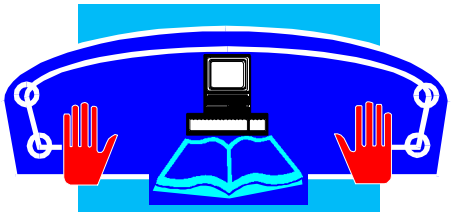

Feasibility Of Modified Atmosphere Packaging Of Fish; A Review
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EXECUTIVE SUMMARY

Modified atmosphere packaging of fish can be very beneficial. It can **extend the shelf** life of the fish, allow greater access to the retail market, reduce shipping costs, decrease problems due to ice melting, prevent cross contamination and give a better quality product. Problems and disadvantages of this technology can include increased costs, **need for a consistent supply of good quality fish, necessity** for choosing the correct mixture of gases, leaking packages, unusual odor upon opening the packages, and need for good control over the storage temperature to keep it at or below 2°C.

The biggest disadvantage is that if this storage temperature is not constantly maintained nonproteolytic types of Clostridium botulinum can grow and produce toxin. Both freshwater and marine fish are contaminated with C. botulinum. Modified atmosphere packaging inhibits the growth o the normal spoilage microorganisms which produce most of the signs of spoilage. Microorganisms which are not inhibited do not grow as quick y and produce less obvious signs of spoilage. As nonproteolytic types of C. botulinum do not produce off-odors, fresh fish can be toxic even though it appears safe to eat. Therefore, until further developments are made vacuum packaging and modified atmosphere packaging of fresh fish should only be allowed where it is possible to have complete control over the storage temperature so that it does not exceed 2°C for any appreciable length of time.

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PART 1. BASICS OF MODIFIED ATMOSPHERE PACKAGING OF FISH

I. INTRODUCTION

The effect of carbon dioxide in extending the shelf life of meat , was known as early as 1882 (Genigeorgis, 1985). In 1932 and 1933, Coyne published articles on the effects of the controlled atmosphere packaging of fish. By 1938, Australia and New Zealand were shipping 26% and 60% respectively of their beef carcasses under a carbon dioxide atmosphere (Genigeorgis, 1985, Wolfe, 1984).

In the 1970's there was a dramatic increase in the use of MAP (Genigeorgis, 1985, Wolfe, 1980, Wolfe, 1984). With this increased usage has come an increased concern about the safety of such products. Outbreaks of botulism, such as those in the United States in 1963 involving smoked fish, have lead to intense study of the safety of both vacuum packaging and modified atmosphere packaging of fish. This report reviews the literature on this subject and discusses the feasibility of vacuum packaging and modified atmosphere packaging of fish.

II. DEFINITIONS

Modified atmosphere packaging (MAP), controlled atmosphere packaging (CAP), and vacuum packaging (VP) refer to when food products are packaged in a gas-impermeable wrapper, and the atmosphere inside this package is altered so that it no longer resembles air. In MAP the atmosphere inside the package is altered at the time of packaging, but no attempts are made to maintain it during the storage life of the product. By contrast, in CAP once the desired atmosphere is obtained, it is maintained for the entire storage life of the product

(Genigeorgis, 1985, Wolfe, 1984). With vacuum packaging the air is almost completely withdrawn from the product to create a vacuum. For meat products, the respiration of meat mitochondria and bacteria can remove the residual oxygen and produce carbon dioxide leading to an enhanced carbon dioxide level in the atmosphere of the package (Clark and Takacs, 1980, Genigeorgis, 1985).

III. GASES USED

A. Oxygen

For most foods the amount of oxygen is usually reduced. However, some oxygen may be necessary to maintain the physiological health of fruits and vegetables (Clark and Takacs, 1980). In addition, oxygen is added to maintain the red color of fresh red meat (Daniels et al, 1985, Langton, 1984, Seideman and Durland, 1984).

Oxygen is removed from the atmosphere to inhibit the growth of aerobic microorganisms, and to slow down the oxidative deterioration of the food such as that due to the oxidation of fats (Wolfe, 1984).

B. Nitrogen

Nitrogen is an inert gas which is used as a filler to replace oxygen. Although nitrogen does not inhibit the growth of micro-organisms, the removal of the oxygen achieves this purpose (Clark and Takacs, 1980, Seideman and Durland, 1984). Nitrogen is also added to prevent the collapse of the packaging material which can occur when carbon dioxide absorbs into the food product (Langton, 1984).

C. Carbon Dioxide

Carbon dioxide inhibits the growth of some microorganisms, but not the growth of others (Clark and Takacs, 1980, Seideman and Durland, 1984). Fortunately it inhibits the growth of the normal spoilage organisms of meat and fish (Pseudomonas, Acinetobacter, and Moraxella). This allows the growth of slower growing organisms (lactic acid bacteria) which are not as sensitive to carbon dioxide. Lactic acid bacteria do not grow as quickly, and produce less offensive changes in the food so a longer shelf life is obtained (Clark and Takacs, 1980, Lindroth and Genigeorgis, 1986, Statham, 1984).

The inhibitory effect of carbon dioxide is affected by the concentration of the carbon dioxide, the period of time before its application, the temperature of food storage, and the water activity of the food product (Clark and Takacs, 1980).

IV. METHODS OF MODIFIED ATMOSPHERE PACKAGING

There are three main methods for the packaging and storage of fish under a modified atmosphere (Bell, 1981, Statham, 1984). The first is the bulk transportation in specially-fitted ships, railcars and trailers. The second method involves the use of a master carton. Fish are packaged in individual retail-type packages using permeable film. These packages are placed into a barrier bag made of gas-impermeable film, and a modified atmosphere is applied. The third method is the use of individual retail-type packages where the packaging film is gas impermeable.

V. ADVANTAGES OF MODIFIED ATMOSPHERE PACKAGING OF FISH

The advantages of modified atmosphere packaging of fish include both those advantages that are obtained for most food products, and some advantages that are more applicable to fish. These advantages are discussed below.

A. Longer Shelf Life and a Better Quality Product

Modified atmosphere packaging of both frozen and fresh fish can extend the shelf life and give a better quality product. The shelf life of frozen fish is limited by the development of rancidity due to the oxidative deterioration of lipids. Vacuum packaging or modified atmosphere packaging where there is little oxygen in the atmosphere can slow down the oxidation of fats. In addition, for both frozen and fresh fish there is less dehydration (Pivnick and Barnett, 1967, Sanders, 1981). The packaging also prevents surface contamination (Biede et al, 1981/82) .

The shelf life of vacuum packaged or modified atmosphere packaged fresh fish is extended as microbial spoilage is slowed down (Hanson and Duckworth, 1982, Pivnick and Barnett, 1967, Statham, 1984). The actual shelf life will vary with fish type and will depend upon the initial microbial load of the fish and the temperature of storage. In many cases the shelf life of fresh fish packaged under carbon dioxide has been extended 1.5 to 2.0 times the shelf life of the same quality of fish stored in air (Villemure et al, 1986, Wilhelm, 1982). Table A in the Appendix gives some examples of shelf lives reported by various researchers. Of these Cl ingman & Hooper (1986), Gauthier et al (1986),

Haard and Lee (1982), Molin and Stenstrom (1984), Partmann (1981), Stier et al (1981), Wang and Brown (1983), and Wilhelm (1981) all reported extensions in shelf life by this factor for fish stored at 4°C or lower. Woyewoda et al (1984) did not, however, and other results are difficult. to evaluate in this manner.

B. Better Access to the Retail Market

A longer shelf life may mean that more fresh fish can be placed on the retail store shelves (Scott et al, 1984). Lanier and Korhonen (1981) mention market studies which found that people preferred fresh fish over frozen fish. In addition, longer transit times are possible (Wolfe, 1980) which may open new markets. Longer transit times also mean that less frequent shipments may be possible (Langton, 1984) with a resulting reduction in cost.

MAP makes fish easier for the retailer to handle. There is less smell, and as fish are prepackaged they can go straight into the cooler for sale (Schwartz, 1981, Whaley, 1981). The packaging also means that there is less cross contamination both with other fish and other meat products (Banks et al, 1980). The consumer gets an attractive package in which the fish are clearly visible, and there is no smell or drip (Tiffney and Mills, 1982). These benefits apply both to the use of master cartons and individual retail MAP packages (Bannar, 1979).

C. Less Problems with Ice

Traditionally fish are packed in ice for storage and shipping. Ice is costly to ship, and the drip from melting ice can cause contamination (Banks et al, 1980, Szetela et al, 1981). MAP or VP prevent dehydration

of the fish (Pivnick and Barnett, 1967, Sanders, 1981) so from this aspect ice may not be necessary. However, both for maintaining quality and for safety the temperature of fresh fish must be maintained at or below 2°C. A 1981 report from the Pace Fish Co. of Brownsville, Texas , (Schwartz, 1981), indicated that they found it necessary to add ice to their MAP master cartons to ensure the temperature remained at O°C. Therefore, ice may still be necessary for temperature control.

However, drip and contamination from melting ice may be less of a problem. Schwartz (1981) placed ice inside the barrier bag of the master carton so that the drip remained inside the bag. This could cause a problem with a large amount of drip, but it may be possible to place the ice outside the barrier bag adjacent to the fish. Biede et al (1981/82) and Szetela et al (1981) stated that fish packaged in either the permeable packages inside the master carton or in individual MAP or VP packages would be protected from contamination from the drip.

VI. PROBLEMS WITH THE MODIFIED ATMOSPHERE PACKAGING OF FISH AND POSSIBLE SOLUTIONS

A. Increased Costs

One of the disadvantages of modified atmosphere packaging is that it can be costly (Wolfe, 1980). The equipment costs money and so do supplies. Cohen (1981) mentions that this can result in companies having to compete with other companies that do not use MAP and can sell fish at a lower price.

B. Need for Good Quality Fish

Good quality fish are essential if the full benefit of MAP is to be obtained. Cohen (1981), Sea Harvest, Inc., New Jersey and Schwartz (1981), Pace Fish Co., Texas both reported that their company had trouble getting a good, consistent supply of high quality fish.

Cann at the Terry Research Station, Aberdeen, Scotland has done a lot of work in this area, and stipulates that all fish should be handled hygienically and kept in ice from the time of catching until processing (Urch, 1986). Fish used for modified atmosphere packaging should be of the same quality as similar fish held for a specific period of time on ice. The Terry recommendations for quality of fish to use for MAP are given in table 1. Fish used for MAP should be of an equivalent quality as that specified in the table below for fresh fish held in ice.

Table 1. Quality of Fish to be Used for MAP

Type of Fish	No. of Days Storage in Ice
White fish	1 to 4 days, no blemishes or visible parasites
Herring and Mackerel	1 to 3 days, at least 8% fat
Smoked fish products	Same quality as that stated for fresh fish of the same type
Salmon and trout	1 to 3 days

(Urch, 1986)

C. Choosing the Correct Atmosphere

The mixture of gases used must be adjusted to suit the individual species of fish. An improper mixture of gases can result in physical

changes in the fish, collapse of packages, excessive weepage, and the development of oxidative rancidity in fatty species with a resulting decrease in shelf 1 ife (Tiffney and Mills, 1982). Woyewoda et al (1984) lists physical changes to fish observed by various researchers working with elevated levels of carbon dioxide. These include clouding of eyes, bleaching of skin, softening of flesh, and development of off-colors.

Carbon dioxide is very soluble in water, and it dissolves readily into the flesh of fish (Guise, 1983 and Tiffney and Mills, 1982). This results in the collapse of packages. Reducing the amount of carbon dioxide to 40 percent can solve this problem (Tiffney and Mills, 1982, Urch, 1986). Less of a problem is encountered with fatty species than white fish as there is less water to dissolve the carbon dioxide due to the high oil content (Urch, 1986).

High concentrations of carbon dioxide can promote excessive weepage from the fish (Guise, 1983, Hanson and Duckworth, 1982, Lindsay, 1981, Tiffney and Mills, 1982). Tiffney and Mills (1982) found that weepage was reduced by decreasing the amount of carbon dioxide to 40 percent. For fillets of white fish they used 40% carbon dioxide, 30% oxygen and 30% nitrogen. However, for fatty species they used 60% carbon dioxide and 40% nitrogen to prevent oxidative rancidity. Urch (1986) recommends the 60/40 mixture of carbon dioxide and nitrogen for salmon, trout, fatty fish such as herring and mackerel, and for smoked fish. If smoked fish turn green, then the mixture for white fish is recommended. Gauthier et al (1986) found that 25% carbon dioxide and 75% nitrogen reduced weepage in bulk turbots. Sodium tripolyphosphate can also be used to retard weepage in fish (Cann et iii, 1967, Lindsay, 1981).

D. Preventing Gas Leakage

Extended shelf lives can only be obtained as long as the integrity of the packaging is maintained. Two causes of packages that leak are: (1) moisture, such as a dip solution, getting on the sealing area and preventing a good seal (Cohen, 1981, Lazio, 1981); and (2) fish being incorrectly placed in the package and the heat sealing bar coming down on the fish (Cohen, 1981).

E. Odor Upon Opening Package

One of the advantages of using MAP for individual packages of fresh fish is that due to the impermeability of the packaging there is no smell. However, once the package is opened there is an odor which can be a disadvantage. Cohen (1981) reported that for a good quality product there was only a brief metallic smell upon opening the package. If the product was temperature abused, however, there was a worse smell that the consumer would notice Upon opening the package. The company he was affiliated with at that time, Sea Harvest, Inc. of New Jersey received complaints about the smell. In addition, the consumer did not know the quality of the fish until the package was opened. Whaley (1981) label led packages of fish to warn consumers about the smell and explain it. Daniels et al (1986), Fey and Regenstein (1982), Gray et al (1983), Hulgaard (1981), Schwartz (1981), Stier et al (1981) and Tomlins et al (1981) all noticed a slight off odor upon opening packages of MAP fish.

F. Need for Good Temperature Control

The volubility of carbon dioxide in water increases as the temperature decreases with maximum volubility being at O°C. The effectiveness of carbon dioxide also increases with decreasing temperature due to the increase in volubility (Ogrydziak and-Brown, 1982). For this reason it is still necessary to refrigerate foods packaged under a modified atmosphere.

Good refrigeration is also necessary from a safety point of view. Fish are often contaminated with nonproteolytic spores of C. botulinum. These microorganisms will grow and produce toxin at temperatures as low as 3.3°C. Therefore, storage at temperatures of 2°C or lower is mandatory to prevent any risk of botulism. Fortunately the best temperature for safe storage is also the one where the maximum effects of MAP are obtained. For example, Molin and Stenstrom (1984) reported that best results for the storage of herring were obtained using 100% C02 and a storage temperature of 2°C.

PART II. MODIFIED ATMOSPHERE PACKAGING OF FISH AND THE POTENTIAL FOR FOOD POISONING

I. PATHOGENS OTHER THAN CLOSTRIDIUM BOTULINUM

There is a concern that vacuum packaging or modified atmosphere packaging utilizing low levels of oxygen could increase the risk of food poisoning. The low amounts of oxygen could favor the growth of anaerobic pathogens. However, recent work with other food products has shown that at least as far as Staphyloccus aureus, enterococci and Salmonella are concerned there is no increased risk from MAP (Goodfellow, 1981, Hintl ian and Hotchkiss, 1986, Nickel son and Finne, 1984, Sill iker and Wolfe, 1980). This information should also apply to fish (Nickelson and Finne, 1984).

Other possible pathogens are Vibrio parahaemolvticus and Yersinia enterocolitica. Vibrio parahaemolyticus is a marine organism and unlikely to occur in freshwater fish (Goodfellow, 1981, Nickel son and Finne, 1984). Yersinia enterocolitica has been isolated from raw seafood products; however, there are no reported outbreaks due to the consumpt· on of fish (Nickelson and Finne, 1984). In 1976, Hanna et al isolated Yersinia enterocol itica-type organisms from vacuum-packaged beef and lamb. This raised some concern especially since this organism has a minimum growth temperature of O°C to 4°C (Banwart, 1981 and Hintl i an and Hotchkiss, 1986). In 1980 it was proposed that the group including Yersinia enterocolitica and the Yersinia enterocolitica-1 ike organisms be divided into four different species. Of these four species only Y. enterocolitica is pathogenic for humans (Feeley and Schiemann, 1984). Since that time both Gill and Tan (1980) and Eklund and Jarmund

(1983) have reported that carbon dioxide inhibited the growth of Yersinia enterocolitica. Although there may still be some need for caution concerning the growth of this organism in VP or MAP fish, it does not appear to be a serious risk.

II. CLOSTRIDIUM BOTULINUM

A. Types and Characteristics

The species Clostridium botulinum consists of anaerobic, sporeforming bacteria that produce neurotoxic proteins called toxins. Consumption of food containing these toxins causes a type of severe food poisoning called botulism that can be fatal (Baird-Parker, 1969, Kautter and Lynt, 1984). As the toxins are heat labile, they can be destroyed if food is properly cooked (Kautter and Lynt, 1984). However, despite this fact, good sanitation and refrigerated storage are still necessary. Uncooked toxic fish can contaminate food contact surfaces with the possibility of contaminating prepared foods that are not cooked (Post et al, 1985) .

The species is divided into seven types, A through G, based on the type of toxin produced (Eklund, 1982, Kautter and Lynt, 1984). There are four types of C. botulinum which can cause botulism in humans as shown in Table 2 below. These types can be further divided on the basis of whether or not they degrade protein.

Lindroth and Genigeorgis, 1986, Silliker, 1981, Sperber, 1982, Statham, 1984, Thatcher et al, 1962).

B. Occurrence

C. botulinum is found in both freshwater and marine fish (Eyles and Warth, 1981, Lindroth and Genigeorgis, 1986, Shewan, 1971). "Type E is the most common; however, other types have also been isolated (Eyles and Warth, 1981). Type E is common in some areas of the world, but not in others. It is rare in Britian and Australia, but prevalent in the United States, Russia, Scandinavia, Japan and Canada (Baird-Parker, 1969, Cann et al, 1966 a & b, 1975, Cann and Taylor, 1979, Eyles and Warth, 1981, Eklund, 1982, Hobbs et al, 1965, Laycock and Longard, 1972, Laycock and Loring, 1972, Shewan, 1970, 1971, Southcott and Razzell, 1973). Huss et al (1974) isolated type E from Danish trout farms. In Canada type E has been found in high numbers in some locations of the Gulf of St. Lawrence and lower reaches of the St. Lawrence River, but is absent at other locations in these same areas (Laycock and Loring, 1972). Type E is also quite prevalent in fish caught in the Great Lakes (Baird-Parker, 1969, Christiansen et al, 1968, Laycock and Loring, 1972). Types B, C and E have been detected off the coast of Nova Scotia, and type E was found off the coast of Newfoundland (Laycock and Longard, 1972). Boyd and Southcott (unpublished data cited in Southcott and Razzell, 1973) detected types A, B and E in lakes in central Canada. Type E is also common in British Columbia (Dolman and Iida, 1963).

Less information is available on natural levels of C. botulinum contamination in fish. Eyles and Warth (1981) summarized the available

literature and stated that freshly-caught fish would most likely have no more than a few C. botulinum per gram of flesh. Huss et al (1974) reported 0.34 to 5.3/g of whole fish in fish from a Danish trout farm. Cann et al (1966a) reported one spore per 16 grams of fish for herring from Norwegian fishing grounds. Haddock fillets in the United States contained up to 17 spores/100g (Eyles and Warth, 1981). Eyles and Warth (1981) caution that despite low levels in freshly-caught fish a build-up of contamination in factories could increase the contain" nation levels of the fish.

C. Outbreaks of Botulism Due to VP or MAP Fish

Up until the 1960}s there were no outbreaks of botulism due to VP fish; however, in 1960 and 1963 there was a total of 3 outbreaks of botulism due to smoked white fish (Pivnick and Barnett, 1967). All were due to type E toxin (Lynt et al, 1982, Pivnick and Barnett, 1967). In 1960 there were two fatal cases of botulism, type E, caused by vacuum packaged smoked fish from the Great Lakes (Dolman and Iida, 1963, Thatcher et al , 1962). The fish had been refrigerated for 5 or 6 weeks between processing and consumption (Dolman and Iida, 1963). In 1963, an outbreak of type E botulism involved 16 people none of whom noticed any off-odors and only 3 of whom noticed unusual flavors. The fish had been temperature abused (Eklund, 1982).

D. Toxin Production

1. Effect of Temperature

Clostridia produce spores which must germinate before vegetative growth and toxin production can occur (Grecz and Arvay, 1982). Optimum

temperatures for spore germination and vegetative growth are different (Grecz and Arvay, 1982). Toxin is produced at all pH values and temperatures at which growth of C. botulinum occurs (Baird-Parker, 1971, Sperber, 1982).

There are three steps in the production of a biologically active toxin. The first is the production of a nontoxic protoxin inside the cell. The second is the autolysis of the cell and the release of the protoxin. The third step is the changing of protoxin to biologically active toxin by proteolytic enzymes (Baird-Parker, 1971, Hobbs, 1976, Sperber, 1982). Proteolytic strains produce enzymes that activate their toxins, and the toxin may be active when it is released from the cell. Toxin produced by nonproteolytic strains is activated by exogenous proteases, such as those produced by other bacteria or enzymes in human intestines (Hobbs, 1976, Sperber, 1982). In the laboratory, samples suspected of containing nonproteolytic types of toxin are often treated with trypsin to activate protoxin (Kautter and Lynt, 1984, Lucke et al, 1981). The time for detectable amounts of toxin to be produced is longer at lower temperatures due to a slower rate of autolysis of the cells and the effect of temperature on enzyme activity (Baird-Parker, 1971, Bonventre and Kempe, 1959).

The most widely quoted reference for theminimum temperature for growth and toxin production of C. botulinum type E is that of Schmidt et al (1961) who used a beef stew substrate. Schmidt et al reported growth of four strains of C. botulinum at 3.3°C with toxin production within 31 to 45 days. No growth or toxin production was observed at 1.1 or 2.2°C. Before this Ohye and Scott (1957) had not detected growth or toxin

production in medium at 2.5°C after 20 weeks storage. In 1982, Grecz and Arvay reported that spores of C. botulinum type E strain VH germinated rapidly in medium at 2°C. However, they did not detect growth at this temperature.

Studies using fish have produced variable results which-may have been affected by the use of trypsin and the duration of the tests (see Tables B and C in the Appendix). Cann et al (1965) found that the time for herring to become toxic increased as temperature was reduced. Stier et al (1981) did not detect type E toxin in salmon fillets stored in either air or 60% $C0_2$, 25% 0_2 and 15% N2 and incubated for 57 days at 4.4°C. However, they did not use trypsin to activate any protoxin present. Lindsay (1981) did not detect type E toxin in rockfish fillets stored in air, vacuum or carbon dioxide at 1.7oC, 4.40C, and 7.2°C during a 29 day test. Toxin was detected at 10°C after 25 days. As methodology was not reported, it is not known if trypsin was used. Even though Lindroth and Genigeorgis (1986) used trypsin, they did not detect toxin after 21 days in rockfish stored under a vacuum or modified atmosphere at 4°C. Possibly toxin would have been detected if the tests had been of longer duration. More recently, Post et al (1985) detected type E toxin in cod stored under carbon dioxide after a minimum of 18 days at 4°C. Toxin was detected in whiting stored under carbon dioxide at 4°C after 27 days. At 8°C toxin was detected after a minimum of 8 days in cod, 5 days in whiting, and 23 days in flounder.

Eklund et al (1967) working with a nonproteolytic strain of C. botulinum type B first detected toxin in cooked-meat medium after 26

Ando and Iida (1970) reported that in a complex medium C. botulinum type E spores can germinate under aerobic or anaerobic conditions, but that outgrowth after germination occurred only at Eh values of +198 or lower. At these Eh values germinating spores could reduce the redox , potential even further. In agreement with this, Parkin et al (1981) and Johnson et al (1981) both at the University of California, found that the redox potential decreased rapidly on the surface of rockfish fillets stored in air. Although the redox potential of fillets stored in 80% C02, 20% air decreased slightly, MAP fillets had a higher Eh. This was felt to be due to aerobic bacteria producing slime on the surface of the fish.

Although C. botul inure is a strict anaerobe, Sperber et al (1982) mention that it is a fallacy to believe that C. botulinum cannot grow in foods with a high redox potential or foods that are exposed to air. Even though fish may be stored in air or in a modified atmosphere containing oxygen, anaerobic sites with a low enough Eh to allow the growth of C. botulinum can develop (Goodfellow, 1981, Lindsay, 1981, Silliker, 1981, Sperber, 1982, Statham, 1984). For example, Huss et al (1980) found that smoked herring individually packaged in air or pure oxygen, and inoculated with 102/g type E spores became toxic after six to nine days at 15°C. Conditions allowing growth of C. botulinum can occur a few millimeters below the surface of fish after death (Baird-Parker, 1969, Genigeorgis, 1985). In addition, although oxygen is included in the initial atmosphere, aerobic bacteria can partially use it to produce more anaerobic conditions (Urch, 1986).

3. Effect of Carbon Dioxide

Research with different species of clostridia has shown that carbon dioxide at 1 atmosphere pressure can stimulate the germination of spores. Germination of C. botulinum types A and B, C. sporogenes, C. bifermentans, and C. perfringens were stimulated by carbon dioxide (Enfors and Mol in, 1978, Foegeding and Busta, 1983, Holland et al, 1970). However C. sporogenes and C. perfringens were inhibited by 10 atmospheres and 25 atmospheres of carbon dioxide respectively (Enfors and Molin, 1978). In contrast to these reports, Doyle (1983) found that in comparison to 100% nitrogen, 100% carbon dioxide at atmospheric pressure delayed the production of type A and B toxin. At higher pressures carbon dioxide was even more inhibitory. All of these tests used growth medium rather than fish.

Tests to determine whether samples stored n carbon dioxide became toxic before samples stored in air have produce(var· able results. Post et al (1985) tested toxin production in cod, whiting and flounder at various temperatures (see Table C in the Appendix). Results varied with temperature, but at 8°C toxin was detected first in cod and whiting samples stored under a mixture of gases containing carbon dioxide as compared to those stored in air. However, toxin production in 100% carbon dioxide was delayed in comparison to samples stored in air. Although, Huss et al (1980) reported that high concentrations of carbon dioxide did not enhance toxin production in smoked herring, toxin was produced two days sooner in three out of the four atmospheres containing carbon dioxide than in air (see Table B in the Appendix). The fourth and safest mixture of gases was 48% carbon dioxide and 52% oxygen.

Second best was 99.7% oxygen. Cann et al (1980) found that fish deeply inoculated with C. botulinum became toxic before surface inoculated fish whether the fish were VP or stored in air.

Further research appears to be necessary before it can be determined whether or not carbon dioxide actually increases the risk of toxin production by C. botulinum. Due to differences in inoculum levels, sites of inoculation and gas atmospheres, direct comparisons cannot be made between different research results (Statham, 1984). Research could determine the influence each of these has on the time for toxin production in air as compared to carbon dioxide.

4. Effect of Contamination Level

The number of C. botulinum present in fish may influence the time for fish to become toxic (Cann et al, 1965, Eyles and Warth, 1981). Cann et al (1965) found that reducing the inoculum size increased the time for samples to become toxic. Eklund et al (1967) reported shorter times to detect toxin in growth medium with an inoculum of 2×10^6 spores of nonproteolytic C. botulinum, type B, than with 2×10^5 spores. Lindsay (1981) studied rockfish samples inoculated with 1 or 10 type E spores per gram and stored at 27°C. Results were generally similar for samples stored in air and carbon dioxide. However, rockfish samples inoculated with 10 spores/g usually became toxic sooner than those inoculated with 1 spore/g. Eklund (1982) found that for salmon carbon dioxide was inhibitory for a short period of time at lower inoculum levels. However, when the inoculum was increased results were similar for storage in air and carbon dioxide (see Table C).

5. Time for Spoilage Versus Toxin Production

MAP inhibits the growth of the faster growing spoilage organisms that usually produce obvious signs of spoilage. The lactic acid bacteria that become the dominant spoilage organisms spoil food slowly and produce less noticeable signs of spoilage (Clark and Takacs, 1980, Lindroth and Genigeorgis, 1986). In addition, MAP could reduce the number of competing microorganisms which could help to inhibit the growth of pathogens (Clark and Takacs, 1980, Lindroth and Genigeorgis, 1986). MAP could, therefore, extend the shelf life long enough for fish contaminated with nonproteolytic C. botulinum to become toxic. As nonproteolytic types of C. botulinum do not produce an off-odor, food may become toxic without obvious signs of spoilage (Bell, 1981, Hintl ian and Hotchkiss, 1986, Lindroth and Genigeorgis, 1986, Silliker, 1981, Sperber, 1982, Statham, 1984, Thatcher et al, 1962). Thatcher et al (1962) found that smoked fish stored at 30°C and inoculated with Type A spores were spoi" ed whether stored aerobically or anaerobically. However, samples inoculated with Type E spores and stored anaerobically were not spoiled when they became toxic.

Numerous studies have been made comparing the time for spoilage with the time for toxin production (see Table C in the Appendix). Such studies are complicated by the fact that different criteria have been used to determine the amount of spoilage (Hintlian and Hotchkiss, 1986). In addition toxin production is affected by the number of spores, temperature and packaging conditions, type of fish, microorganisms present on the fish, and the site of the inoculation (Genigeorgis, 1985). Some researchers (Eyles and Warth, 1981, Genigeorgis, 1985,

L ndroth and Genigeorgis, 1986) believe that at storage temperatures at or below 10°C spoilage usually preceeds toxicity. However, there are reports of toxin production before obvious spoilage at these temperatures (Eklund, 1982, Lee and Solberg, 1983, Post et al, 1985). ' More research appears to be necessary before such a generalized statement can be made.

E. Residual Effect of Carbon Dioxide

While MAP fish often have a longer shelf life than fish stored in air, once the atmosphere is removed the shelf life is reduced. Atmospheres are lost when packages are punctured or have faulty seals. Research has been conducted to determine whether there is any residual effect of the carbon dioxide after it is gone. Wang (1984) measured the amount of carbon dioxide retained in rock cod flesh. The carbon dioxide concentration in fillets that had been stored in 80% carbon dioxide and 20% air for 7 days and 14 days decreased by 36% and 56% respectively after 1 day storage in air. After 3 days in air carbon dioxide concentrations were similar to that found in fresh fish.

Wang (1984) attributes the residual effect of carbon dioxide to the response of the microbial population to the changes in atmosphere. While Pseudomonas spp. predominate in air, their growth is inhibited under MAP. Wang found that it took 6 days storage in air after removal from the MAP for these organisms to again become the dominant species. Banks et al, 1980 and Finne, 1981, both at Texas A & M University, also found that the normal gram-negative spoilage flora started to grow again once the fish were removed from the MAP.

Other studies include that of Silliker (1981) who found a residual effect of carbon dioxide on the shelf life of fish. Barnett et al (1982) found that salmon that had been stored in bulk in a modified atmosphere for two weeks remained in good condition for one week after removal from the modified atmosphere. Eklund (1982) found that salmon stored first under cabon dioxide for nine days at 2°C and then in air for 7 days at 10°C were spoiled before they become toxic.

F. Smoked Fish

Although there are reports that in some instances smoking may delay the growth of C. botulinum (Baird-Parker, 1969, Cann et al, 1965, Cann et al, 1984)), spores of C. botulinum can survive both the cold- and hot-smoking process (Christian et al, 1968, Eklund, 1982, Hobbs et al, 1969, Nickelson and Finne, 1984, Southcott and Razzell, 1973). As the surface Eh of smoked fish is low enough to permit sporulation and growth (Huss et al, 1980, Nickelson and Finne, 1984), smoked fish with low salt concentrations can become toxic (Christiansen et al, 1968, Pelroy et al, 1985). Smoked fish are often eaten without prior cooking, which increases the risk of botulism (Cann et al, 1966a, 1967, Southcott and Razzell, 1973).

Additional risks are created by the fact that smoked fish is handled more than fresh fish (Sill iker, 1981), and there are less competing microorganisms (Lindsay, 1981, Nickel son and Finne, 1984). In addition, Lindsay (1981) felt that there was an increased risk of smoked fish becoming toxic before they spoiled due to the cooked flesh being less susceptible to spoilage.

G. Possible Solutions

1. Sorbates

Sorbates, consisting of sorbic acid and potassium sorbate, are one of the least harmful preservatives (Sofas and Busta, 1983). In the U.S. sorbates may be used in any food to which preservatives can be added as well as in 70 other foods that have federal standards of identity (Liewen and Marth, 1985, Sofas and Busta, 1983). The Canadian Food and Drugs Act regulations for marine and fresh water animal products allows the use of sorbates in dried fish that is smoked or salted, but not in fresh or frozen fish (Food and Drug Act, 1985).

Although research in the 1950>s indicated that sorbates could act as a selective agent for clostridia, this is now believed to be wrong. Instead at pH values below 6.0 to 6.5, sorbate can act as an anti-clostridial agent (Liewen and Marth, 1985, Sofas and Busta, 1983). This pH range is compatible with fish (Regenstein and Regenstein, 1981).

Some researchers have investigated the use of potassium sorbate on the shelf life of fish. Fey and Regenstein (1982) tested dips of 1 to 5% potassium sorbate alone without MAP, but did not find them too effective in extending shelf life. Regenstein (1982) found that potassium sorbate dips plus MAP gave the longest shelf life for haddock. Debevere and Voets (1972) and Ampola and Keller (1985) both reported that potassium sorbate extended the shelf life of cod fillets.

Lindsay (1981) reported that a 5% sorbate dip delayed the development of *C. botulinum* type E toxin for 72 hours in whitefish fillets inoculated with 10^4 spores/g and stored at 21°C in carbon

dioxide. lie did not include the times for toxin to develop in untreated fillets. He also cautioned that 5% sorbates dips were not always that effective possibly due to uneven absorption of the sorbate. Dips of a mixture of sodium tripolyphosphate and potassium sorbate were also effective, but could cause off-flavors. Polyphosphate dips are used to reduce weepage or drip from the fish. Cann et al (1967) found that they did not increase the potential for toxin production.

While research into the effectiveness of sorbates as antibotulinal agents in fish is limited, reviews by Sofos and Busta (1983) and Liewen and Marth (1985) state its effectiveness in other food products. More research will be necessary, however, to determine effective concentrations to inhibit the development of toxin. Also the potential for off-flavors, and whether or not use of such a dip is economically feasible should be investigated before it is used commercially. In Canada the use of potassium sorbate is presently only allowed in dried fish that have been smoked or salted, therefore, the Food and Drug Act would have to be changed before it could be used in fresh or frozen fish.

2. Use of Master Cartons

Due to the inability to ensure that the consumer stores the MAP fish safely, many researchers feel that MAP should not be applied to retail-sized packages (Genigeorgis, 1985, Hauschild - personal communication, Lindsay, 1981, Wilhelm, 1982). Instead some are suggesting the use of master cartons. Lindsay (1981) feels that there would be less risk of temperature abuse as consumers would still receive fish in the packages they are familiar with. While the packages of fish

were in the master carton, the benefits of MAP would still be obtained. In addition, there would be less risk of leaking packages and greater control over temperature (Bell, 1981).

There is little information available on what effect storing fish in master cartons has on shelf life either while in the master carton or after removal from the carton. Mauser (1981) reported that after removal from a master carton fish were acceptable for 3 to 6 days depending on the initial quality of the fish. Finne (Banks et al, 1980, Finne, 1981) reported that normal spoilage organisms started to grow again as soon as individual packages were removed from the master carton. They did not give an estimate of shelf life. Barnett et al (1982) stored bulk salmon in a modified atmosphere and found that they were still acceptable one week after removal from the modified atmosphere. However, they did not wrap the fish in retail-type packages. Llobrera (1983) inoculated flounder with a mixture of types A, B and E spores and stored them in master cartons. At all temperatures samples were spoiled when toxin was detected (see Table C). Tests were not made on samples that had been removed from the master carton for a period of time. Wang (1984) reported a shelf life of at least 2 weeks for rock cod fillets stored in 80% $C0_2$/20% air at 4°C. A further 2 to 4 days shelf life was possible after fillets were removed from the carbon dioxide and stored in air.

Woyewoda et al (1984) stored bulk cod under a controlled atmosphere, and reported that carbon dioxide did not penetrate to all of the fillets. Regenstein (1982) also expressed this concern for tightly packed fish stored in bulk. This inefficient penetration of carbon

d oxide may also occur with master cartons. It would appear that more research is necessary before it is known whether or not master cartons are really beneficial.

3. Areas of Future Research and Technological Development

Researchers are currently working on ways to make the MAP packaging of fish safer. Hintlian and Hotchkiss (1986) are investigating the effects of different atmospheres to determine if it is possible to get enough growth of spoilage microorganisms so that food products are spoiled before they are toxic and still get an extended shelf life. The most promising work appears to be that of Dr. Genigeorgis at the University of California some of which is soon to be published in both the International Journal of Food Microbiology and Journal of Food Protection. He has developed models for certain species of fish that would allow prediction of the time for fish to become toxic at a specified temperature and inoculum level (personal communicant on). This could help in establishing pull dates for products.

Time-temperature indicators could also prove to be usefu" . However, Dr. R.C. Lindsay of the University of Wisconsin, who has worked in this area, indicated that with the existing technology two indicators would be necessary (personal communication). One would be necessary to determine temperature abuse at IO°C for a period of days, and the other would be necessary to detect high temperature abuse at 21°C for a number of hours.

111. CONCLUSIONS

Many researchers have stated that while there is no risk if MAP fish are kept below 3.3oC, there is a potential risk due to C. botulinum for storage at any temperature above this (Bell, 1981, Eklund, 1982, Genigeorgis, 1985, Hintlian and Hotchkiss, 1986, Post et al, 1985, Sill iker, 1981, Wilhelm, 1982). Researchers who have worked in this area have made the following comments: the risk of botulism is "remote, but still possible" (Lindsay, Haard, Finne - personal communications); MAP is not safe for consumer-sized packages (Hauschild, Eklund-personal communications); MAP of fresh fish is not prudent at this time (Hotchkiss-personal communication); and VP or MAP "would be an undue risk to consumers" (Sperber - personal communication). The noteable exception to the belief that there is some risk is that of researchers from Australia . These researchers feel that due to the low numbers of C. botulinum in Australian waters, consumers would be adequately protected as long as packages are clearly labelled with instructions on proper storage (Eyles and Warth, 1981).

It is questionable whether strict enough temperature controls can be maintained to eliminate any risk of botulism. Bell (1981) cites a study done by the American Meat Science Association which found that of the retailers studied only 47.5% stored all products below 4.4°C, 35.8% had some products stored over 7.2°C, and 20% had products stored at or over 10°C. Bell (1981) also cites a 1974 USDA study which found that 32% of the 2,503 householders surveyed kept their refr· gerators at or above 7.2°C. Eklund (1982) mentions that temperatures in refrigerated display cases in retail outlets can be as high as 10°C,

While, time-temperature indicators may not prevent temperature abuse they would possibly give some indication of how high the temperature went and for how long. Unfortunately even if such devices were avai" able, strict controls would be necessary in monitoring them and ensur' ng that any product that had exceeded the time-temperature limit was thrown out. This would require extremely conscientious personnel. In addition, it is likely that due to economic considerations their use would be limited to bulk commercial shipments, such as with master cartons. While the use of master cartons is worth further consideration, extensive testing would still be necessary to determine how effective they are.

C. botulinum can grow in fish stored in air, VP or MAP (Cann et al, 1980, Goodfellow, 1981, Lindsay, 1981, Silliker, 1981, Sperber, 1982, Statham, 1984). However, VP and MAP inhibit the growth of the microorganisms that produce indications of spoilage. As nonproteolytic types of C. botulinum do not produce an off-odor, food may become toxic without obvious signs of spoilage (Bell, 1981, Hintlian and Hotchkiss, 1986, Lindroth and Genigeorgis, 1986, Silliker, 1981, Sperber, 1982, Statham, 1984, Thatcher et al, 1962). Therefore based on the literature surveyed for this report and until such a time as research comes up with a solution to this problem, it appears that the VP or MAP of fresh fish should only be allowed where there is absolute control over the storage temperature. Unfortunately, until further developments are made this appears to preclude the use of this technology at the retail level.

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APPENDIX

Table A. Effect of MAP on Shelf Life

Type of Fish	Storage Temp. (°C)	Atmosphere	Shelf Life
Herring[1]	0	Air	7 d
	15	Air	1 d
	0	100% C02	33 d
	15	100% C02	3 d
Turbot2-bulk			
Whole	0	Air	7 d
Fillets	0	Air	4 d
Whole	0	25% CO_2/75% N2	15 d
Fillets	0	25% CO_2/75% N2	9 d
Cod[3]	1	Air	<9 d
	1	60% CO_2/40% air	<12 d
Salmon[4]	22.2	Air	<1 d
	22.2	60% CO_2/25% O_2/15% N2	<2 d
	4.4	Air	<6 d
	4.4	60% CO_2/25% O_2/15% N2	<12 d
Shark[5]	4	Air	9 d
	4	Vacuum	15d
Monk[5]	4	Air	3 d
	4	Vacuum	10 d
Grouper[5]	4	Air	6 d
	4	Vacuum	10 d
Sole[5]	4	Air	6 d
	4	Vacuum	10 d
Salmon[6]	3	Air	6-8 d
	3	C02 (hypobaric)	>20 d
	3	C02 (atmospheric pressure)	<16 d

Type of Fish	Storage Temp. (°C)	Atmosphere	Shelf Life
Cod[7]	2	Air	6-8 d
	2	Vacuum	d
	2	CO_2	. 8-1: d
Rockfish8	2	Air	<10 d
	2	80% C02/20% air	>14 d
Trout[9]	1	Air	7 d
	1	C02	11 d
	-12	Air	3 mon
	-12	Co_z	3 mon
Snapper[10]	3	No vacuum, no barrier	3 d
	3	Vacuum, no barrier	3 d
	3	Vacuum, medium barrier	3 d
	3	Vacuum, high barrier	6 d
	3	C02, medium barrier	8 d
	3	C02, high barrier	6-8 d
Crayfish[11]	4	Air	14 d
	4	80%, C02/20% air	21 d
Haddock[12]	0,4	Air	9 d
	0,4	Vacuum	12 d
Cod[12]	0,4	Air	12 d
	0,4	Vacuum	17 d
Rock cod[13]	4	Air	< 7 d
	4	80% C02	>21 d
Trout[14]	o	60% $C0_2$/40%N_2	8 d
	o	Vacuum	9 d
Salmon Steaks[14]	0	60% $C0_2$/40%N_2	12.9 d
	0	Vacuum	11.8d

continued

Type of Fish	Storage Temp. (°C)	Atmosphere	Shelf Life
Smoked Salmon[14]	0	Both 60% CO_2/ 40% N_2 and Vacuum	25 d
	5		21 d
	10		14 d

[1]Molin and Stenstrom (1984)
[2]Gauthier et al, 1986
[3]Woyewoda et al, 1984
[4]Stier et al, 1981
[5]Clingman and Hooper, 1986
[6]Haard and Lee, 1982
[7]Jensen et al, 1980
[8]Parkin et al, 1981
[9]Partmann, 1981
[10]Scott et al, 1984
[11]Wang and Brown, 1983
[12]Cann et al, 1967
[13]Mokhele et al, 1983
[14]Cann et al, 1984

NS = Not Stated
< = Only Day of Spoilage Given, Shelf Life Less Than This Date.
> = Not Spoiled By End of Test.

Table B. Time for Samples Inoculated with C. botul inure to Become Toxic

Type of Fish (°C)	Storage Temp.	Inoculum Level	Atmosphere	Time to Toxicity
Rockfishl	4	1 spore/ sample	MA or VP[2]	>21 d
	8			12 d
	12			9 d
	17			6 d
	30			2 d
	4	10 spores/ sample	MA or VP[2]	>21 d
	8			9 d
	12			6 d
	17			3-6 d
	30			1-2 d
Herring3 Viscera4	15	10 spores/g	VP	2 d
Fillet				3 d
Fillet	15	100 spores/g	VP	l d
Gut				2 d
Fillet or Gut	10	100 spores/g	VP	8 d
Rockfish5	27	10 spores/g	Air	24 h
			Vacuum	24 h
			Excess C02	24 h
			Partial vacuum with C02	24 h
	27	1 spore/g	Air	48 h
			Excess CO_2	48 h
			Partial vacuum with CO_2	24 h
Smoked herring[6]	15	$5x10^1$/g	Air	6 d
			Vacuum	4 d
			99.6% CO_2	4 d
			48% C02/52% 02	10 d
			46% CO_2/54% N2	4 d
			23% C02/77% N2	4 d
			99.9% N2	4 d
			99.7% O_2	9 d

Type of Fish	Storage Temp. (°C)	Inoculum Level	Atmosphere	Time to Toxicity
Herring[7]	3.3	10^6/100g	Vacuum	21 d
Scallops[8]	20	5x107/pack	Vacuum	6 d
Smoked haddock[8]	20	5x107/pack	Vacuum	3 d
Kipper[8]	20	5x107/pack	Vacuum	2 d
Herring[8]	20	5x107/pack	Vacuum	1 d
Smoked haddock[8]	10	5x107/pack	Vacuum	29 d
Kipper[8]	10	5x107/pack	Vacuum	9 d
tierring[8]	10	5x107/pack	Vacuum	7 d
Trout[g] Types B&E 10^2/g	20	Deep muscle	60% CO_2/40%N_2	2 d
		Surface	60% CO_2/40%N_2	2 d
		NS	Vacuum	2 d
	15	Deep muscle	60% CO_2/40%N_2	3 d
		Surface	60% CO_2/40%N_2	4 d
		NS	Vacuum	3 d
	10	Deep muscle	60% CO_2/40%N_2	8 d
		Surface	60% CO_2/40%N_2	8 d
		NS	Vacuum	8 d
Salmon Steaks[g] Types B&E 10^2/g	20	Deep muscle	60% CO_2/40%N_2	2 d
		Surface	60% CO_2/40%N_2	3 d
		NS	Vacuum	3 d
	15	Deep muscle	60% CO_2/40%N_2	4 d
		Surface	60% CO_2/40%N_2	4 d
		NS	Vacuum	5 d
	10	Deep muscle	60% CO_2/40%N_2	8 d
		Surface	60% CO_2/40%N_2	11 d
		NS	Vacuum	8 d

Type of Fish	Storage Temp. (°C)	Inoculum Level	Atmosphere	Time to Toxicity
Smoked Salmon[9] Types B&E 10^2/g	20	Deep muscle	60% CO_2/40%N_2	NT
		Surface	60% CO_2/40%N_2	5 d
		NS	Vacuum	6 d
	15	Deep muscle	60% CO_2/40%N_2	NT
		Surface	60% CO_2/40%N_2	14 d
		NS	Vacuum	10 d
	10	Deep muscle	60% CO_2/40%N_2	NT
		Surface	60% CO_2/40%N_2	>42 d
		NS	Vacuum	>42 d

[1]Lindroth and Genigeorgis, 1986

[2]Earliest time to detect toxinin samples stored under vacuum, 100% C02 or 70% C02 + 30% air.

3Huss et al, 1979

[4]Site of inoculum

[5]Lindsay, 1981

6Huss et al, 1980

[7]Cann et al, 1967

[8]Cann et al, 1965

'Cann et al, 1984

NS = Not Stated

NT = Not Tested

> = Not Toxic by End of Test Period

Table C. Time for Samples Inoculated with Spores of C. botulinum, type E to Become Toxic Compared to Time for Spoilage

Type of Fish	Storage Temp. (°C)	Atmosphere	Inoculum	Time to Toxicity	Spoiled[a]
Salmon[1]	10	Air	10^2	7 d	+
		60% C02/25% O_2/15% N2	10^2	10 d	-
		90% C02/10% N2	10^2	10 d	
	10	Air	10^3	7 d	+
		60% CO_2/25% O_2/15% N2	10^3	7d	-
		90% CO_2/10% N2	10^3	7 d	
	10	Air	10^4	7 d	+
		60% CO_2/25% O_2/15% N2	10^4	7d	-
		90% CO_2/10% N2	10^4	7d	-
Smoked fish[2]	30	Air	4000/g	8 d	+
		100% H2	4000/g	8 d	-
Salmon[3]	22.2	Air	10^4/g	2 d	+
		60% C02/25% O_2/15% N2	lo4/g	2 d	+
	4.4	Air	10^4/g	Not Toxic	+
		60% CO_2/25% O_2/15% N2	10^4/g	Not Toxic	+
Cod[4]	26	Air	50/g	2 d	+
		Vacuum	50/g	2 d	+
		N_2	50/g	2 d	+
		CO_2	50/g	2,2 d	+,-
		90% C02/8/% N2/1% 02	50/g	3 d	+
		65% CO_2/31% N2/4% 02	50/g	ld	-
	12	Air	50/g	> 9 d	+
		Vacuum	50/g	14 d	+
		N_2	50/g	6 d	
		CO_2	50/g	11 d	+
	8	Air	50/g	>10 d	+
		Vacuum	50/g	20 d	+
		N_2	50/g	17 d	+
		CO_2	50/g	19 d	-
		90% C02/8/% N2/1% 02	50/g	8d	-
		65% CO_2/31% N2/4% 02	50/g	9 d	
	4	CO_2	50/g	18,21 d	-,-

Type of Fish	Storage Temp. (°C)	Atmosphere	Inoculum	Time to Toxicity	Spoiled[a]
Whiting[4]	26	Air	50/g	3 d	+
		Vacuum	50/g	2 d	+
		N_2	50/g	2 d	+
		CO_2	So/g	2,2 d	+,+
		90% C02/8/% N2/1% 02	50/g	1 d	
		65% CO_2/31% N2/4% 02	50/g	2 d	+
	12	Air	50/g	> 8 d	+
		Vacuum	50/g	12 d	+
		N_2	50/g	12 d	+
		CO_2	50/g	12 d	+
	8	Air	So/g	>12 d	+
		Vacuum	So/g	17 d	+
		N_2	50/g	17 d	+
		CO_2	50/g	20 d	+
		90% C02/8/% N2/1% 02	50/g	8 d	-
		65% CO_2/31% N2/4% 02	50/g	5d	-
	4	CO_2	50/g	27 d	+
Flounder[4]	26	Air	So/g	2 d	+
		Vacuum	50/g	2 d	+
		N_2	50/g	2 d	
		CO_2	50/g	2 d	+
	12	Air	50/g	l l d	+
		Vacuum	50/g	15 d	+
		N_2	So/g	14 d	+
		CO_2	50/g	10 d	+
	8	Air	50/g	>12 d	+
		Vacuum	50/g	>21 d	+
		N_2	So/g	>21 d	+
		CO_2	So/g	23 d	+
Rockfish[5]	10	Air, vacuum, partial C02, 100% C02	5 & 50/g	25 d	+ +
	7.2	Air, vacuum, partial C02, 100% CO_2	5 & So/g	Not toxic after 29 d	+ +

continued

Type of Fish	Storage Temp. (°C)	Atmosphere	Time to Inoculum Toxicity	Spoiled[a]
	4.4	Air, vacuum, partial CO_2, 100% CO_2	5 & 50/g	Not toxic + after 29 d +
	1.7	Air, vacuum, partial CO_2, 100% CO_2	5 & So/g	Not toxic + after 29 d +
Cod[6] Types B&E	10	40% CO_2/30% 02/30% N2	Deep muscle	11 d +
		Vacuum	Surface	10d +
10^2/g	10	Vacuum	NS	8d +
Herring[6] Types B&E	10	40% CO_2/30% O_2/30% N2	Deep muscle	7 d +
		40% CO@% O_2/30% N_2	Surface	9d +
10^2/g	10	Vacuum	NS	7d +
	10	60% CO_2/40% N2	Deep muscle	6 d +
		60% CO_2/40% N2	Surface	8d +
		Vacuum	NS	6d +
Smoked mackere16 Types B&E	10	40% CO_2/30% 02/30%" N2	Deep muscle	- +
		40% CO_2/30% 02/30% N2	Surface	- +
		Vacuum	NS	+
10^2/g	10	60% CO_2/40% N2	Deep muscle	- +
		60% CO_2/40% N2	Surface	+
		Vacuum	NS	12-d -
Flounder[7] Types A, B & E	4.4	100% co_2	10- looo/g	>21 d +
		70% C02/30% air	lo-l ooo/g	>21 d +
	10	100% co_2	10- looo/g	<6d +
		70% C02/30% air	10- looo/g	<6d +
	26.6	100% co_2	10- looo/g	<ld +
		70% C02/30% air	10- looo/g	<ld +

[a] + = spoiled, - = acceptable

[1]Eklund, 1982

2Thatcher et al, 1962

[3]Stier et al, 1981 - Samples stored at 4.4°C were not toxic after 57 days.

[4]Post et al, 1985

[5]Lindsay, 1981

[6]Cann et al, 1983

[7]Llobrera, 1983