MAP at 0 C for 3 weeks. Correlation coefficients for the other two treatments, MAP-2 weeks and MAP-0 weeks, were 0.677 and 0.391, respectively.

Table 12. ANOVA for post-MAP aerobic plate count.

Source of Variation	df	SS	MS	F	
Storage Time in MAP (M)	2	9.286	4.643	23.9863	**
Post-MAP Storage Time (P)	3	22.387	7.462	38.5494	**
M x P	6	3.616	0.603	3.1130	*
Error	12	2.323	0.194		
Total	23	37.611			

Coefficient of variation = 12.54 %

* is significantly different at a level of $P < 0.05$

** is significantly different at a level of $P < 0.01$

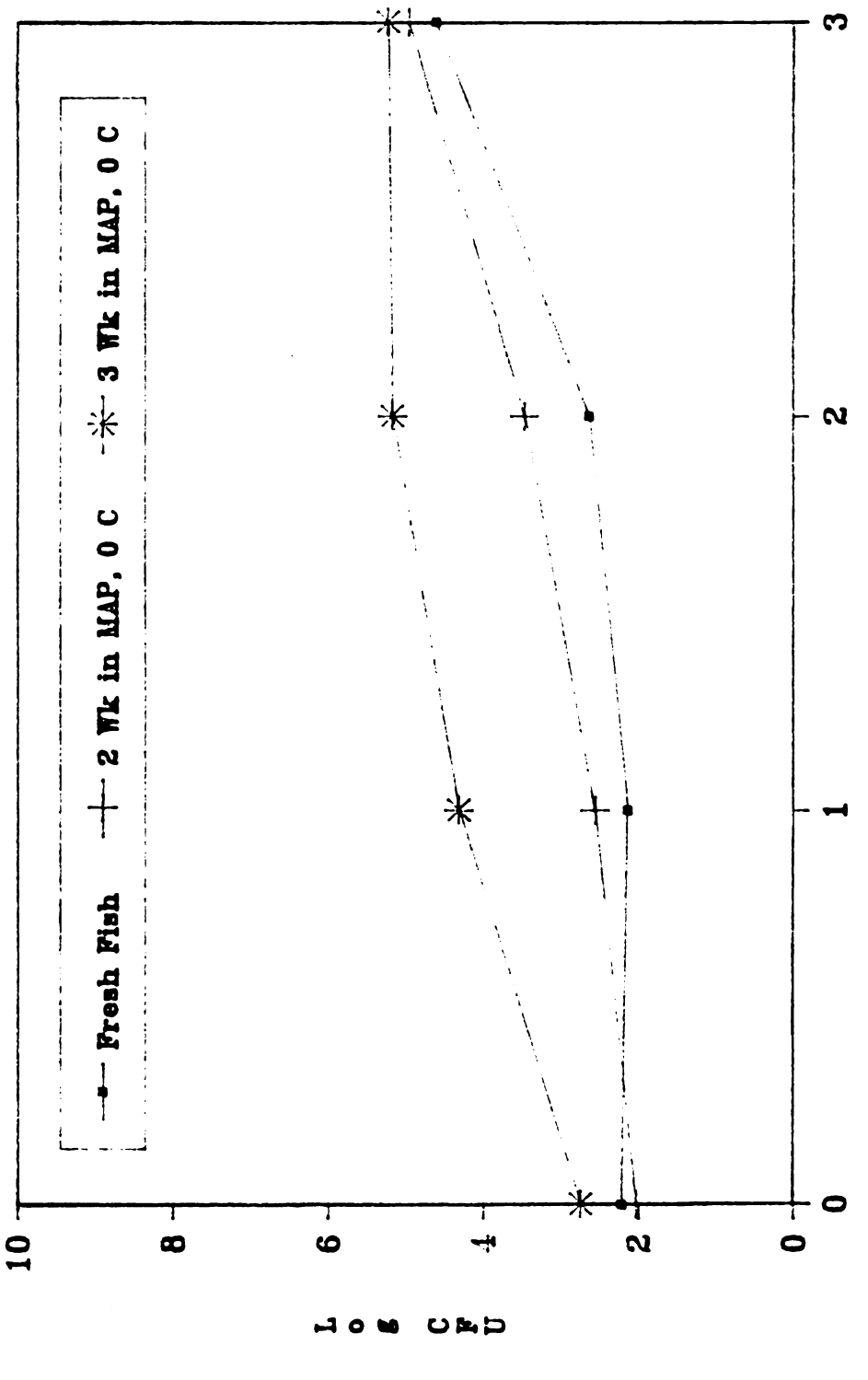
df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

Fig. 15. Effect of Storage Time in MAP On Post-MAP Aerobic Plate Count



Log CFU = 6 is the end of shelf life.

Table 13. Parameters of linear regression* of post-MAP sensory score against APC.

Treatment	r	a	b	SE Y
MAP - 0 WEEK	0.391	1.001	0.014	0.050
MAP - 2 WEEKS	0.677	1.078	0.108	0.184
MAP - 3 WEEKS	0.879	0.462	0.360	0.276

* Y = a X + b , where Y is Sensory Score
X is APC (log CFU/g).

Number of observations is 4 in each treatment .

r is correlation coefficient.

SE Y is Standard error for Y.

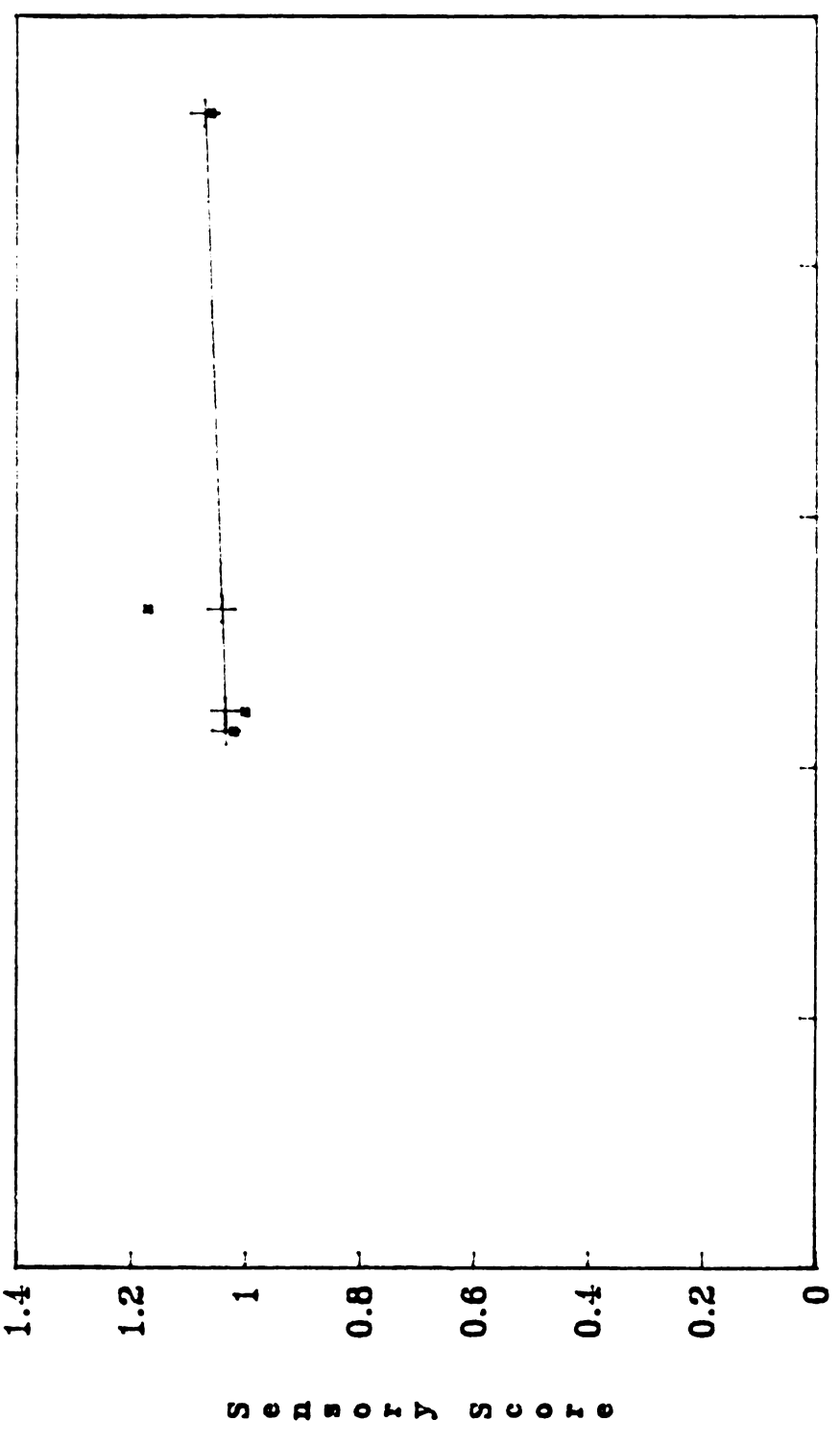


Fig. 16. Linear Regression of Post-MAP Sensory Score vs. APC After 0 Weeks, MAP

Correlation coefficient = 0.391
Y = 1.001 X + 0.014

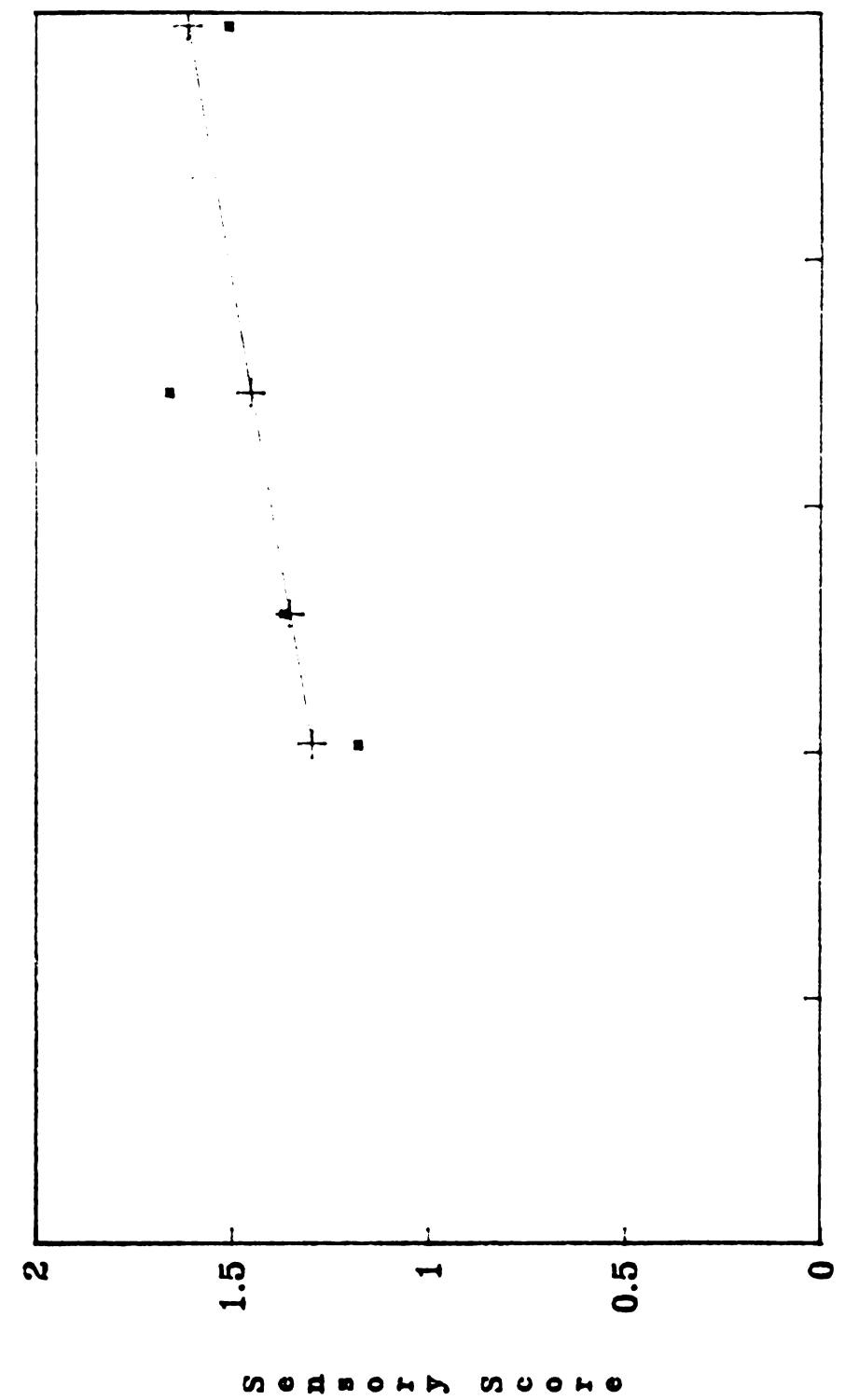
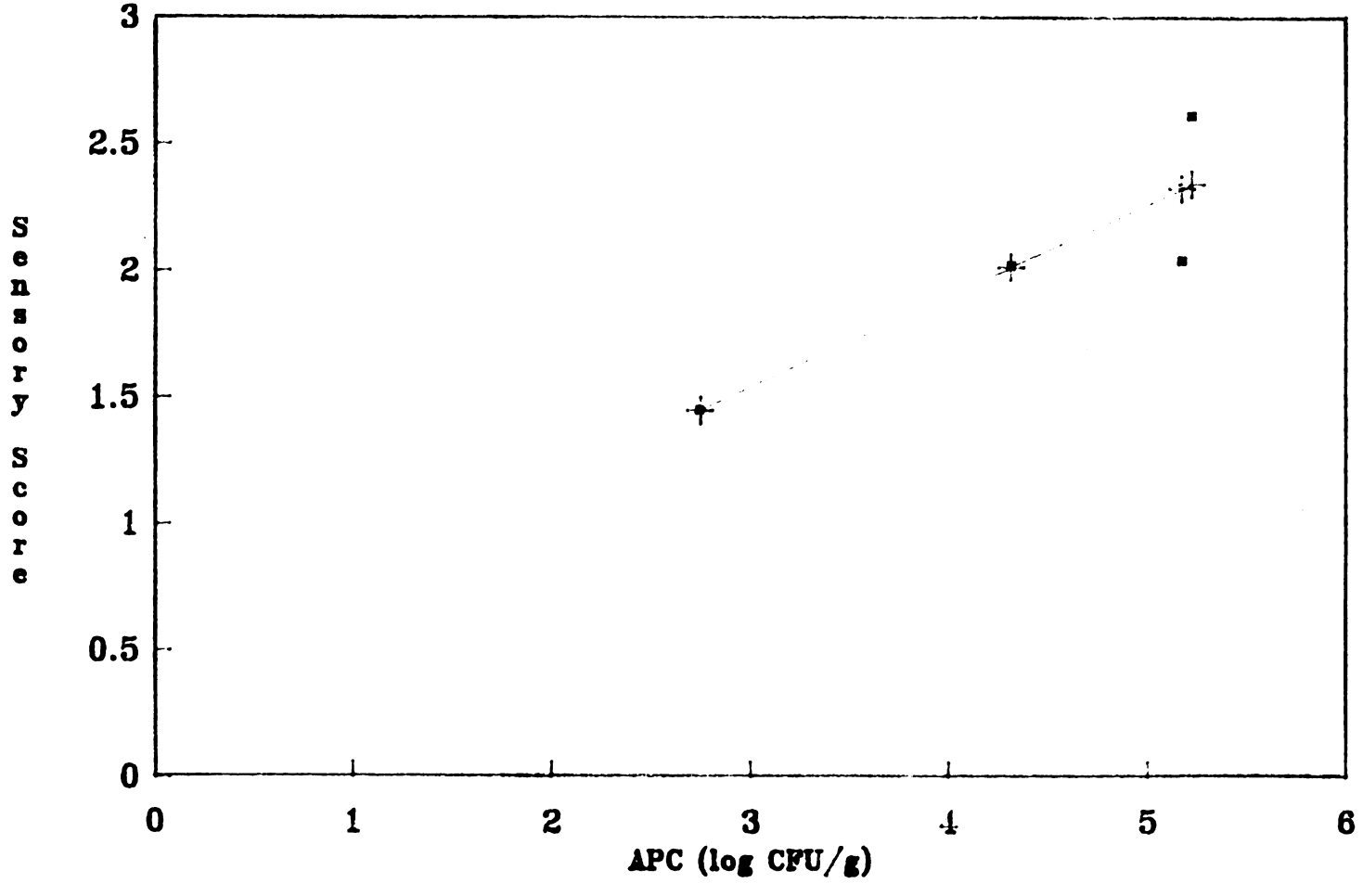


Fig. 17. Linear Regression of Post-MAP Sensory Score vs. APC After 2 Weeks, MAP

Correlation coefficient = 0.677
Y + 1.078 X + 0.108

Fig. 18. Linear Regression of Post-MAP Sensory Score vs. APC After 3 Weeks, MAP



Correlation coefficient = 0.879
Y = 0.462 X + 0.360

CONCLUSIONS

The effect of temperature on shelf life of fresh rainbow trout was investigated. Shelf life of fish stored at 0 C was consistently higher than that of fish stored at 5 C. The shelf life of fish at 0 C was more than 2.6 weeks if judged by APC value, or more than 3.1 weeks by sensory score.

The effect of modified atmosphere on shelf life of refrigerated fish was also investigated. The shelf life of fish under MAP was significantly longer than that of fish under air atmosphere packaging (AAP). Fish under MAP retained their quality for more than 2.5 weeks if judged by APC value, or more than 2.2 weeks by sensory score. MAP extended shelf life of chilled fish by a factor more than 1.5.

A combination of low temperature (0 C) and MAP was the most effective way to extend the shelf life of chilled freshwater rainbow trout. Shelf life of the fish under MAP at 0C was more than four weeks. Fish retained their acceptability when stored in air-permeable packaging at 5 C for 3 additional days following removal from 0 C MAP containers in which they had been stored for either 2 or 3 weeks.

Log CFU/g for anaerobic bacteria after storage for four weeks under MAP at 0 C increased from 1.45 to 2.14, indicating that these bacteria were not completely inhibited

under the test conditions. Future research would be desirable to examine a greater range of MAP and test conditions, with the goal of optimizing storage conditions for longer shelf life. Safety issues must also be satisfactorily resolved, particularly the question of Cl. botulinum growth and toxin production. For this purpose, inoculation of fish samples with Cl. botulinum prior to the start of storage would appear to be essential.

Results of post-MAP storage showed that all samples of rainbow trout remained acceptable (by sensory panel measurement) after 3 days at 5 C. Since post-MAP storage was concluded after 3 days, future tests will be required to determine the number of additional days of 5 C storage (beyond 3 days) that are possible before the fish become unacceptable. Post-MAP storage tests, similar to those reported upon in the present study, but at higher-than-5 C temperatures, would also be desirable because refrigerated cabinets in retail establishment are sometimes at temperatures that exceed 5 C.

Results of this study indicate that the acceptability of fresh rainbow trout can be retained for more than 4 weeks under MAP at 0 C. It may be both technically and commercially feasible to use a Master Packaging concept (Lindsay, 1981) in which a small quantity of fish is overwrapped or otherwise packaged in air-permeable film, then placed in a larger container (the Master Package) under MAP conditions for shipment and for storage under closely-

controlled, low temperature conditions. Once delivered under the above conditions to the retailer or food service establishment ,the Master Package concept requires that the seal be broken and air allowed into Master Package. In this way, air enters the atmosphere of the individual packages and will not allow growth of Cl. botulinum despite the higher refrigerated temperatures likely to prevail in retail and food service facilities.

According to Hobbs (1979), temperatures of 3.3 C and below prevent Cl. botulinum growth and toxin production, regardless of storage atmosphere. Storage life at 0 C reported in the study would be expected to diminish slightly at 3.3 C but protection against Cl. botulinum growth should be unaffected.

APPENDICES

Appendix A

Score sheet for sensory evaluation used in this study

Name : _____
Date : _____

Odor intensity evaluation rainbow trout.

Please sniff samples in the order listed across the top of the score sheet. There will be a total of 8 to 10 samples. Instructions shown below should be followed exactly as written.

a. When ready to begin, remove lid from first container;
b. WITHOUT DELAY, inhale deeply from the open top of the sample container using nostrils only (mouth should be closed);
c. Replace the lid quickly;
d. Place a check mark in the box (any box, 1 through 7) that best matches your odor evaluation;
e. Take at least five deep breaths (through nostrils only) before going on to the next sample
f. Repeat steps a through e for each of the additional samples.

RATING

Rating	Odor description	Sample #				
1	No off odor					
2	Between 1 and 3					
3	Slight off odor					
4	Between 3 and 5					
5	Moderate off odor					
6	Between 5 and 7					
7	Strong off odor					

This form has been reduced in size for illustrative purposes.

Appendix B

Score sheet for the organoleptic characteristics of white fish *

Raw fish

Odor (10 marks)	Score marks
Fresh 'seaweedy' odours	10
Loss of freh 'seaweediness', shellfish odours	9
No odours, neutral odours	8
Slight musty, acetamide-like, miky or caprylic acid-like odours	7
"Bready', 'malty', 'yeasty', odours	6
Lactic acid, ' sour milk', or oily ofours	5
Some lower fatty acid (e.g. acetic or butyric acids), or 'grassy'. slightly sweet, fruity odours	4
Stale, sour, 'cabbage water', 'turnipy', or phosphine-like odours	3
Ammoniacal (trimethylamine and other lower amines) with strong 0-toluidiine-like odours	2
Hydrogen sulphide, other sulphide and strong ammoniacal odours	1
Nauseating, putrid, faecal odours :indole, ammonia, etc.	0

Cooked fish (approx. 6 - 8 oz. middle cut of fish steamed in casserole in resistance-glass dishes (7 in diameter) over boiling water for 35 minutes.

Odor (10 mark)	
Strong fresh'seaweedy' odours	10
Some loss of fresh 'seaweediness'	9
Lack of odour, or neutral odours	8
Slight strengthening of the odour but no sour or stale Odour,'wood shaving', 'woodsap', vanillin or terpene-like Odours; slight salt-fish or cold storage odours	7
'Condensed milk', caramel or toffee-like odours	6
'Milk jug,'boiled-potato' or 'boiled clothes', or metallicodours	5
Lactic acid, 'sour milk' or o-toluidine-like odours	4
Some lower fatty acid (e.g acetic or butyric acids) 'grassy', 'soapy', 'turnipy', or /taaowy' odours	3
Ammoniacal (trimethylamine and lower amines) odours	2
Strong ammoniacal (trimethylamine etc.) and some sulphide odours	1
Strong putrid and faecal odours (ammonia, indole etc	0

* Taken from Shewan et al. (1953) pp 293 - 294, as abridged by the author.

APPENDIX C

Table 14. Effects of temperature and atmosphere on sensory score* and TBA number*.

Tmt +	Sensory score++	TBA #
C	1.20	0.08
LMW1	1.77	0.12
LMW2	2.27	0.22
LMW3	2.44	0.54
LMW4	1.41	0.31
LM@	1.97	0.30
LAW1	1.91	0.13
LAW2	1.85	0.47
LAW3	2.81	2.32
LAW4	4.08	2.20
LA@	2.66	1.28
HMW1	2.52	0.19
HMW2	2.97	0.30
HMW3	3.05	0.25
HMW3	4.50	0.34
HM@	3.26	0.27
HAW1	3.77	0.17
HAW2	5.12	0.36
HAW3	6.31	0.28
HAW4	6.97	0.32
HA@	5.54	0.28
Standard error	0.155	0.107

* Mean value from 2 replications.

+) Tmt is treatment;
C is control, freshly-killed fish (without storage)
L is low temperature storage (0 C).
H is high temperature storage (5 C).
M is modified atmosphere packaging
A is air atmosphere packaging.
W followed by 1, 2, 3, and 4, is time storage in week.
@ is mean of all samples under that condition.

++)Score 1 is no off odor (the best quality).
Score 7 is strong off odor (the worst quality).

#) mg malonaldehyde/kg sample.

Table 15. Effects of temperature and atmosphere on bacterial count$

Tmt+	APC ***	Psychro **	Anaerob *
C	2.06	2.66	1.45
LMW1	2.07	2.04	1.23
LMW2	1.81	1.65	1.77
LMW3	2.46	2.44	1.99
LMW4	2.89	2.67	2.14
LM@	2.30	2.20	1.78
LAW1	3.25	3.23	2.45
LAW2	5.10	3.06	3.86
LAW3	6.53	6.63	5.98
LAW4	8.45	9.39	6.73
LA@	5.83	5.58	4.75
HMW1	2.69	2.30	1.93
HMW2	4.57	4.59	4.83
HMW3	7.02	6.51	6.89
HMW4	8.81	8.89	8.71
HM@	5.78	5.57	5.59
HAW1	6.51	6.65	6.35
HAW2	8.29	9.40	7.47
HAW3	8.02	9.20	7.37
HAW4	6.75	9.19	6.38
HA@	7.39	8.61	6.89
Std error	0.179	0.364	0.266

$ Mean value from 2 replications.

\+ Tmt is treatment

C is control, freshly-killed fish (without storage)
L is low temperature storage (0 C)
H is high temperature storage (5 C)
M is modified atmosphere packaging
A is air atmosphere packaging.
W is time storage in week.
@ is mean of all samples under that conditions.

*** Aerobic plate count (log CFU).

** Psychrotrophic plate count (log CFU).

* Anaerobic plate count (log CFU).

Table 16. Effect of storage time in MAP on post-MAP sensory score$ and bacterial plate count$.

Tmt+	Sensory++	Psychro *	Anaerobe **	APC ***
M0D0	1.00	1.38	1.75	2.22
M0D1	1.02	1.99	1.30	2.14
M0D2	1.10	2.92	1.96	2.63
M0D3	1.06	6.37	3.68	4.61
M2D0	1.18	1.94	1.53	2.03
M2D1	1.37	2.29	2.29	2.56
M2D2	1.66	3.89	4.17	3.46
M2D3	1.51	5.17	4.06	4.95
M3D0	1.45	2.42	2.08	2.74
M3D1	2.02	3.81	3.58	4.31
M3D2	2.04	5.32	3.89	5.17
M3D3	2.61	6.54	5.34	5.22
Std error	0.108	0.339	0.273	0.311

$ Mean value from 2 replications.

+) Tmt is treatment
M is modified atmosphere packaging
D followed by 0, 1, 2, and 3 is storage time post-MAP in air atmosphere.

++ Score 1 is no off odor;
Score 7 is strong off odor.

*** Aerobic plate count (log CFU).

** Anaerobic plate count (log CFU).

* Psychrotrophic plate count (log CFU).

Table 17. Analysis of variance (ANOVA) for initial carbon dioxide content of MAP containers.

Source of Variation	df	SS	MS	F
Replication	1	1.11	1.108	0.79 ns
Initial CO2	7	9.48	1.354	0.97 ns
Error	7	9.80	1.400	
Total	15	20.39		

Coefficient of variation = 2.09 %

Table 18. ANOVA for initial oxygen content of MAP containers.

Source of Variation	df	SS	MS	F
Replication	1	0.04	0.037	3.58 ns
Initial O2	7	0.14	0.021	2.00 ns
Error	7	0.07	0.010	
Total	15	0.25		

Coefficient of Variation = 5.90 %

ns is not significantly different at a level of $P < 0.05$

df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

Table 19. ANOVA for final carbon dioxide content of MAP containers.

Source of Variation	df	SS	MS	F
Replication	1	1.25	1.254	1.90 ns
Finaal CO2	7	14.08	2.011	3.05 ns
Error	7	4.61	0.659	
Total	15	19.95		

Coefficient of Variation = 1.57 %

Table 20. ANOVA for final oxygen content of MAP containers.

Source of Variation	df	SS	MS	F
Replication	1	0.00	0.002	0.04 ns
Final O2	7	0.37	0.052	0.98 ns
Error	7	0.37	0.053	
Total	15	0.74		

Coefficient of Variation = 37.35 %

ns is not significantly different at a level of $P < 0.05$

df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

Table 21. ANOVA for initial carbon dioxide content of MAP containers from which fish were removed for post-MAP storage in air.

Source of Variation	df	SS	MS	F
Replication	1	7.99	7.995	4.21 ns
Initial CO2	7	1.84	0.264	0.14 ns
Error	7	13.28	1.897	
Total	15	23.12		

Coefficient of Variation = 2.49 %

Table 22. ANOVA for initial oxygen content of MAP containers from which fish were removed for post-MAP storage in air.

Source of Variation	df	SS	MS	F
Replication	1	0.06	0.061	9.32 *
Initial O2	7	0.08	0.011	1.70 ns
Error	7	0.05	0.007	
Total	15	0.19		

Coefficient of Variation = 4.56 %

ns is not significantly different at a level of $P < 0.05$

* is significantly different at a level of $P < 0.05$

df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

Table 23. ANOVA for final carbon dioxide content of MAP containers from which fish were removed for post-MAP storage in air.

Source of Variation	df	SS	MS	F
Replication	1	3.60	3.601	1.22 ns
Final CO2	7	1.84	0.263	0.09 ns
Error	7	20.64	2.949	
Total	15	26.08		

Coefficient of Variation = 3.37 %

Table 24. ANOVA for final oxygen content of MAP containers from which fish were removed for post-MAP storage in air.

Source of Variation	df	SS	MS	F
Replication	1	0.00	0.005	0.66 ns
Final O2	7	0.09	0.012	1.67 ns
Error	7	0.05	0.007	
Total	15	0.14		

Coefficient of Variation = 11.67 %

ns is not significantly different at a level of $P < 0.05$

df is degree of freedom.

SS is sum of squares

MS is mean square.

F is calculated F value.

BIBLIOGRAPHY

BIBLIOGRAPHY

Amalacher, E. 1961. Rigor mortis in fish,. in " Fish as Food Vol. 1 : Production, Biochemistry and Microbiology", ed. Brogstrom, G. 1961. Academic Press, New York and London.

Amerine, M.A., Pangborn, R.M., and Roessler, E.B., 1965. Principle of sensory evaluation of food. Academic Press. New York and London.

Anonymous, 1974. Microorganism in foods, sampling for microbiological analysis : Principle and specific application; International Commission on Microbiological Societies, Univ. of Toronto Press, Toronto, Canada.

Azam, K., Mackie, I.M., and Smith, J. 1989. The effects of slaughter methods on the quality of rainbow trout (Salmo gairdneri) during storage on ice. Int. J. Food Sci. and Technol., 24: 69 - 79.

Baig, I.A. and Hopton, J.W. 1969. Psychrophilic properties and the temperature characteristics of growth of bacteria. J. Bacteriol. 100: 552 -553.

Baker, D.A. and Genigeorgis, C. 1990. Predicting the safe storage of fresh fish under modified atmosphere with respect to Clostridium botulinum toxigenesis by modeling length of the log phase of growth. J. Food Protec. 53(2) 131 - 140.

Banks, H., Nickelson II, R., and Finne, G. 1980. Shelf-life studies on carbon dioxide packaged finfish from the Gulf of Mexico. J. Food Sci. 45: 157-162.

Barile, L.E., Milla, A.D., Relly, A., and Villodsen, A. 1985. Spoilage patterns of mackerel (Rastrelliger faughni Matsui). 1. Delay in icing. Asean Food J. 1(2): 70.

Barile, L.E., Milla, A.D., Relly, A., and Villodsen, A. 1985. Spoilage patterns of mackerel (Rastrelliger faughni Matsui). 2. Mesophilic and psychrophilic spoilage. Asean Food J. 1(3).

Barnett, H.J., Nelson, R.W., Hanter, P.J., and Groningen, H., 1971. Studies on the use of carbon dioxide in refrigerated box for preservation of whole fish. Fish, Bull. 69: 433 -441.

Barnett, H.J., Nelson, R.W., Hanter, P.J., and Groningen, H., 1978. Use of carbon dioxide dissolved in refrigerated brine for preservation of pink shrimp (Pandalus spp.) Mar., Fish. Res. 40: 24 - 28.

Becker, Z.E. 1933. A comparison between the action of carbonic acid and other acids upon the living cell. Protoplasma, 25 : 161 - 175.

Bendal, J.R., 1969. Muscles, molecules and movement, and essay in the contraction of muscle. American Elsevier, New York.

Benedict, B.C. Strange. E.D.. Palumbo, S., and Clifton, E.S., 1975. Use of packaging controlled atmosphere for extending the shelf-life of meat products. Food Chem., 23 : 1208 - 1211.

Bisset, K.A. 1948. Seasonal changes in the normal bacterial flora of freshwater fish. J. Hyg. 46: 94 - 97.

Brody, A.L., 1989. Controlled/ modified atmosphere/ vacuum packaging of foods. Food and Nutrition press. Inc. Trumbul, Conecticut, 06611, USA.

Brown, E.E. and Gratzek, J.B. 1981. Fish farming handbook. Avi Publishing Comp. Inc., Conneticut.

Brown, W.D., Albright, M., Watts, D.A., Heyer, B., Spurce, B., and Price, R.J. 1980. Modified atmosphere storage of rockfish (Sebastes miniatus) and silver salmon (oncorhynchus kisutch). J. Food Sci. 45: 93-96.

Burt, J.R., Gibson, D.M., Jason, A.C., and Sanders, H.R. 1975. Comparison of methods of freshness assessment of wet fish. Part I, Sensory assessment of boxed experimental fish. J. Fd. Technol. 10 : 645 - 656

Busta, F.F., Peterson, E.H., Adam, D.M., and Johnson, M.G. 1984. Colony Count Methods, in, Speck, M.L. 1984. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.

Cann, D. 1985. Developments in the modified atmosphere packaging of fish. Institute of Food Sci. Technol. (UK), Juni : 15 - 16.

Cann, D.C., Smith, G.L., and Houston, N.C. 1983. Further studies on marine fish stored under modified atmosphere packaging. Aberdeen, Torry Research Station.

Callow, E.H., 1932. Gas storage of pork and bacon. Part I. Preliminary experiment. J. Soc. Chem. Ind. (London) 51: 116T - 119T.

Castell, C.H. 1971. Some fundamental problems in the quality assessment of fishery products. In "Fish Inspection and Quality Control". Fishing News (Books) Limited, London, England.

Castell, C.H. and Anderson, G.W. 1948. Bacteria associated with spoilage of cod fillets. J. Fish. Res. Bd. Can. 7 (6): 370

Castell, C.H., and Mapplebeck, E.G.. 1952. The important of Flavobacterium in fish spoilage. J. Fish. Res. Bd. Can. 9 (3):148

Chow, C.J., Ociai, Y., Watabe, S., and Hashimoto, K. 1987. Autoxidation of bluefin tuna myoglobin associated with freezing and thawing. J. Food Sci. 52 (3): 589-591.

Chung, J. 1968. Post mortem degradation of fish muscle : The role of proteolytic Pseudomonas spp; Their mechanism action, Ph.D Thesis, Univ. of Washington, Seatle, WA.

Coyne, F.P. 1932. The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish, Part 1. J. Soc. Chem. Ind. 51: 119

Coyne, F.P. 1933. The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish, Part 2: Gas storage of fresh fish. J. Soc. Chem. Ind. 52: 19T.

Clark, D.S. and Lenz, C.P. 1972. Use of carbon dioxide for extending shelf life of prepackaged beef. Can. Inst. Food Sci. Technol. 5: 175.

Crackel, R.L. 1986. Stability of lipid in restructured beef steaks, Masters thesis, Dept. of Food Science and Human Nutrition, Michigan State University.

Daniels, J.A., Krishnamurthi, R., and Rizvi, S.H. 1985. A review of effects of carbon dioxide on microbial growth and food quality. J. Food Sci. 48: 532-537.

Danwood, A.A., Roy, R.N., and Williams, C.S. 1986. Effects of delayed icing on the storage life of rainbow trout. J. Food Technol, 21: 159 - 166.

Downes, T.W., and Giacin, J.R. 1990. Permeability and shelf life, Course Notes PKG-820. School of Packaging, Michigan State University.

Dugan, L. 1976. Lipids . In "Principle of Food Science: Part I, Food Chemistry". Marcel Dekker, Inc., New York and Basel. p. 178

Dyer, W.J., 1952. Amine in fish muscle I: Colorimetric determination of trimethylamine as the pictrate salt. J. Fish. Res. Bd. Can. 6: 351 - 358.

Eklund, M.W. 1982. Effect of carbon dioxide modified atmospheres and vacuum packaging on Clostridium botulinum and spoilage organism of fishery products. In proceeding of the First National Conference on Seafood Packaging and Shipping (R. Martin, ed.) p. 298 - 331, National Fishery Institute, Seaattle, WA.

Eklund, T., and T. Jarmund. 1983. Microculture model studies on the effect of various gas atmosphere on microbial growth at different temperatures. J. Appl. Bacteriol. 55:119-125

Elden, S.R. 1938. The effect of carbon dioxide on the production of succinic acid by Bact. Coli Commune. Biochem. J. 32: 187 - 193.

Enfors, S.O., and Molin, G. 1980. The influence of temperature on the growth inhibitory effect of carbon dioxide on Pseudomonas fragi and Bacillus cereus. Can. J. Microbiol., 27: 15.

Fanestil, D.D., Hasting, A.B., and Mohowald, 1963. Environmental carbon dioxide stimulation of mitochondrial adenosine phosphate activity. J. Biol. Chem., 238: 836 - 842.

FAO. 1973. Code of practice for fresh fish, FAO fisheries Circ. C318. Food and Agric. Org. of the United Nations, Rome, Italy.

Farber, L. 1965. Freshness test, in Fish as Food Vol. IV, Processing, Part 2, ed. Borgstorm, G. 1965. Academic Press, New York, London.

Fey, M.S., and Regenstein, J.M. 1982. Extending shelf-life of fresh wet red hake and salmon using carbon dioxide-oxigen modified atmosphere and potassium sorbate ice at 1 C. J. Food Sci. Vol.47: 1048 - 1054.

Finne, G. 1981. The effects of carbon dioxide on the bacteriological and chemical properties of foods : a review. In Proceedings of the first national conference on modified and controlled atmosphere packaging of seafood products, Ed. Martin, R.E., San Antonio, Texas. p. 2 - 25.

Finne, G. 1982. Modified and controlled-atmosphere storage of muscle foods. Food Technol. (February): 128-133

Foster, J.W., and Davis, J.B., 1949. Carbon dioxide inhibition of anaerobic fumarate formation in molds Rhizopus nigricans. Arch. Biochem. 21: 135 - 142.

Fukuda, H. 1955. Application of anti oxidant to shikokora. Bull. Jap. Soc. Scient. Fish. 21 (8): 934 - 936.

Frankel, H.R. 1882. As cited in : Killeffer, D.H., 1930. Carbon dioxide preservation of meat and fish. Ind. Eng. Chem. 22 : 140 - 143.

Gee, A.H. 1930. Bacteria; concerned in the spoilage of haddock, III: Further observation on the flora of live fish. Contribs. Can. Biol. and Fisheries (N.S) 5: 433 - 439.

Georgala, D.L. 1958. The bacterial flora of the skin of North Sea Cod. J. Gen. Microbiol., 18: 84 - 91.

Gibson, D.M. 1985. Predicting the shelf life of packaged fish from conductance measurement. J. Appl. Bacteriol. 58: 465 - 470.

Gibson, D.M., Ogden, I.D., and Hobbs. G. 1984. Estimation of the bacteriological quality of fish by automated condectance measurements, Int. J. Fd. Microbiol. : 127 - 134

Gill, C.O., and Newton, K.G. 1977. The development of aerobic spoilage flora on meat stored at chilled temperatures. J. Appl. Bact., 43: 189 - 195.

Gill, C.O. and Tan, K.H. 1979. Effect of carbon dioxide on the growth of Pseudomonas fluorescens. Appl. Environ. Microbial. 38: 237 - 240.

Gilliland, S.E., Michener, H.D., and Kraft, A.A. 1984. Psychrotrophic microorganisms, in Speck, M.L. 1984. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.

Gorga, C., and Ronsivalli. L.J. 1988. Quality assurance of seafood. An AVI Book Publishing by Van Nostrand Reinhold. New York.

Gram, L., Trolle, C., and Huss, H.H. 1987. Detection of specific spoilage bacteria from fish stored at low (0 C) and high (20 C) temperatures. Int. J. Food Microbiol., 4: 65 - 72.

Gray, J.I. 1978. Measurement of lipid oxidation. J. Am. Oil Chem. Soc. 55 : 39.

Gray, R.J.H., Hoover, D.G., and Muir, A.M. 1983. Attenuation of microbial growth on modified atmosphere-packaged fish. J. Food Protection, Vol 46(7): 610 - 613.

Groninger, H.S. 1959. The occurrence and significance of trimethylamine oxide in marine animals. U.S. Fish and Wild. Serv. Spec. Sci. Rep. Fish, 333.

Greene, B.E. and Cumuzu, T.H. 1981. Relationship between the TBA numbers and inexperienced panelists assessment of oxidized flavor in cooked beef. J. Food Sci. 47 : 52.

Guise, B. 1983. Controlled atmosphere packaging. Food Process. April: 29.

Haines, R.B. 1933. The influence of carbon dioxide on the rate of multiplication of certain bacteria, as judged by viable counts. J. Soc. Chem. Ind. 52: 13T.

Hansen, P. 1963. Fat oxidation and storage life of iced trout, Part I; Influence of gutting . J. Sci. Fd. Agric. 14: 781 - 786.

Hansen, P. 1972. Storage life of prepacked wet fish at 0 C II; Trout and herring. J. Food Technol. 7: 21 - 26.

Hanus, F.J. and Morita, R.Y. 1968. Significance of the temperature characteristic of bacterial growth. J. Bacteriol. 95 (2): 736 -737.

Harder, W. and Veldkamp, H. 1971. Competition of marine psychrophilic bacteria at low temperatures. Antonie Van Leeuwenhoek. 37 : 51.

Hardy, R. 1980. Fish Lipids, part II, In "Advances in Fish Science and Technology", (J. Connel. ed) p. 138 - 157, Fishing News Books Ltd, Farnhan, Surrey, England.

Harper, R. 1987. A guide to sensory analysis and its development. Food Sci. and Technol. Today; Vol 1(2): 73 - 76.

Hebard, C.E., Flick, G.J., and Martin, R.R. 1982. Occurrence and significance of trimethylamine oxide and its derivates in fish and shellfish. In "Chemistry and Biochemistry of Marine Good Products, p.: 149 - 272. ed. Martin, R.E., Flick, G.J., Hebard, C.E., and Ward, D.R. Avi Publishing Co., Wesport, CT.

Herbert, R.A., Hendrie, M.S., Gilson, D.M., and Shewan, J.M. 1971. Bacteria active in the spoilage of seafood. J. Appl. Bacteriol., 34 :41 - 38.

Herbert, R.A., and Shewan, J.M. 1976. Roles played by bacteria and autolytic enzymes in the production of volatile sulphide in spoiling Noth Sea Cod (Gadus morhua). J. Sci. Food Agric. 27: 89-94.

Hintlian, C.B., and Hotckiss, J.H. 1986. The safety of modified atmosphere packaging : A review. Food Technol. Desb.: 70-76.

Hintlian, C.B., and Hotchkiss, J.H. 1987. Comparative growth of spoilage and pathogenic organisms on modified atmosphere-packaged cooked beef. J. Food Protect. 50 (3): 218-223.

Hobbs, G. 1976. Clostridium botulinum and its importance in fishery products. In, Advance in Food Res., 22: 135 - 185.

Hobbs, G. 1982. Changes in fish after catching. In Fish Handling and Processing second ed. p.20 - 27. (A. Aitkin, et.al. eds) Torry Station, Edinburg.

Huffman, D.L. 1974. Effects of gas atmospheres on microbial quality of pork. J. Food. Sci., 39: 723.

Huss, H.H. 1972. Storage life of prepacked wet fish at 0 C. J. Fd. Technol. 7: 13 - 19.

Huss, H.H., Dalsgaard, D., Hansen, L., Ladefoged, H., Peterson, A., and Zitton. 1974. The influence of hygiene in catch handling on the storage life of iced cod and plaice. J. Food Technol. 9: 213.

Ingraham, J.L. 1958. Growth of Psychrophilic bacteria. J. Bacteriol. 76: 75 - 80.

Insalata, N.F. 1952. Carbon dioxide versus beverage bacteria. Food Eng. 24 : 84-85, 190.

James, D.G. 1986. The prospects for fish for the undernourished. Food and Nutrition (FAO), 12: 20 - 27.

Jaquat, 1961. Organic constituents of fish and other aquatic animal foods, in " Fish as Food Vol. 1 : Production, Biochemistry and Microbiology", ed. Borgstrom, G. 1961. Academic Press, New York and London.

Johannsen, A. 1965. Public health aspects of prepackage fish for retail. p. 271 - 278, In "Fish Handling and Preservation". OECD, Paris.

Kadar, A.A. 1980. Preservation of ripening in fruits by controlled atmospheres . Food Technol. 34 : 51 - 54.

Karel, M. 1975. Protective packaging of foods, in "Principle of Food Science, Part II: Physical principles of food preservation " ed. by Fennema, O.R. M.Dekker Inc. pp: 422 -466.

Killefer, D.H. 1930. Carbon dioxide preservation of meat and fish. Ind. Eng. Chem. (22): 140.

King, A.D., and Nagel, C.W. 1967. Growth inhibition of a Pseudomonas by carbon dioxide. J. Food Sci. (40): 362 - 366.

Klein, T. and Knorr, D. 1990. Oxygen absorption properties of powdered iron. J. Food Sci., 55(3): 869 - 870.

Knocche, W. 1980. Chemical reaction of CO2 in water. In "Biophysiology of carbon dioxide", ed. Bauer, C. Gros, G, and Bartel, H. P. 3; Spring- Verlag, New York.

Kolbe, H. 1882. As cited in :Killeffer, D.H., 1930. Carbon dioxide preservation of meat and fish. Ind. Eng. Chem.22: 140-143.

Koski, D. 1984. Cost and benefit of contolled atmosphere packaging. Presented at controlled atmosphere packaging conference, Univ. of Del., March :25 - 26.

Laird, L.M., and Needham, T. 1988. Salmon and trout farming. Ellis Harwood Limited, Chichester.

Lannelongue, M., Finne, G., Hanna, M.O., Nickelson, R., and Vanderzant, C. 1982a. Microbiological and chemical changes during storage of swordfish (Xiphias gladius) steaks in retail packages containing CO2- enriched atmospheres. J. Food Protection. 45 (13): 1197-1203.

Lannelongue, M., Finne, G., Hanna, M.O., Nickelson, R., and Vanderzant, G. 1982b. Storage characteristics of brown shrimp (Penaeus aztecus) stored in retail packages containing CO2-enriched atmospheres. J. Food Sci. 47: 911-913.

Laycock, R.A., and Legier, L.Q. 1970. Pseudomonas and Achromobacter in the spoilage of irradiated haddock of different preirradiation quality. Appl. Microbiol. 20: 333-334.

Lee, J.S. 1973. Commercial Catfish Farming. The Interstate Printer and Publishers, Inc. Danville, Illinois.

Lee, J.S., Willet, C.L., Robinson, S.M., and Sinnhuber, R.O. 1967. Comparative effects of chlortetracycline, freezing and gamma irradiation on microbial population of ocean perch. Appl. Microbiol. 15:368-372.

Lerke, P., Adam, R., and Forber, R. 1965. Bacteriology of spoilage of fish muscle. Appl. Microbiol. 13(4): 625.

Licciardello, J.J., Nickerson, J.T.R. Rablich, C.A., and Goldblith. 1967. Thermal inactivation of type E botulinum toxin. Appl. Microbiol. (15): 249 - 256.

Lindsay, R. 1981. Modified atmosphere packaging systems for refrigerated fresh fish providing shelf-life extension and safety from Cl. botulinum toxigenesis. In Proceedings of the first national conference on modified and controlled atmospere packaging of seafood products, Ed. Martin, R.E., San Antonio, Texas.

Lindsay, R.C., Josephson, D.B., and Olafsdottir, G. 1986. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmosphere. in "Seafood Quality Determination, Proceedings of an International Symposium"., Coordinated by the University of Alaska Sea Grant Collage Pr ogram, D.E. Kramer and J. Liston (eds). pp 221-234.

Lioutas, T.S. 1988. Challenges of controlled and modified atmosphere packaging : A food company's perspective. Food Technol. Sept.: 78 - 86.

Liston, J. 1980. Microbiology in fishery sciences. In "Advances in Fish Science and Technology", (J. Connel. ed) p. 138 - 157, Fishing News Books Ltd, Farnhan, Surrey, England.

Liston, J. 1982. Recent advances in the chemistry of iced fish spoilage. In Chemistry and Biochemistry of Marine Food Products, (Martin, R.,, Flick, G., Hebard, C.E., and Ward, D.R., Eds) p. 27 - 36. AVI Publishing Co., Westport, CT.

Liston, J., Stansby, M.E., and Olcott, H.S. 1963. Bacteriology and chemical basis for deteriorative changes. In "Industries Fishery Technology." Robert, E. Krieger Publishing Co. Huntington, New York, p.345.

Love, R.M. 1988. The food fishes, their intrinsic variation and practical implications. Farrand Press, London, an AVI book, Published by Van Nostrand Reinhold Company, New York.

Maclean, D.P., and Welander, C. 1960. The Preservation of fish with ionizing radiation : Bacterial Studies. Food Technol. May: 251-255.

Martin, R.E., Gray, J.H., and Pierson, M.D. 1978. Quality assessment of fresh fish and the role of the naturally occurring microflora. Food Technol. Vol. 32(5): 188.

McGill, A.s., 1976. As cited in Hardy, R. 1980. Fish Lipids, part II, In "Advances in Fish Science and Technology", (J. Connel. ed) p. 138 - 157, Fishing News Books Ltd, Farnhan, Surrey, England.

Meilgaard, M., Civille, G.V., and Garr, B.T. 1987. Sensory Evaluation technique, Vol. I. CRC press, Inc. Boca Raton, Fl.

Meilgaard, M., Civille, G.V., and Garr, B.T. 1987. Sensory Evaluation technique, Vol. II. CRC press, Inc. Boca Roton, Fl.

Melton, S.L. 1983. Methodology for following lipid oxidation in muscle foods. Food Technol. 37(7): 105.

Miller, A.III., Scanlan, R.A., lee. J.S., and Libbey. L.M. 1973. Volatile Compounds produced in sterile fish muscle (Sebastes melonops) by Pseudomonas putrefaciens, Pseudomonas fluorescens, and an Achromobacter species. Appl. Microbiol, 26: 18 - 21.

Mitsuda, H., Nakajima, K., Mizuno, H., and Kawai, F. 1980. Use of sodium chloride solution and carbon dioxide for extending shelf-life of fish fillets. J. Food. Sci. (45): 661-666.

Murray, C.K., Gibson, D.M., and Shewan, J.M. 1971. Quality control aspects of prepacked fresh and smoked fish, In "Fish Inspection and Quality Control" ed. Kreuzer, R. pp:66 - 70.. Fishing News (Books) Ltd., London.

Nakamura, H. and Hoskino, J. undated. Techniques for preservation of food by employment of an oxygen absorber. Mitsubishi Gas Chemical Company, Inc. Tokyo Plant, Ageless Division.

Neil, C.W. 1977. As cited by Favrie, M.L. Storage characteristics of fresh fish packed in modified gas atmosphere containing carbon dioxide. Masters Thesis, Texas A & M University.

Nickelson II, R. and Finne, G. 1984. Fish, crustaceans, and precooked seafoods, in Speck, M.L. 1984. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C. p: 62 - 83.

Novikov, V.M. 1983. Hand Book of Fishery Technology Vol. 1. p.64., A.A. Balena, Rotterdam.

Ogilvy, W.S., and Ayres, J.C. 1951. Post mortem changes in meat II. The effects of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. Food Technol. 5: 97 - 102.

Ogilvie, J.M.G. and Warren, A.A. 1957. The occurrence of trimethylamine oxide in Fundulus heteroclitus. Can. J. Zool. 35: 735 - 745.

Ogrydziak,D.M., and Brown , W.D. 1982. Temperature effects in modified atmosphere storage of sea food. Food Technol. May: 86 - 88, 94 -91, 90 - 91.

Olley, J., and Ratkowsky, D.A. 1973. The role of temperature function integration in monitoring of fish spoilage. Food Technol. N.Z. 8: 13, 15, 17.

Olsen, R.H. and Jezeski, J.J. 1963. Some effects of carbon source, aeration, and temperature on growth of a psychrophilic strain of Pseudomonas fluorescens. J. Bacteriol. 86: 429 - 433.

Owen, D. and Nesbit, 1984. A versatile time temperature function integrator . Laboratory Pactice, 33: 70 - 75.

Parkin, K.L., and Brown, W.D. 1983. Modified atmosphere storage of Dungeness crab (Cancer magister). J. Food Sci. (48): 370 - 374

Parkin, K.L., Wells, W.J., and Brown, W.D. 1982. Modified atmosphere storage of rosk fish fillets. J. Food Sci. (47): 47 - 181.

Pedrosa-Menabrito, A., and Regenstein, J.M. 1988. Shelf-life extension of fresh fish : A review, Part I - Spoilage of fish. J. Food Quality, 11: 117 - 127.

Petersen, A., Jensen, M.H., Roge, E.H., and Jepsen, A. 1980. Storage of chilled cod under vacuum, and at various concentrations of carbon dioxide, In, Advances in Fish Science and Technology; ed. Connell, J.J. Fishing News Book Ltd.; Farnham, Surrey, England.

Post, L.S., Lee, D.A., Solberg, M., Furgang, D., Specchio, J., and Graham, C. 1985. Development of botulinal toxin and sensory deterioration during storage of vacuum and modified atmosphere packaged fish fillets. J. Food Sci. 50: 990 - 996.

Poulter, N.H. and Nicoloides, L. 1985. Studies of the iced storage characteristics and composition of a variety of Bolivian freshwater fish. 1. Altiplano fish. J. Food Technol. 20: 437 - 449.

Pryor, W.A., Stanley, J.P., and Blair, E. 1976. Autoxidation of polyunsaturated fatty acids. II. A suggested mechanism for the formation of TBA-reactive materials from prostagladine-like endoperoxides. Lipids. 11: 370.

Ratkowsky, D.A., Olley, J., McMeekin, T.A., and Ball, A. 1982. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149: 1 - 5.

Ravesi, E.M., Licciardello, J.J., Tuhkunen, B.E., and Lunstrom, R.C. 1985. The effect of handling or processing treatment on storage characteristics of fresh spiny dogfish, Squalus acantkias. Mar. Fish. Rev. 47 (1): 48.

Regenstain, J.M. 1983. What is fish quality ? INFOFISH Marketing Digest, 6 : 26- 28.

Reppond, K.D., and Collins, J. 1983. Pacific cod (Gadus macrocephalus): Changes in sensory and chemical properties when held in ice and in carbon dioxide modified refrigerated seawater. J. Food Sci. 48: 1152 - 1154.

Ruiter, A. 1971. Trimethylamine and the quality of fish. Voedingmiddelen Technol. 2 (43) : 1 - 10.

Sagarin, E. 1954. Odor : A proposal for some basic definitions. ASTM Spec. Tech. Publ> 164 : 3 - 8.

Samuel, R.O, DeFeO, A., Flick, G.J., Ward, D.R., Rippen, T., Riggins, J.K., Coale, C., and Smith, C. 1984. Demonstration of a quality maintenance program for fresh fish products, A report sumitted to Mid-Atlantic Fishery Development Foundation, Virginia Polytecnic, Int. and State Univ., Sea Grant, VPI-SG-84-04R, Blacksburg.

Schmidt. C.F., Lechowich, R.V., and Folinazzo, J.F. 1961. Growth and toxin production by type E Clostridium botulinum below 40 F. J. Food Sci. 26: 626 - 630.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada, Ottawa. p. 184 - 191.

Sears, D.F., and Eisenberg. R.M. 1961. A model representing a physiological role of carbon dioxide at the cell membrane. J. Gen. Physiol. 44: 869 - 887.

Sedgwick, S.D. 1978. Trout farming hand book. Seely Service & Co, London.

Sedgwick, S.D. 1982. The salmon hand book : the life and cultivation of fishes salmon family; Streat, London. WCI.

Seiler, D.A.L. 1985. Controlled atmosphere shelf-life extension of bakery products. Baking Ind. Feb., 152.

Shaw, M.K. 1967. Effect of abrupt temperature shift on the growth of mesophilic and psychrophilic yeasts. J. Bacteriol. 93(4): 1332 - 1336.

Shewan, J.M. 1949. Some bacteriological aspects of handling, processing, and distribution of fish. J. Roy. Sanit. Inst. 59 : 394 - 421.

Shewan, J.M., Ruth, G.M., Tucker, C.G., and Ehrenberg, A.S.C. 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J. Sci. Food Agric., 4: 283 -298.

Shewan, J.M. 1950. Improving the quality of "white" fish by use of gas storage. Fish News No: 1946, p. 14.

Shewan, J.M. 1961. The microbiology of seawater fish. In "Fish as Food", ed. G. Borgstrom, 1961, p. 487; Academic Press, New York.

Shewan, J.M. 1971. The microbiology of fish and fishery products - a progress report. J. Appl. Bacteriol. 34: 299 - 315.

Sikorski, Z.E. 1990. Chilled Storage of fresh fish, in "Seafood Resources, Nutritional Composition, and Preservation", ed. Sikorsky, Z.E. CRC Press Inc., Boca Raton, Florida. p. 93 - 109.

Sikorski, Z.E., Kolokowwska, A., and Burt, J.R. 1990. Postharvest biochemical and microbial changes, in "Seafood Resources, Nutritional Composition, and Preservation", ed. Sikorsky, Z.E. CRC Press Inc., Boca Raton, Florida.

Simmonds, C.K., and Lamprecht, E.C. 1980. Correlation of microbiological status with organoleptic quality of chilled cape hake; In, Advances in Fish Science and Technology; ed. Connell, J.J. Fishing News Book Ltd.; Farnham, Surrey, England. pp 297 - 299.

Singh, B., Yong, C.C., and Salunkle, D.K. 1972. Controlled atmosphere storage of lecttuce heads. J. Food. Sci. 37:48 - 51.

Sinnhuber, R.O., Yu, T.C., and Yu, T.C. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Res. 23: 626.

Smith, W.H. 1963. The use of carbon dioxide in transport and storage of fruits and vegetables. p. 96 - 99. In "Advance in Food Research", Vol. 12. Academic Press.

Smith, J.G.M., Hardy, R., and Young, K. 1972. Autoxidation in very unsaturated fats. Proc.: SAAFOST Symp., Food and their fats., Pretoria, October: 79 - 105.

Smith, G.C., Seideman,S.C., Sewell, J.W., Dill, C.W., and Vanderzant, C. 1983. Vacuum packaging versus modified atmosphere packaging of lamb loin. J. Food. Prot. 46 : Jan.

Smith, G.R. and Stearley, R.F. 1989. The classification and scientific names of rainbow and cutthroat trouts. Fisheries 14: 4 - 10.

Speck, M.L. 1984. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.

Spencer, R. and Baines, C.R. 1964. The effect of temperature on the spoilage of wet white fish. Food Technol. 18: 769.

Standby, M.E. and Griffiths, F.P., 1935. Carbon dioxide in handling fresh fish (Haddock). Ind. Eng. Chem. 27:1452.

Standby, M.E. 1976. Industrial Fishery Tecnology : A survey of methods for domestic harvesting, preservation, and processing of fish used for food and for industrial products. Robert E. Krieger Publishing Company, Huntington, New York.

Statham, J.A., Bremmer, H.A., and Quarmby, A.R. 1985. Storage of morwong (Nemadactylus macropterus Black and Schneider) in combination of polyphosphate, potassium sorbate and carbon dioxide at 4 C. J. Food Sci. 50: 1580.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistic, second edition. McGaw-Hill Book Company, New York.

Stenstrom, I.M. 1985. Microbial flora of cod fillets (Gadus morhua) stored at 2 C in different mixtures of carbon dioxide and nitrogen/oxygen. J. Food Protec. 48(7): 585 - 589.

Stevenson, J.P. 1987. Trout farming manual second edition, Fishing News Books Limited, Farnham, Surrey, England. p. 143 - 164.

Stone, J. and Sidel, J. 1985. Sensory evaluation practice. Academic Press, Inc. Orlando, Fl.

Storey, R.M. and Owen, D. 1985. As cited in Gibson, D.M. 1985. Predicting the shelf life of packaged fish from conductance measurement. J. Appl. Bacteriol. 58: 465 - 470.

Tarr, H.L.A. 1954. Microbiological deterioration of fish post mortem; its detection and control; Bacteriol. Rev. 18 : 1 - 15.

Tarladgis, B.G., Watts, B.M., Younathan, M.T., and Dugan, L.R.Jr. 1960. A distillation method for quantitative determination of malonaldehyde in rancid odor. J. Am.Oil Chem. Soc. 37: 44.

Tarladgis, B.G., Pearson, A.M., and Dugan, L.R. Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in food II: Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric. 15: 602.

Taylor, A.A., and McDougall, D.B. 1973. Fresh beef packed in mixture of oxygen and carbon dioxide. J. Food Technol. 8:453.

Thomson, J.E., and Risse, L.A. 1971. Dry ice in various shipping boxes for chilled poultry : Effects on microbiological and organoleptic quality. J. Food Sci. 36: 74 - 77.

Toyama, K. 1956. Protection of marine products from deterioration due to oxidation of the oil. Bull. Jap. Soc. Sci. Fish, 22 (6): 383 - 385.

Van Veen, A.G. 1965. Fermented and dried sea-food products in South East Asia. In "Fish as Food". Vol. 3, ed. G. Borgstrom, Academic Press, N.Y.; p. 107 - 131.

Verant, J.F., and Robe, K. 1979. CO2- enriched atmosphere keeps fish fresh more than twice as long. Food Process. 40: 76.

Venugopal, V. 1990. Extracellular proteases of contaminant bacteria in fish spoilage : A review. J. Food Protec. Vol. 53 (4): 341 - 350.

Villareal, B.P. and Howgate, P. 1987. Spoilage of European hake (Merluccius merluccius) in ice. J. Sci. Food Agric., 41: 335 - 350.

Villemure, G., Simard, R.E., and Picard, G. 1986. Bulk storage of cod fillets and gutted cod (Gadus morhua) under carbon dioxide atmosphere. J. Food Sci. 51 (2): 317-320.

Vyncke, W. 1975. Evaluation of the direct thiobarbituric acid: extraction method for determining oxidative rancidity in mackerel (Scomber scombrus L); Fette, Seifen, Anstrichmittel 77: 329.

Wang, M.Y., and W.D. Brown. 1983. Effect of elevated CO2 atmosphere on storage of freshwater crayfish (Pacifastacus leniusculus). J. Food Sci. 48:158-162

Ward, D.R. and Baj. N.J. 1988. Microbiology of marine food products. Food Technol. March, p. 85 - 89.

Willer, W.B. 1981. Trout Biology an angler's guide. The University of Wisconsin Press.

Withelm, K.A. 1982. Extended fresh storage of fishery products with modified atmospheres. A survey, Mar. Fish. Rev. 44: 17 - 20.

Wolfe, S.K. 1980. Use of CO and CO2-enriched atmosphere for meats, fish, and produce. Food Technol. 34(3): 55-58.

Woyewoda, A.D., Bligh, E.G., and Shaw, S.J. 1984. Controlled and modified atmosphere storage of cod fillets. Can. Inst. Food Sci. Tecnol. J. 17: 24

Yamada, K. 1967. Occurrence and origin of trimethyl amine oxide in fishes and marine invertebrates. Bull. Jpn. Soc. Sci. Fish, 33: 591 - 603.