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Criteria for selecting materials suitable for application to modified atmosphere packaging of fish

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CRITERIA FOR SELECTING MATERIALS SUITABLE FOR APPLICATION TO MODIFIED ATMOSPHERE PACKAGING OF FISH

By

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ABSTRACT

The process of preservation is an important step in stabilizing perishable items in order for them to withstand distribution, handling, and storage. Preservation can be achieved by several methods, but the method of concern in this paper is that of modified atmosphere storage which is reported to extend the shelf-lives of perishable products.

Studies on modified atmosphere storage have emphasized the preservating action of carbon dioxide (CO_2) in reducing (1) the extent of microbial spoilage, and (2) the tissue pH of the products so stored in comparison to those stored aerobically. Low tissue pH creates unfavourable environment which negatively influences the growth of spoilage bacteria.

This paper will deal only with modified atmosphere packaging and reduction of aerobic microbial action. The emphasis is on preserving fresh fish for resale. This work explores modified atmosphere packaging and cold storage as two methods used in conjunction to accomplish fresh fish marketing.

As a first step in this investigation, this study attempts to:

(1) establish criteria for selecting materials for primary packages for modified atmosphere preserved fish, and
(2) describe a test to evaluate the suitability of flexible materials for application to modified atmosphere packaged fish.

Suitability of materials for application to modified atmosphere packaged fish is determined by their rate of permeability to CO_2 nitrogen (N_2), and oxygen (O_2) - the gases commonly used in modified atmosphere preservation. The rate of permeability of the materials to these gases is determined by gas chromatography.

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INTRODUCTION

Man's interest in the consumption of fish is based on the fact that fish is an excellent source of protein of high biological value. The protein found in fish provides the essential amino acids required for good nutrition for both human and animals (Geiger and Borgstrom, 1962). Unfortunately, fish is one of the most perishable of food items (Parkin et al, 1981). It is so highly succeptible to microbial and chemical deterioration (Lannelongue et al, 1982) that Ogrydziuk and Brown (1982) reported that of all the commercial seafood landed in the United States in 1977, only about 3% was marketed as fresh product (NMTS, 1978), the rest being processed, frozen, or marinated, to prevent deterioration. The problem of providing effective preservation has long been a cause of concern in the marketing of fresh (unprocessed) fish. Effective preservation can ensure better keeping quality of the fresh product which is desired by both consumers and retailers. The fresh product fetches premium price from the consumer, who in turn derives nutritional benefits from the product. Further, fresh fish is rarely involved in food poisoning (Paine and Paine, 1983).

The preservating effect of modified atmosphere of carbon dioxide (CO_2) on perishable products such as pork, poultry, beef (Coyne, 1932; revised in Banks et al, 1980); freshwater crayfish (Wang and Brown, 1983); dungeness crab (Parkin and Brown, 1983) have been demonstrated. By experimental investigations, a number of studies have reported the benefits of CO_2 for the preservation of fish and fishery products against spoilage organisms which can withstand the low temperature (Wolfe et al, 1980) in which fish are generally stored.

Modified atmosphere utilizes a selection of gases which selectively inhibits microbial growth in perishable products, thereby prolonging the storage period. The technique normally involves evacuation of air from an oxygen-impermeable container, followed by an injection of the desired gas mixture, then sealing the container (Banks et al, 1980).

In the technique of modified atmosphere system, an initial charge of the selected atmospheric concentration of gases is made, consistent with the expected requirements of the commodity during transportation. It differs from "controlled-atmosphere" systems in which the selected concentrations of gases are maintained throughout the entire storage process (Wolfe, 1980).

EARLY STUDIES

The concept of using gaseous CO_2 as a food preservative is not new. From as early as 1882, the beneficial effects of modified atmospheres were reported when Kolbe (1882, reviewed in Coyne, 1933), observed the preservative effects of CO_2 on meat. Since then a number of studies have corroborated Kolbe's report.

Early studies by Killeffer (1930), Haines (1933), Coyne (1933), and Stansby and Griffiths (1935) indicated that the shelf-life of meat, and fish could be extended in an atmosphere containing CO_2. Further work in the field led Windsor and Thoma (1974) to investigate the use of CO_2 atmosphere in conjunction with a variety of chemical preservatives for use with commercial fishery products.

Barnet et al (1971), and Hiltz et al (1976) indicated that CO_2 dissolved in refrigerated brine improved the keeping quality of rockfish, and chum salmon (Barnett); silver hake (Hiltz et al, 1976); and pink salmon (Bullard and Collins, 1978; Barnett et al, 1978).

More recent studies by Sander and Soo (1978) showed the shelf-life of freshly-packed chicken to be markedly increased by using CO_2 at low temperature; and Veranth and Robe (1979) reported that CO_2-enriched atmosphere (60% CO_2 - 25% O_2 - 15% air) doubled the shelf-life of fresh salmon.

GAS COMPOSITION AND TECHNIQUE OF MODIFIED ATMOSPHERE

Hanson and Dulchworth (1982) reported that gases were chosen to maintain the visual appearance of the product, and to prevent spoilage. Following tests on a number of different gas compositions, pure CO_2, or combinations of CO_2 with O_2 and or N_2, were determined to be the most effective in extending the shelf-life or preserving fresh foods (Finne, 1982). Nitrogen was used to cushion the effect of gas absorption by the product; O_2 to maintain the oxidation state of the product, and to preserve the product's color and appearance; and CO_2 to restrict the growth of aerobic bacteria which cause spoilage.

Coyne (1932) observed that one of the factors in the efficacy of CO_2 in extending the shelf-life of fish was its selectivity in bacterial inhibition. Carbon dioxide attacks gram-negative bacteria which are primarily responsible for spoilage at low temperatures. Another factor, according to Aickin and Thomas (1975), and Turnin and

Warner (1977) (reviewed in Wolfe, 1980) is the gas' ability to penetrate the bacterial membranes and directly lower the pH within the cells of the spoilage organisms. This results in inhibiting metabolic activities of the microbes and a marked reduction in the number of organisms in the muscle of the fish. Intracellular pH change disrupts the internal enzymatic equilibrium and cannot be effectively buffered by the organisms. Since the change occurs internally, its effect would be of a greater magnitude than if it had occurred externally (Wolfe, 1980).

Studies with seafoods have shown low-temperature modified storage to be very effective (Barns et al., 1980, reviewed in Parkin and Brown, 1980). Temperatures frequently used in the experiments were 35±2°F (Parkin et al., 1981); 3.5°C (Lannelongue et al., 1982); and 4°C (Wang and Brown, 1983). At low temperature CO_2 is more soluble, and consequently more mobile in the fish tissue. Carbon dioxide dissolves in water to give the acid reaction:

$$\underset{\text{water}}{H_2O} + CO_2 \longrightarrow \underset{\text{Carbonic acid}}{H_2CO_3} \longrightarrow \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Bicarbonate ion}}{HCO_3^-}$$

which creates an unfavourable environment for the growth of bacteria. Reducing the temperature further will increase the solubility of CO_2 and the concentration of hydrogen ions (H^+) available to penetrate the bacterial wall. This is deleterious to the bacteria. However, it should not be assumed that reduced temperature increasesthe bacteriocidal action of CO_2. The reason for this is unclear. According to Enfors and Molin (1981): and Gill and Tan (1980), in their studies on the effect of CO_2 on the growth of spoilage bacteria,

> the relative inhibitory effect and solubility of CO_2 decreased as the temperature increased, ...if the solubility of CO_2 was taken in account, then the inhibitory effect was largely independent of temperature.

Another factor influencing the effectiveness of storage is the composition of the modified atmosphere. The maximum effective level of CO_2 selected for bacteriostatic effect, varied according to the type of products being packed. High levels of CO_2 could be used successfully on fish and other seafoods with little risk of heme[1] protein discoloration due to their low pigment concentration. Ogrydziah and Brown (1982) reported drastic inhibition of microbial growth on rockfish stored at 4°C in atmosphere of 80% CO_2 and 20% air. Parkin and Brown (1983) confirmed that their trained panelists could not detect discoloration on rockfish fillet held in 80% CO_2 atmosphere for 13 days. However, high concentration of CO_2 was not recommended for use with fresh red meats as it caused a brown coloration due to the formation of metmyoglobin on the tissue surface.

Experiments by Lannelongue et al. (1982) to determine the effect of different levels of CO_2 on the microbiological and chemical characteristics of fresh sheephead fillet during storage have indicated that:

> Bacterial growth on fish stored in 40% CO_2: 60% O_2 was similar to that stored in 60% CO_2: 40% O_2 combination and that bacterial growth was much less extensive than that in 20% CO_2: 80% O_2 combination. They concluded that the optimum inhibitory CO_2 concentration was reached somewhere between 20 - 40% CO_2 and that no significant advantage was obtained by increasing the CO_2 level beyond this point.

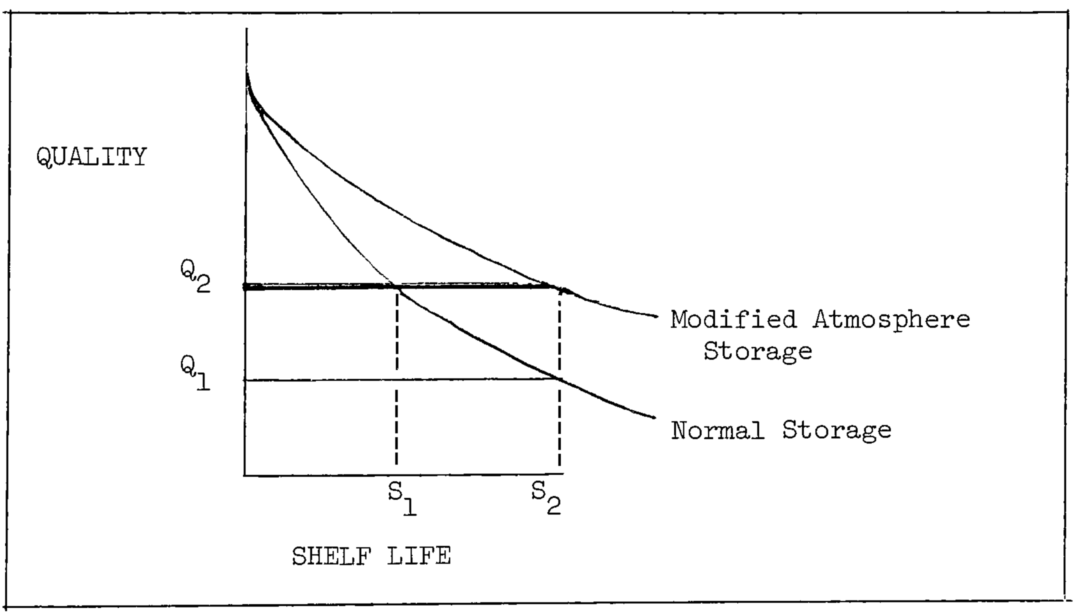
Gill and Tan (1980) also supported this finding by demonstrating

1. Heme is a deep-red pigment, $C_{34}H_{32}N_4O_4Fe$, obtained from hemoglobin.

that increased partial pressure of CO_2 above 200mm (26% CO_2) did not increase the degree of inhibition for a number of common spoilage bacteria. Wolfe (1980) confirmed that CO_2 could inhibit microbial growth for several weeks under optimum conditions, even under elevated O_2 levels.

Wolfe (1980) also observed that under similar conditions, for a given storage period, the quality of a product held in modified atmosphere was higher than for one held in air. He illustrated this by the following diagram.

Diagram illustrating TRADE-OFF OPTIONS between quality and shelf life with and without the use of modified atmosphere



The diagram illustrates some of the trade-offs which are optimal with modified atmosphere. For example, for a given shelf-life S_2, quality Q_2 can be obtained with modified atmosphere; whereas under equivalent transit conditions without modified atmosphere, quality Q_1 would result. Alternatively Q_2 would result at S_1 under normal storage, but can be maintained until time S_2 under modified atmosphere.

This suggested that fish packaged in modified atmospheres was capable of maintaining its freshness over a wider area of distribution, or for a longer storage period. Commercially applied, modified atmosphere packaged fish could therefore reduce some of the problems faced by the fish industry by:

(1) Increasing the availability of 'fresh' high quality fish for which premium price can be charged;

(2) Allowing for wider distribution of fish to more distant consumers since the storage period could be prolonged;

(3) Reducing the number of spoiled fish thereby reducing loss to spoilage and increasing the amount for sale;

(4) Providing a better alternative to bulk transportation of fish on ice since:

   (a) bulk transportation offers limited distribution (short distances) as ice provides only limited extension of shelf-life,

   (b) freight cost of bulk ice transportation is expensive,

   (c) with ice, cross contamination and other forms of abuse are possible since the surfaces of the fish are exposed, not protected by overwrapping, and

   (d) the drip loss from the melting during transportation can result in unhygenic conditions (Banks et al., 1980).

Wolfe (1980) summarized the benefits of modified atmosphere storage as extending the transit time; maintaining high quality; and actively inhibiting bacteria, molds and fungi, and reducing economic losses.

The disadvantages of modified atmosphere storage include:

(1) added cost of equipment, training requirements, and materials for creating the atmospheric condition;

(2) undesirable changes in fish such as:
clouding of the eyes; bleaching of the skin (Coyne, 1933; Stansby and Griffiths, 1935); softening of the flesh of some types of fish - such as cod, whiting, and haddock (Coyne, 1933); and development of off-colors (browing) in salmon (Tarr, 1948; Veranth and Robe, 1979). These problems could probably be reduced by using modified atmospheres for fish fillets instead of whole fish, atmospheres of 40 - 60% concentration of CO_2 to lessen the softening of flesh (Coyne, 1933), and atmospheres of high oxygen concentration in combination with CO_2 to overcome the discoloration in salmon;

(3) uncertainty of marked increase in shelf-life, - dramatic benefits do not result from gaseous atmospheres if:-

(a) the initial quality of the fish is poor, and handling conditions are unsanitary (Coyne, 1933; Lannelongue, 1982). While the handling conditions can be controlled, the initial microbial quality of the fish is unpredictable because the bacteria population varies with species, area, method of catch, seasons, and other environmental conditions (Shewan, 1968; Bramsnaes, 1965; Nair and Lahiry, 1968; Castell, 1971; Cox and Nickleson, 1977; reported in Banks et al., 1980).

(b) the fat content of the fish is less than 1.0%.

The preservative action of CO_2 in extending the shelf-life of proteinaceous products is based on the control of oxidative deterioration (Sainsbury, 1969; reviewed in Woyeda et al., 1984) and it would not apply to lean fish.

Experiments showing beneficial results with gaseous atmospheres have used fatty fish such as salmon, trout, or croaker (Brown et al., 1980; Veranth and Robe, 1979), or beef, or pork (Huffman et al., 1975; Baran et al., 1970; Silliker and Wolfe, 1980; reviewed in Woyeda et al., 1984).

(4) maintaining the atmosphere.

> Efficient packaging of the fish is in many respects responsible for preserving the fish, for unless the gas barrier of a package is adequate the whole effort of modified atmosphere packaging would be lost. An appropriate package can produce a desirable micro-atmosphere around the product that contributes in reducing spoilage.

Although investigators have adequately shown modified atmosphere storage to be an effective technique in extending shelf-life of fish and other seafoods, complementary studies identifying packaging materials suitable for maintaining modified gaseous atmospheres systems for fish have been lagging.

The role of packaging in fish preservation would be essentially one of protection against gas permeation, and unsanitary handling in order for the product to withstand transport, handling, and storage over a longer period of time.

Identification of materials appropriate for maintaining gaseous systems is an important step in packaging design. Consequently, such studies on material identification would contribute significantly to the design of retail packages. Retail packages are required to apply the technology of modified atmosphere preserved fish from the laboratory conditions to practical applications where the benefits previously described would be enjoyed.

In the food industry, retail packages have been successfully employed to control factors such as changes in moisture and gas contents, interaction with oxygen (gases), intrusion of foreign odors and vapors, and mechanical damage which can lead to spoilage in other proteinaceous products such as bacon.

In addition to their protective function, these retail packages have been designed to function smoothly, efficiently and economically on the packaging line. They are convenient to the consumer and distributor. Adopting a similar style of prepackage for modified atmosphere preserved fish would broaden the scope of convenient packages, and make self-service possible. Self-service is an important and predominant means of retail distribution in most countries today.

The packages used for other proteinaceous products may not be suitable for modified atmosphere preserved fish, therefore development of a successful retail pack for fish preserved by CO_2, depends on determination of several criteria:-

(a) The product;

- its composition,
  fat content, dietary benefits,
- factors affecting its stability/quality,
  microbial attack, enzymatic degradation,

(b) The product/package/environment system;

- the effect of the material on the product's intrinsic quality, and shelf-life due to inadequate barrier properties,
- the material's compliance with federal regulations,
- the availability of the material,
- the ease of processing of the material, and
- the cost.

The objectives of this study were to:-

(1) determine the criteria for selecting materials for primary packages for modified atmosphere preserved

fish; and

(2) describe a test to evaluate the suitability of flexible materials for application to modified atmosphere packaging of fish.

SECTION 1

MODIFIED ATMOSPHERE PACKAGING

THE STATE OF THE ART

Preserving perishable food items by using modified atmosphere is not just a laboratory exercise. The benefits of modified atmosphere in both improving the quality and extending the shelf life of perishable food items have been experienced in practical applications. The types and concentrations of gases (modified atmosphere) vary according to the produce as illustrated in the following table.

TYPICAL MODIFIED ATMOSPHERES FOR VARIOUS COMMODITIES

Commodity	Relative Concentrations of Modified Atmosphere Components			
	Nitrogen	Oxygen	Carbon Dioxide	Carbon Monoxide
Meat, fish, poultry	to bal.	20+%	20+%	-
Tomato	"	0 - 10%	-	0 - 10%
Cauliflower, mushroom	"	10 - 20%	0 - 10%	"
Cantaloupe	"	0 - 10%	10 - 20%	"
Citrus (most)	"	0 - 10%	10 - 20%	"
Navel oranges	"	10 - 20%	0 - 10%	"
Strawberries	"	10 - 20%	10 - 20%	"

Wolfe, 1980.

Carbon monoxide (CO) is a modified atmosphere agent in effectively reducing the aging process in certain fruits and vegetables. It does this by reacting with cytochromes (enzymes which catalyze intracellular oxidation), thereby blocking oxidation. Carbon monoxide is also active in inhibiting some yeast and mold growth on produce, possibly by the same reaction. Carbon monoxide is not commonly used, but has been used in low concentrations to prolong shelf-life of produce indicated in the above table.

Carbon dioxide is another agent used in modified atmosphere systems. It is a byproduct of the respiratory process and has a direct action in inhibiting decay process of a number of commodities. Increased concentrations of CO_2 has the effect of decreasing the rate of metabolism of spoilage organisms, thereby inhibiting the growth of certain micro-organisms responsible for decay. Carbon dioxide is also effective in controlling spoilage organisms capable of withstanding low temperature.

Other theories on the mechanism for the inhibition of microbes by CO_2 includes:

(1) toxicity of carbonic acid in its undissociated form (Silliker, 1981). Carbon dioxide dissolves in water to form carbonic acid which lowers tissue pH. This in turn inhibits the activity of gram-negative bacteria,

(2) King and Nagel (1975) further elaborated that CO_2 enters into the mass action equilibrium for enzymatic decarboxyaltion. A high concentration of CO_2 would inhibit the metabolic activity of the microflora (Finne, 1982), and

(3) Sander and Soo, (1978) suggested that change in the surface pH, due to the absorption of CO_2 in the food surface and subsequent ionization of the

carbonic acid, inhibits microbial activity (Finne, 1982).

PRACTICAL APPLICATION OF MODIFIED ATMOSPHERE

Carbon dioxide has been used for many years for preserving apples on a large scale. Carbon dioxide delays ripening and aging of harvested fruits in cool storage, by depressing the rate of metabolism of microbes. Apples can be stored for over six months in order to be available in the off-seasons. In the late 1930's, large transoceanic shipments of fresh meat in CO_2 atmosphere were made from New Zealand to Britain. In 1956, Yokoseki et al., reported the practical application of CO_2 in extending the shelf-life of fish cakes, and Lannelongue (1982), reported the use of CO_2 in preserving marine food prepared for bulk shipment.

These early applications of the technology of modified atmosphere storage involved bulk shipments. Over the years the number of applications increased, and later with improved technical packaging developments, the use of modified atmospheres spread to less bulky shipments such as pallets enclosed in large plastic bags treated with gaseous atmospheres (Annoymous, 1979; reviewed in Wolfe, 1982). Wolfe (1982), reported that these palletized shipments were successful in handling strawberries, and mixed loads of other produces in gas-flushed bags, by trucks.

Eventually, commercial applications of CO_2 atmosphere were made to smaller units. They were used successfully to ship fresh chicken in bag-in-a-box form of packages. This method of shipping extended the shelf life, and advantageously reduced freight cost by elimination of chill-pack ice. Recently pork, and 10-pound institution packs of prepared vegetables have been shipped in modified atmosphere successfully (Wolfe, 1982).

In spite of the increased commercial applications of CO_2 - enriched atmosphere to various food items, investigations dealing with both laboratory research and commercial application to fresh fish are limited (Woyewoda, 1984).

Disadvantages

The use of high CO_2 atmosphere in fresh meat storage can lead to the undesired development of color darkening related to metmyoglobin on the tissue surface. However, fish contain much lower levels of myoglobin than do meats, and higher levels of CO_2 can be used with seafood before discoloration becomes a problem (Parkin et al., 1982). With respect to meat, carbon monoxide (CO) has the potential to retard the above process, but application of CO has not yet been approved by regulatory agencies for commercial use with meats (Wolfe, 1980). Carbon monoxide is a poisonous gas and its use raises many questions with regards to safety as residue (less than 0.09 ppm) of CO was left on cooked ground beef which was exposed to CO storage (Watts et el., 1978 reviewed in Wolfe, 1980).

Wolfe (1980) cautioned that the use of modified atmosphere is not a panacea. The decision to implement a system of gaseous atmosphere depends on the benefits to be gained, and loss claims minimization through its use. However, for premium and highly perishable products such as fresh fish, the cost would be justified. Modified atmosphere stored fish can be sold as 'fresh', and fresh unprocessed fish offers higher monetary returns (Ogrydziah and Brown, 1982).

SECTION II

THE PRODUCT - FISH

The physical, chemical, and biochemical characteristics of fish vary according to the species, sex, seasons, method of catching, location and depth of the fishing areas. Nevertheless, the following generalizations have been observed of the physical features, the changes that take place after death, spoilage, and for fish totally unfit for human consumption.

Physical Features

The surface of freshly caught fish is shiny and thinly covered with slime, which is almost transparent. The eyes are bright, and protruding. The gills are bright red and free from visible slime. The flesh which is soft and flabby just before and at death, becomes firm and hard, and elastic on the onset of rigor mortis. There are two kinds of muscle tissues in fish, red and white. Their proportions vary according to the species. The odor is non-offensive and usually described as 'marine', or 'fresh seaweedy'.

Chemical Composition

The chemical composition of fish is extremely varied, being affected by their genetics, morphology, physiology, enrivonmental, and feeding conditions/factors. The main components and approximate proportions of the muscle tissue are water, 66 - 84%; protein, 15 - 24%; lipids, 0.1 - 22%; and mineral substance, 0.8 - 2%. In minute quantities are sugars; glycogen, reaching a maximum of 0.3%; insignificant quantities of ribose in nucleic acids, and traces of numerous water - or fat-soluble vitamins. Again, one encounters great variations in the composition of the muscle depending on its anatomical location, the species of the fish, seasons, sex, etc.

AVERAGE COMPOSITION OF EDIBLE PARTS OF FISH, CATEGORIES OF FISH, AND SOME EXAMPLES

CATEGORY	WATER %	PROTEIN %	LIPID %	ASH %	EXAMPLES
Fat fish	68.6	20.0	10.0	1.4	herring, mackerel, shad, tuna, salmon
Semi fat Fish	77.2	19.0	2.5	1.3	barracuda, mullet, perch, shark
Lean Fish	81.8	16.4	0.5	1.3	cod, haddock, hake, plaice

Raymond Jacquot 'Organic constituents of fish' in Fish as Food, Vol. 1

According to the quantity of the fat content, fish are categorized as 'fat fish', 'semi fat fish', and 'lean fish'. It is possible for the fat content to vary within a species of fish, depending on when the fish is caught. For example, at times salmon and herring (fatty fishes) are lean as their fat content vary between 0.35 - 14%, and 2 - 22% respectively depending on the time of the year they are caught. In the summer, the fat content of herring in northern waters is highest. In recognizing this variation, it is important to note that it is not usual for the fat content to vary to the extent where lean fish are classified as fatty.

Nutritional Value

The literature indicates that fish are good sources of protein and energy. Sardines, for example, have a protein value of 25.7% and an energy value of 937 calories per pound. Amino acids such as leucines, lysine, and valine, which are essential for normal human metabolism, can be obtained from fish. The oil in fish provides an ample source of fats required for good human nutrition. A rich source of phosphorous, iron, sulfur, flourine, and iodine, can also be obtained from fish (Clark and Berglund, 1965). So that the nutritional values can be enjoyed, it is necessary that the fish be adequately preserved.

Distribution and retailing of fish

Unprocessed fish for the retail market are usually brought from landing points to a packaging house where they are iced or re-iced and prepared for transportation to the wholesaler or retailer (Bramsnaes, 1965). The factors influencing the amount of ice to be used, and the method of applying it during transportation will depend among other things, on the type of vehicle, its insulation, the outside air, temperature, and the length of the trip. Preparation for transport requires conditioning of the fish by reducing the temperature to about 0°C. To assist the retention of the low temperature, the boxes containing the iced fish, are closely stacked (Bramsnaes, 1965), to form one large mass of packaging, thereby, reducing the surface area exposed to air.

Fish for retail sale are placed on trays with ice, or on blocks of ice slabs. The preserving action of ice is very limited as ice only slows the activities of spoilage bacteria, it does not arrest their growth. Retailing fish in modified atmosphere packages would maintain the "fresh" quality of the fish over a longer shelf-life because it would arrest microbial growth not just slow it down. This is demonstrated by Coyne (1933) where in modified atmosphere, fish kept 2 - 3 times as long as that held in air; and 3 days more than that stored in air according to Lannelonque et al., (1982).

Causes of Spoilage

Spoilage of fish involves changes which render the fish unattractive, unsalable, and unwholesome. It is evidenced by changes in the appearance, odor, texture, and flavor. When fish are improperly or inadequately preserved, microbial decomposition may affect the amino acids content of the fish and in some cases lowers the value of the fish protein. Further, under such unfavourable conditions, autolysis due to digestive action of tissue enzymes can occur. This has the capacity of altering the texture, flavor, and appearance of the fish, but seldom affects its nutritive value (Geiger and Borgstrom, 1965). In fatty fish such as herring and sprat, there is a considerable amount of fish oil in their flesh. This oil contains highly unsaturated fatty acids which are susceptible to oxidation and undergo rapid deterioration when the fish are stored at room temperature. Fat oxidizing enzymes, present in the muscles of the flesh itself, participate in the oxidation process. Some of the products resulting from this oxidation process, e.g. trimethylamine from trimethylamine oxide, have unpleasant flavors, and contribute to the development of unpleasant odors. This oxidative deterioration is referred to as rancidity.

Rancidity becomes apparent in the advanced stages of the chemical deterioration. Theoretically, the degree of rancidity increases as the chemical deterioration progresses, but its assessment is difficult and likely to be inaccurate since it does not develop uniformly in all species of fish.

Nearly all bacteria associated with fish can withstand a temperature range from -7.5 to 30°C, and carry out their destructive activities between 0 and 90°C. Fish spoilage proceeds at about twice as fast as 2.8°C as at 0.3°C. A method of retarding fish spoilage is to store it at temperatures as close as possible to the freezing point of the muscle, -1.1°C (Salle, 1973). Many types of spoilage bacteria require oxygen, and the rate of spoilage therefore may also be a function of the oxygen available (Wang and Brown, 1983).

Changes in Appearance, Odor in Raw Fish

As the fish spoils its surface progressively loses its bright sheen and color. It becomes covered with a thick slime, which gets increasingly turbid, lumpy, and finally changes from clear to a yellow or brown coloration. The eyes gradually sink and shrink, the pupils become cloudy, and the cornea opaque. The gills assume a bleached, pale red color which turns to greyish brown, and then become covered with a thick slime.

The flesh gradually softens until it is very easily stripped from the backbone and exudes liquid under pressure. Its original translucent sheen changes to a dull milky appearance. The digestive tracts of freshly caught fish usually contain food. If the fish are not gutted soon after being caught, the powerful digestive enzymes attack the viscera and belly walls, causing discoloration (so called "belly-burn") or disruption, giving rise to so called "torn bellies".

As spoilage proceeds, there is a gradual change in odor of the raw fish, from 'seaweedy' to 'sweetish', sometimes 'fruity', and later to 'ammonical' or 'fishy'. The limit of edibility in fish usually occurs when the odor consists of a mixture of 'ammonical' (ammonia, trimethylamine, and other amines) and putrid elements (hydrogen sulfide). The following table shows the sensory, chemical, and bacteriological changes in haddock that have been carefully gutted, washed, and stowed in ice.

Methods by which deteriorative changes can be detected

The undesirable flavors, odor, and textures resulting from spoilage can be detected by microbial, chemical, and organoleptic (sensory) test methods.

The microbial method involves the counting of viable bacteria. Many investigators have used aerobic plate count as an index of spoilage for sea foods. Brown et al., (1980) used it to detect spoilage in modified atmosphere storage of rockfish and salmon; Lannelongue et al., (1982) for brown shrimp; Banks et al.,(1980) for finfish; Wang and Brown (1983) for freshwater crayfish, and Parkin and Brown (1983) for dungeness crab. In all these cases, the aerobial count of products stored in CO_2 atmospheres was lower than those stored in air. Parkin et al., (1982) suggested that the CO_2 inhibited the growth of bacteria through the formation of carbonic acid.

Table showing Changes during spoilage

Storage time in ice (days)	0 1 2 3 4 5	6 7 8 9 10 11	12 13 14 15	16 17 18 19 20
Organoleptic changes	<------------> Phase I No marked spoilage	<------------> Phase II First definite signs of spoilage, softer flesh, staler appearance, strengthening of odor	<------------> Phase III Definite stale appearance and odor, and soft flesh	<------------> Phase IV Rapid deterioration from staleness to putridity
Storage time in ice (days)	0 1 2 3 4 5	6 7 8 9 10 11	12 13 14 15	16 17 18 19 20
Chemical changes	<------------ Dimethylamine increases steadily	------------> (to 11) | Trimethylamine increases rapidly (from 8)	Trimethylamine increases rapidly (to 16) | Ammonia increases rapidly (from 14)	Ammonia increases rapidly
Storage time in ice (days)	0 1 2 3 4 5	6 7 8 9 10 11	12 13 14 15	16 17 18 19 20
Changes in bacterial numbers		| bacterial numbers rapidly increase	bacterial numbers rapidly increase	| (to 17)

The organoleptic, chemical, and bacteriological changes in haddocks, carefully gutted and washed and stowed in plenty of ice (Cutting et al., 1953).

Source: Bramsnaes (1965) in Fish as Food, Vol 4. Edited by Georg Borgstrom.

Chemical methods involve measurement of surface pH, and volatile basic nitrogen compound. A rise in pH in a fresh product is usually associated with spoilage as it indicates production of amine by bacteria. However, the majority of studies on pH as a determinant of fish spoilage indicates that pH is not a good index of spoilage. The main reason being that the usefulness of pH determination is often greatly restricted by its variability from sample to sample by its cyclic fluctuations during the storage process (Faber, 1965). Nevertheless it is still used since a change in pH reflects bacterial activity (Wang and Brown, 1983) and, under restricted conditions, pH limits for definite levels of spoilage may be set.

Volatile basic nitrogen compounds include ammonia, trimethylamine, thiobarbituric acid, and total volatile nitrogen. Ammonia production is due to the bacterial breakdown of muscle protein and is expected to be directly proportional to the bacteria population. Trimethylamine is produced by bacterial action on trimethylamine-oxide in marine fish. Thiobarbituric acid is an indicator of lipid oxidation (rancidity).

Total volatile nitrogen includes the measurement of trimethylamine and ammonia. The reliability of total basic nitrogen compounds as an index of spoilage is questionable as conflicting results have been reported by several authors. Some investigators find it useful in estimating the freshness of lean fish such as cod, and useless for fat fish such as herring.

Bank et al. (1980) concluded that total volatile nitrogen had little value in his study on shelf life of CO_2 packaged finfish from the Gulf of Mexico, as total volatile nitrogen values were low enough for spoiled fish to be rated as good.

Total volatile nitrogen values for the degree of freshness are:

< 12	fresh fish out the water
12 - 20	good quality - very slightly decomposed
20 - 25	decomposed and inedible (Lannelongue et al., 1982).

Organoleptic (sensory) methods involve the evaluation of odor, visual appearance and textural changes. It is the oldest and most widely used method of evaluating the qualities of fresh fish. Its main advantage is based on the fact that no special laboratory equipment is needed. Its main disadvantage is that it is subjective. Nevertheless, it is the criterion against which the usefulness of the other methods are judged (Farber, 1965).

SECTION III

PRESERVATION OF FISH

The rapid perishability of fish has made preservation against putrefaction an urgent necessity (Cutting, 1965). This section attempts to summarize some of the mostly used methods of preservation.

Fish preservation includes any method extending the storage life by retarding or preventing deterioration of flavors, odor, color, texture, and appearance. Deteriorative changes may result from microbial growth, and or enzymatic (chemical) reactions. The main objectives of food preservation are to extend the shelf life, retain nutritive value, and ensure safety by delaying or preventing microbial decomposition by supressing the growth of pathogenic microorganisms. Packaging plays an important part in preservation as the integrety of the package can influence the degree to which these objectives are realized.

Basically, there are two methods of preservation:

(1) Chemical - involves the use of chemical compounds in a food system to inhibit microbial activities, e.g. salting; and

(2) Physical - involves control of microbial prolification by actual treatment of fish such as dehydration, and low temperature storage.

Sometimes the physical methods are used in combination with the chemical ones to allow the preserving agents to be used at lower concentrations and retain good keeping quality with only minor changes in the product's properties.

Dehydration

Dehydration refers to the process of drying by controled, artificial means. Several different methods can be used, but whatever the method used, the conditioning of the mass - heat transfer and certain thermodynamic properties of the system must be considered. The outward movement of water in the sequence:

migration within material ⟶ removal from surface ⟶ mixing with surrounding atmosphere material ⟶ removal from vicinity of surface

must, in convential methods of drying, be accompanied by the inward transfer of heat indicated by the sequence

emission from the source ⟶ transfer to surface ⟶ conduction within material ⟶ provision of latent heat of evaporation and partial enthalpy of dilution of system regarded as a solution (Jason, 1965).

Dehydrated fish are inferior in quality to fresh fish. They require a considerable time for reconstitution, and are rubbery in texture, difficult to chew, and have a characteristic 'cured' flavor. Dehydrated fish has not reached perfection because of the severe damage suffered by the cells of the fish and the irreversible changes that take place during drying.

Salt Curing

Salt curing, an age-old method of preserving fish, is losing ground to other methods of preservation due to major improvements in such new methods, increasing use of fresh fish distribution, and the disadvantages of salting. In the salting method, dry salt is used for non-fatty species such as cod; and pickling is used for fatty species such as mackerel.

The salt sensitive group of bacteria (halophobic) which includes most putrefactive types in relation to fish, does not grow in salt concentrations greater than 6%. They may, however, remain viable for long periods of time. The halotolerant group of bacteria which includes some anaerobes, can grow in brine concentrations greater than 6% and even up to saturation. The growth rate varies inversely with the concentration of salt. The salt loving group of bacteria (halophilic) which includes those microbes causing "pink" and "dun" in salted fish, grow best in the presence of salt. They require concentration usually greater than 2%.

The disadvantage of salting lies in the facts that the halotolerant and halophilic can grow at temperatures of +5°C and above, and that their growth can be accelerated by impurities in the salt. Under favorable conditions to the bacteria spoilage will occur. In addition, if the salting is inefficiently done by using too little salt or poor quality fish, then the normal putrefactive bacteria may become active (Shewan, 1965). Over extended period of time, brine may cause denaturing and dissolution of some muscle protein and may combine with the muscle tissues to such an extent that its removal is impossible. Further, with some fish, brine tends to cause dehydration. This results in a temporary or permanent loss of weight. The effects of these changes are influenced by the temperature, pH, and concentration of the brine.

Antioxidants

The highly unsaturated fatty acids in fish are very susceptible to oxygen and to their tissue enzymes which act as pro-oxidants. The use of antioxidants, (such as ascorbic acid, butylated hydroxyanisole, liquid smoke, and tocopherol) on oxidative changes in fat and lean fish in preserving the nutritive value of fish have been investigated. The results are not conclusive. Heen and Karsti (1965) found that antioxidants which were highly active in autoxidation of unsaturated fatty acids in isolation, may fail when the lipids are in situ. Again, Banks (Banks, 1952b reviewed in Heen and Karsti, 1965) reported that antioxidants showed varying results in different species as well as in the same species due to the difference in fat distribution which is influenced by the time of the year (season) the fish is caught. For example, herring have its highest fat content in the summer. More research is needed on the use of antioxidant as a preservation before its 'merits' can be accepted.

Radiation

While radiation offers the advantage of allowing storage of fish without refrigeration, it has a significant disadvantage. It causes unwanted chemical changes which may adversely affect the quality, odor, flavor, texture, and color of the fish. Fish treated by radiation have been variously described as 'metallic', 'burnt-feather-like', and 'rubbery'. This method has limited usefulness in that the sterilizing does of radiation only partially inactivate the enzymes of the fish tissue. Supplementary processing may be necessary to prevent enzymatic deterioration.

Low Temperature

Low storage temperature is used to prevent oxidation of fatty acids, and bacterial spoilage. Common methods are icing, refrigeration, and freezing which are used both commercially and in the home to preserve fish during storage and transporation. Icing refers to packing fish on ice, refrigeration to storage of fish below 15°C and above freezing, and freezing is the storage at temperatures below the freezing point of the fish tissue.

Icing, refrigeration, and freezing lower the storage temperature which in turn affects the rate of spoilage by reducing the rate of chemical reactions, and microbial metabolisms. Generally, the lower the temperature the slower the rate of reactions and bacterial multiplication. Of the three, freezing is the most unsuitable for the growth of micro-organisms, but it causes certain physical changes in the fish such as crystalization of ice with expansion of volume, and dehydration as the water in the muscle is replaced by ice. The extent to which the temperature is lowered and the degree of dehydration achieved will contribute to the preserving effects of the freezing method. This method, however, has certain disadvantages.

The disadvantages/shortcomings of freezing are:

(1) Rate of Freezing.

If freezing is too slow, large crystals are formed which cause porosity in the tissue, and perforation of the tissue which may then become spongy upon thawing;

(2) Frozen fish is frequently dry, tough, and flavorless.

Severe dehydration causes the fish surface to become dry and fibrous. In some cases the surface may change color - resulting in 'freezer burn'. The toughness of the fish tissue increases progressively during freezing;

(3) Thawing of frozen fish results in drip and free water, causing weight loss.

Certain precautions can be taken to reduce the above, but spoilage by organisms capable of withstanding extremely low temperature still remains a problem.

MODIFIED ATMOSPHERE PACKAGING

Since CO_2 is very effective against such spoilage organisms, which can withstand low temperatures, the use of modified CO_2 atmosphere is considered to be a useful alternative method of preservation. In its function as preservative, it displaces oxygen, a vital requirement for aerobic organisms. It also intervenes in the respiratory metabolism of various micro-organisms, in that increased levels of CO_2 and reduced levels of O_2 have the effect of decreasing the respiratory process.

Modified atmosphere packaging involves evacuation of air from an oxygen-impermeable container, injection of a desired gas mixture, then sealing of the container. The anti-microbial activity depends on the gas concentration, the microorganisms in the system, the storage temperature, and the water activity of the system (Block, 1983).

Concentration of CO_2

Coyne (1933) observed that concentrations of CO_2 up to 100% were very effective in preserving fresh fish, with optimum conditions being observed with 40 - 60% CO_2. Carbon dioxide lowers the pH of the system by forming carbonic acid (Koser and Skinner, 1922; Hays et al., 1959) as CO_2 is absorbed on the fish surface and ionized according to the equation:-

$$\underset{\text{carbon dioxide}}{CO_2} + \underset{\text{water}}{H_2O} \longrightarrow \underset{\text{carbonic acid}}{H_2CO_3} \longrightarrow \underset{\text{hydrogen ion}}{H^+} + \underset{\text{bicarbonate ion}}{HCO_3^-}$$

Since the result of the reaction is acidic, acid-sensitive micro-organisms are inhibited while the acid tolerant ones are stimulated (Banks et al., 1980).

The Microorganisms

Carbon dioxide restricts the growth of most objectionable putrefactive, off-odor producing bacteria (Sander and Soo, 1978). Others, such as lactic acid bacteria, will thrive and predominate in CO_2 atmosphere, but these cause less noticeable sensory changes in fish (Ogrydziak and Brown, 1982; Souder, 1980) and are not considered much of a problem.

The Storage Temperature

Enfors and Molin (1981) investigated the influence of temperature on the inhibitory growth effect of CO_2 on the spoilage bacteria. The results indicated that the relative inhibitory effect decreased as the temperature increased. Carbon dioxide applied under pressure with low temperature reduced the rate of spoilage reactions of bacteria and enzymes, and the rate of bacteria multiplication (Lueck, 1980).

The Water Activity of the System

The water provides a medium to transport the gas into the system. The CO_2 molecule is more soluble in water than other gases. In water, more than 99% CO_2 exists as dissolved gas and less than 1% exists as carbonic acid, which partly dissociates to give the afore mentioned equation.

LABORATORY SYSTEM OF CO_2-ENRICHED ATMOSPHERE

Investigators have demonstrated that modified CO_2-enriched atmosphere was effective in extending the storage life of fish and other seafoods (Lannelongue et al., 1982; Parkin and Brown, 1983). The efficacy of CO_2 in extending the shelf-life of fish was demonstrated in laboratories under controled conditions.

The test containers were made from polyvinyl chloride, nylon - surlyn, and polyvinylidene chloride. Low density polyethylene was used to facilitate sealing of flexible containers by heat sealing.

Banks et al., (1980) used plastic molded meat trays overwrapped with a 75 guage polyvinyl chloride (PVC) stretched film. This package was then placed in a nylon-surlyn bag with the following characteristics:-

(1) Oxygen Tramission Rate (OTR) = $60cc/m^2/24\ hr/23°C/10\%$ RH

(2) Moisture Vapor Transmission Rate (MVTR) = $10g/m^2/$ 24 hr/23°C/0% RH

(3) Carbon dioxide Transmission Rate (CTR) = $250cc/m^2/$ 24 hr/23°C/0% RH

Lannelongue et al., (1981, and 1982) used extruded laminated polyvinylidene chloride to provide a barrier to gases; and low density polyethylene to facilitate heat sealing. Woyewoda et al., (1984) used cylindrical storage chamber with plexi glass ends made from polyvinyl chloride pipe to contain fish packaged in 3 mil polyethylene/1 mil nylon laminate. The characteristics of the laminate used were:

(1) OTR = 88cc/24 h/100 in^2/mil thickness/25°C/50% RH

(2) CTR = 549cc/24 h/100m^2/mil thickness/25°C/50% RH

In the studies reviewed, the investigators were less concerned in determining the appropriateness of the test containers to practical application, rather they were concerned with determining the following:

(1) Gases, and their concentrations to be used in a modified atmosphere system,

(2) The maximum storage periods that can be achieved,

(3) The storage characteristics giving maximum storage, and

(4) The microbiological and chemical changes of the fish stored in modified gaseous atmospheres.

Since the investigators have proven, by laboratory testings, that the technique of modified atmosphere is feasible then the next stage is to move this technique to the practical application of the market place where its merits would be tested.

The first step in this stage would be to develop an appropriate package to maintain the favorable environment and to function in the storage conditions dictated by the product. The package should be economically and commercially produceable.

This study aims to identify the criteria for materials suitable for making packages for modified atmosphere preserved fish.

SECTION IV

CRITERIA FOR MATERIAL SELECTION

It should be possible for the beneficial preservative effects of modified atmosphere storage to be continued in an appropriate consumer package. In this instance, the consumer package would be substituted for the laboratory containers used in the referenced studies which have been conducted up to this time. In modified atmosphere, however, the package would become an integral part of the product/package system, as it would be required to provide the enclosure to protect the atmosphere especially selected to preserve the fish. It is imperative that the atmosphere be maintained during packing, storage, and distribution to the consumer. This makes the package inseparable from the product. Any inadequacy, removal, or damage of the package will destroy the selected preserving atmosphere by introduction of additional oxygen (air), and microorganisms.

Based on the requirements of the product, the criteria for selecting appropriate materials for applying to modified atmosphere packaging of fish are:-

(1) High barrier properties - for gases, water vapor, odors,

(2) Chemical inertness - to the product, and weak acids,

(3) Good mechanical strength - against physical damages,

(4) Transparency - sale of the product through consumer appeal,

(5) Inexpensive,

(6) FDA acceptance.

Selection of materials for this package is important and would be made according to the requirements dictated by the product/package system. To determine the requirements of the product/package systems, the following must be defined:-

(1) The product - its characteristics, causes of spoilage, damage, etc.,

(2) The market considerations - display method, consumer, price of product, size of package, etc.,

(3) Distribution of the product - means and types of transportation, distance, warehousing, climate, etc.,

(4) Production of the product/package system - method of processing, filling, etc., and

(5) Properties of the packaging materials.

The above conditions are interrelated and should all be taken into account when designing the optimum package. However, for the purpose of this study, these conditions will be separated and the properties of the packaging materials will be considered in order to identify the criteria for selecting materials suitable for the packaging of modified atmosphere preserved fish. In establishing these criteria, it will be necessary to identify the product and the functions required of the package.

The product is fish in combination with the selected gaseous atmosphere. The product is new, and the possibilities to capture new markets, and to penetrate new areas of distribution exist because of the product's extended shelf life. Success in the market is critical to the packaging material's ability to maintain the shelf life of the product. Modified atmosphere preserved fish is expected to have a shelf life of 16 to 25 days (Coyne, 1933) and 28 days (Parkin et al., 1982). Therefore, a packaging material that can maintain a desirable gas mixture, with little or no change in its composition, will be required to obtain maximum preservation of 28 days.

In the process of selecting a primary packaging material for modified atmosphere preserved fish, the following basic questions must be answered:

(1) What functions must the package performed?

(2) What types of packaging materials are available, have been used, or being used for similar products?

(3) What are the advantages and disadvantages of each alternative material?

(4) Cost of the materials, machinery, machine efficiency, labor?

Packaging plays many functions but for modified atmosphere preserved fish, emphasis is placed on the function of safe delivery of the product through the distribution system. The package is required to protect the product against external microbial contamination, handling, and to control the passage of water vapor, odor, and gases such as CO_2, O_2, and N_2. In addition, the package is required to protect the product against mechanical damage.

Introduction or escape of CO_2, O_2, and N_2, will disturb the selected levels of concentration of the gaseous atmosphere. The optimum levels of concentrations for CO_2 lie between 40 and 60% (Coyne, 1933; and Lannelongue et al., 1982). A decrease of CO_2 due to the escape of gas from the system will reduce the CO_2 concentration.

Bacterial count increases rapidly in atmospheres of less than 20% concentration of CO_2 (Lannelongue, 1982).

Increased levels of CO_2 due to introduction of gas into the system will not have any significant advantage, but may affect the color of the fish' skin. Coyne (1933) concluded that high concentrations of CO_2 have a tendency of bleaching the skin.

An introduction of O_2 would increase the level of O_2 over time. Increased levels of O_2 could become a problem in causing rancidity. Rancidity develops from the oxidation of the poly-unsaturated fatty acids, particularly in fatty fish (Lannelongue, 1982).

Introduction of moisture has the potential of bringing in gases and undesirable foreign organisms, which could be injurious to the system. Loss of moisture would influence the mobility and solubility of CO_2 within the system. A reduction in the availability of water would increase the salt concentration of the fish, and in increased salt concentration, the solubility of CO_2 decreases. In water CO_2 dissolves to form carbonic acid which is unfavorable to the spoilage bacteria associated with fish. Consequently, loss of water would affect the preserving action of CO_2.

The gaseous atmosphere is sensitive to temperature changes and acidic environment. Barnes et al., (1980) have shown that low temperature modified atmosphere storage was effective. High temperatures increase bacterial activities which in turn accelerate the spoilage rate of fish. The material selected should be able to perform in low temperature range such as $35 \pm 2^{o}F$ (Parkin et al., 1981) in which the product/package would be stored. The reaction of CO_2 and the water in the system is acidic viz:

H_2O	+	CO_2	--->	H_2CO_3	--->	H^+	+	HCO_3^-
water		carbon dioxide		carbonic acid		hydrogen ion		bicarbonate ion

This reaction increases the pH of the system which is deleterious to the microbes. The material should therefore be resistant to weak acids and functional in low temperature.

Another factor to be considered in preserving the gaseous atmosphere for the product/package system is the nature of any damaging event that can occur during the time the product needs to be protected. If whole fish is being packed, damage to the package by the fins is likely, and the selected material should not be easily punctured. Puncture of the material will cause the fish to be exposed to bacteria, oxygen (gases), and vapors of the air, which are active agents in the spoilage of fish. Secondly, the adequacy of the gas barrier provided by the material is a function of the soundness of the seal. A poor seal will allow two-way movement of gases and vapors which will disturb the levels of concentration of the gases, and will render the gaseous atmosphere ineffective in preserving the fish. The selected material should therefore, possess good mechanical strength to protect the product from exposure due to mechanical damages, and should be capable of making a good heat seal.

The method of estimating the freshness of the product by the consumer would be sensory analysis, therefore the material should be transparent, allowing the customer to see the product. Opaque materials could engender distrust for this new product and consequently sale resistance. Cost should also be considered since it could be the deciding factor in selecting materials with the optimum features. Cost is eventually passed on to the consumer, and will influence the final cost of the finished product.

Finally, packaging materials that are not accepted by the FDA for food packaging application would not be considered. FDA regulations do not permit packaging material to give up chemical compounds to food products.

SECTION V

MATERIALS

Many food items, over the years, have been distributed in packages composed of paper, glass, metals, and plastics. This study attempts to examine the applicability of these various materials in satisfying the criteria required to packaged modified atmosphere preserved fish. Modified atmosphere preserved fish primarily requires high gas barrier materials to provide resitance to passage of gases, vapors, odor, and oils and fats.

Consequently, paper being porous will not be considered. Laminated products of paper and plastic, and paper treated with various chemicals are used in some high barrier applications. However, paper remains opaque and would not fulfil the transparent criteria. Therefore it is eliminated from the material evaluation.

Metal containers, although offering good barrier properties, do not allow for visual inspection and are generally used for processed foods. This fact that metal containers have long been the standard for heat-sterilized, preserved food-stuffs is disadvantageous in that the average homemaker/consumer being out of habit would stack tins of modified preserved fish on the same shelf used for regular canned foods.

Glass is transparent and allows visibility of its contents. It is imperious to odor, flavors, and gases, is chemically inert, and can maintain long shelf life. However, glass suffers certain disadvantages which rule against its selection as a material for modified atmosphere packaging. Glass is readily broken and with a low resistance to shock treatment, it will not always withstand sudden changes in temperature.

The potential of plastic as a high barrier material is increasing. The chief advantages in using plastics as a packaging material is that most polymers possess excellent physical properties such as strength, and toughness, combined with low weight and cost. It is flexible and may be resistant to cracking. In addition plastic film materials can be laminated and coextruded. Laminations and coextrusions can significantly improve the barrier properties desired, and other functional properties such as mechanical strength required from the material. They allow for the combination of thin layers of materials which by themselves would be either too expensive for the required thickness, or inadequate in their function.

Successful applications of high barrier plastic materials have been made to proteinaceous foods such as prepacked bacon and cheese. Use of plastic in modified packaging of fish would be consistent with standard plastic packaging used for the other proteinaceous products. Similar machines and technique for making and filling packages as in the case of cheese could be used in the case of the fish. Having a package for modified atmosphere preserved fish would assist in the distribution of the product in areas of needs.

By combining the protection needed for the product with the properties of high barrier materials, a judgement can be made of the suitability of each material to the application of modified atmosphere packaging. The table, on the following page, gives the product/material matrix.

This product/material matrix contrasts the characteristics of the various plastics with those required to protect and maintain the quality of the product. It indicates that nylon, polyester, and saran (PVDC) offer the best product/material combinations.

SUMMARY OF MATERIALS

(1) Nylon - provides good barrier properties but is a poor water vapor barrier. This can be improved by coating the nylon with polyethylene, which has excellent water vapor barrier property and is inexpensive. In this combination, the nylon is used for the substrate. It provides the strength to protect the product against physical abuses.

(2) Polyester - although only a fair barrier to gases, it can be useful because it is very strong. Consequently, thin gages can be used which, in effect, reduces the cost of the material for a given application. It is a good base for laminates, it has good water vapor properties, and when coated makes a good heat seal.

(3) Saran (PVDC) - an excellent gas barrier material - is expensive, but it has good mechanical properties which allows thin gages of the material to be used. This in effect reduces the cost. Its disadvantages is that

Table showing properties of flexible plastic materials and those required for modified atmosphere packages fish

Desired Features	Nylon	Polyester	Polyethylene	Polypropylene	Saran (PVDC)	Styrene
Low Temperature				thin film better	suitable	
Weak acid resistant	good	good	good	excellent	good	good
Transparency		clear	controlled	good	good	brilliant
CO_2 Barrier	good	poor	fair	good	very good	poor
Oxygen Barrier	good	poor	fair	good	very good	poor
Nitrogen Barrier	good	poor	fair	good	very good	poor
Water Vapor Barrier	poor	mod. good at low temp.	excellent	good	very good	poor
Heat Seal	good - 85% strong	good when coated	difficult			difficult
Odor Barrier	good		poor			poor
Tensile Strength	good	good	varies with temp.			
Price	9¢/1000 in^2	9¢/1000 in^2	2.5¢/1000 in^2	3¢/1000 in^2	12¢/1000 in^2	8¢/1000 in^2
FDA Approval	yes	yes	provided no additives used in manufacture	provided no additive used in manufacture	yes, for most types	provided no additives are used in manufacture
Suitable for Gas Package	yes, gas/vacuum					used for 95% N_2 & 5% O_2
Aging/stability	shrinks & expands, changes/ humidity	good				ages well
Substrate for Laminate	yes; $\frac{1}{2}$ml nylon coated 2ml PET moisture barrier	yes, $\frac{1}{2}$ml PE with $1\frac{1}{2}$ml PET				

Source: Hanlon J. F., Handbook of Package Engineering. 2nd. Edition. McGraw-Hill Book Company. 1984.

it has clinging properties which is problematic on machines. This can be overcome by sandwiching saran in multilayered material.

CONCLUSION

It appears that no one material possesses all the desirable properties required for modified atmosphere packaging. Therefore coextrusions and laminates of materials having different properties may be used to provide the desired functions.

Theoretically, the selected materials seem adequate for the product/package system, but before any one can be confirmed suitable for the system, it needs to be tested to evaluate its ultimate performance. Hanlon (1984) recommends that materials be tested over several weeks, at extreme temperatures which the product/package system is expected to undergo in commercial use and at room temperatures.

SECTION VI

MATERIALS TESTING

Materials testing is an important stage in the development of packages. It is used to evaluate the performance of materials and serves to confirm the suitability of selected materials for the specific applications. In the case of modified atmosphere packaging of fish, the package material is required to have high barrier properties and good mechanical strength. It should be chemically inert, transparent, inexpensive, and accepted by the FDA.

Assuming that the selected material is chemically inert where the product is concerned, then the tests to evaluate the suitability of materials for use in the product/package system would include:

(1) Test to evaluate clarity - visibility of the product is an important consideration in marketing the product, and the relative transparency of the different plastics is of interest.

(2) Shipping and abuse testing - In order to maintain the atmosphere, the product/package system must arrive at its destination undamaged. Some tests for the durability during transportation and handling are vibration, shocks, and drop.

(3) Test to evaluate water vapor transpiration rate (WVTR) - the system is likely to encounter variation in humidity and a measure of the gain or loss of water vapor through the

packaging materials would also be of interest.

(4) Test to evaluate gas permeability - this measures the rate of transmission of gases through the material. The rate of transmission of gases in proportional to the thickness of the material. That is, twice the thickness will give about half the rate. Gases used in combination act independently and transpire through the material as though they were alone. The value of permeability is also a function of temperature and pressure, and will have little meaning without specifying the experimental conditions, under which the permeability is measured.

Apart from mechanical strength, barrier to gases and vapors is the most critical property of the materials packaging produce, preserved in modified gaseous atmospheres. Two-way passage of gases and vapors through packages for modified atmosphere preserved items will interfere with the effectiveness of CO_2 as a preservative as explained in section IV, and as a result spoilage will be hastened. Identifying packaging materials suitable for gas packaging is of concern to this study, and a test to determine gas passage or gas permeability will be described in the next section.

SECTION VII

TEST FOR GAS PERMEABILITY

Gas permeability refers to the passage of gas through a material (membrane). It can occur in two ways. The first way is a capillary flow through small openings (pinholes) in a material. In the second method, the gas dissolves in the exposed surface of the membrane, diffuses through the material to the opposite surface and escapes. The second method, proposed by Graham, is of greater interest, since with modern materials pinholing is considered to be a defect.

The properties of the membrane (material), the properties of the gas or vapors, and the degree to which interaction between the gas and the material may occur, will influence the rate at which the gas or vapor passes through the membrane.

Investigators of gas and vapor permeability have concluded that the amount of gas or vapor transmitted is directly proportional to the:-

(1) Area of material exposed to the gas and/or vapor,

(2) The duration of the exposure, and

(3) The pressure differential across the thickness of the barrier material. This is inversely proportional to the thickness of the material.

One method of determining the rate of gas/vapor permeability of a material is by gas chromatography. Gas chromatography is a method that separates components of mixtures of volatile compounds and quantifies them. This method is selected for this study because:-

(1) The process is well documented and widely used,

(2) Gas chromatography gives quantitative analysis, and variations in the percentage composition of the gases can be detected, and

(3) The interpretation of the data is simple.

Gas chromatograph can be used to analyze mixture/s of volatile compounds boiling at any temperature and for any substance which can be heated sufficiently without decomposing to give a vapor pressure of a few millimeters of mercury (Littlewood, 1970).

Equipment:-

(1) Gas chromatograph - used for gas analysis,

(2) Heat Sealer - used for sealing pouches,

(3) Cylinders of oxygen, nitrogen, and carbon dioxide - for creating the atmospheres.

METHODOLOGY

Materials - The materials selected for testing will be flexible, and rigid high barrier ones which are commercially available.

Procedure - Pouches are to be made from the samples of each flexible material, and tested to ensure seal integrety. Prior seal testing will establish optimum heat seal conditions which provide maximum strength.

(2) Pouches will be divided into three groups and

labeled with the gas to be contained - that is CO_2, N_2, O_2; Air from each pouch would be evacuated, the gas indicated by the label injected under 15 in^3 atmospheric pressure, then the pouch would be sealed;

(3) Each of the three sets of pouches (above) would be separated into two groups, one group would be stored at $4 \pm 2^{o}C$, and the other at room temperature; and

(4) After 1, 3, 7, 14, 21, and 28 days of storage the gas from 6 pouches (three from the set stored at room temperature, and three from the refrigerated set) will be analysed by gas chromatography.

The average result from each set will be used for the record.

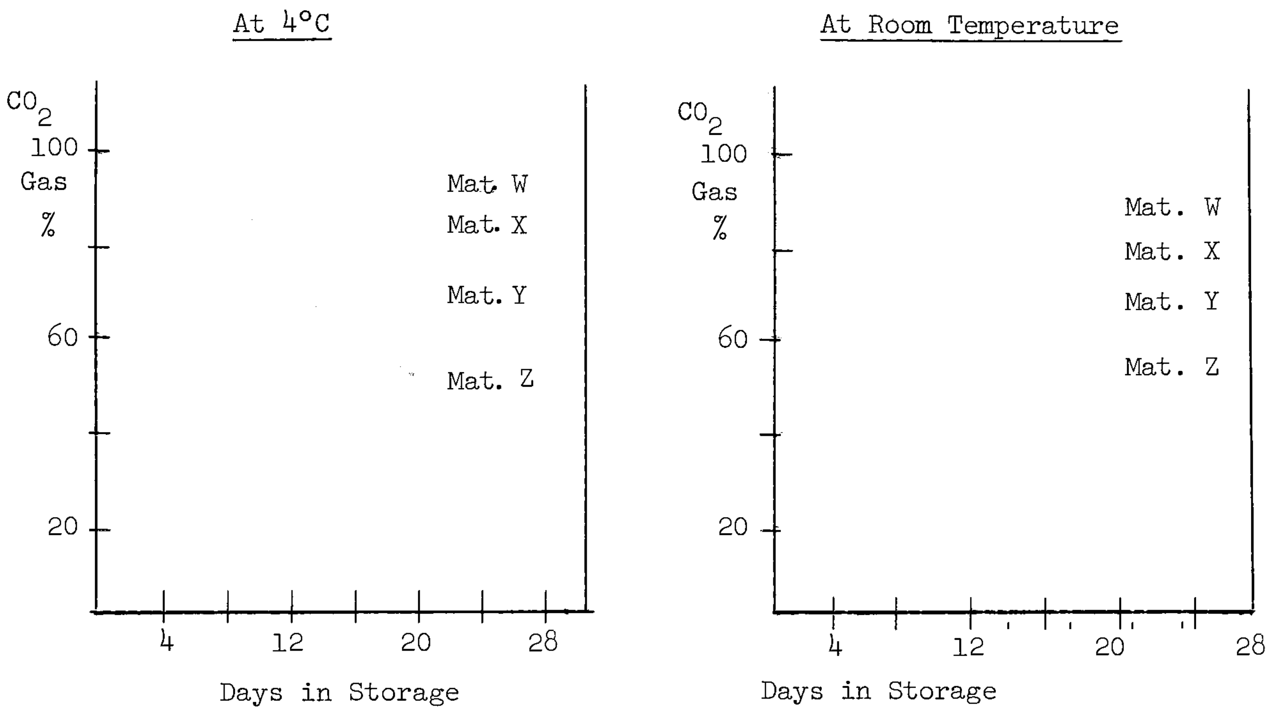
Recording of the data:-

The following table and figures are hypothetical so far as the data they exhibit, but they are illustrative of the way the data will be treated.

Table showing analysis of gas content in pouches made from the selected materials, W,X,Y,Z.

Storage Intervals (days)	Gas %	Material W 4°C	Material W Room Temp.	Material X 4°C	Material X Room Temp.	Material Y 4°C	Material Y Room Temp.	Material Z 4°C	Material Z Room Temp.
Start	CO_2	100	100	100	100	100	100	100	100
	O_2	100	100	100	100	100	100	100	100
	N_2	100	100	100	100	100	100	100	100
Day 1	CO_2	a_1	a_2	b_1	b_2	c_1	c_2	d_1	d_2
	N_2	m_1	m_2	n_1	n_2	o_1	o_2	p_1	p_2
	O_2	q_1	q_2	r_1	r_2	s_1	s_2	t_1	t_2
Day 3									
'	"	"	"	"	"	"	"	"	"
'									
'									
Day 28									

Figures showing changes in the percentage of gases in the pouches



ANALYSIS OF DATA

The data obtained from the tests would be analyzed statistically. Based on the results, the optimum materials would be the ones offering low gas permeability and suitable low temperature performance in regards to handling and distance.

SECTION VIII

CONCLUSION, SUMMARY, AND RECOMMENDATION

The extension of shelf life of fish, a highly perishable premium product which provide valuable dietary benefits, has been a serious concern of the fish industry.

Traditional methods of preservation have either limited effects, undesirable effects, or both in protecting the product biological attack and enzymatic degradation. Modified storage emmerges as an improved preservative method because of its efficiency in bacterial inhibition, and subsequent reduction of the microbial population.

The introduction of high barrier plastic containers, possessing low gas and vapor permeation rates, has encouraged the use of these high barrier materials as alternatives to glass or metal in the packaging of foods requiring protection from gas and vapor transmission. Successful application of high barrier materials have been used in packaging of proteinaceous foods such as bacon. A similar packaging type and technology could be adopted in the case of modified atmosphere preserved fish.

The feasibility of packaging modified atmosphere preserved fish in high barrier flexible materials, from a permeation point of view, has been demonstrated in this study. Although low permeability is one of the chief parameters leading to an acceptable package for modified atmosphere preserved fish, other parameters such as mechanical strength, and good seals can affect the overall quality of the fish and should also be examined. A material of low mechanical strength is susceptible to punctures and breakages. Breaks in the materials will permit passage of gases and vapor in and out of the package, which will in turn alter the optimum concentration of gases in the system of modified atmosphere, and introduce foreign items (bacteria). This will adversely affect the preservating ability of the system and spoilage will result. A poor seal will have similar results.

Identification of a package capable of maintaining gaseous atmosphere is an important step in transfering the technique of modified preserved fish from the laboratory to the practical application of the market place, where the benefits of prolonged storage life of the product can be applied and appreciated.

Prolonged storage life would allow more time for the distribution of the product over longer distances before spoilage occur. This would result in making the 'fresh' product available to consumers in areas remote from the catching areas. Further, this package could play a great part in expanding the variety of proteinaceous food donated to areas with food problems. In this way it helps in alleviating food problems and in reducing the inadequacy in the supply of 'fresh' and nutritious foods in area experiencing food problems.

Such a package could also be of benefit to the economies of developing countries where fishing is practised. Export of fish to areas (within distances determined by the shelf life of the product) could make an important contribution to that country's food industry as a valuable export item.

Selection of the packaging material is a basic step in the development of packages for the application to modified atmosphere preserved food items. The criteria for selecting materials for the package constrution were identified by this study. Testing will be required to confirm any selection made. Since gas stability is critical to the preservative effect of the system, a test is described for gas permeability in this study.

Further tests, including abuse testing are recommended before any selected material can be confirmed suitable for application to modified atmosphere packaging of fish. Having identified suitable packaging materials, subsequent studies are needed on appropriate package designs and evaluation of package performance.

BIBLIOGRAPHY

Aickin, C.C. & Thomas, R.C. 1975. Micro-electrode Measurement of the internal pH of Crab Muscle Fibre. J. Physiol. 52. 803

Banks, H., Nickelson II,R., & Finne, G. 1980. Shelf-life Studies on Carbon Dioxide Packaged Finfish From the Gulf of Mexico. J. Food Sci. 45 (2), 157 - 162

Barnett, H., Nelson, P., Hunter, P., Bauer, S., & Groninger, H. 1971. Studies On The Use of Whole Fish. Fish Bull. 69: 433

Block, S.S., Lea, & Febiger. 1983. Disinfection, Sterilization and Preservation. 3rd Edition

Borgstrom, G. Fish as Food Vol. I, II, III, & IV. Academic Press. AP 1965

Bramsnaes, F. 1965. Handling of Fresh Fish. Fish as Food Vol. IV, Edited by George Borgstrom

Brown, W.D., Albright, M., Watts, D.A., Heyer, B., Spruce, B., & Price, R.J. 1980. Modified Atmosphere Storage of Rockfish (Seabstes Miniatus) and Silver Salmon (Oncorhynchus kisutch) J. Food Sci. 45: 93

Bullard, F., & Collins, J., 1978. Physical and Chemical Changes of Pink Shrimp, Pandalus borealis, held in carbon dioxide modified refrigerated seawater compared with pink shrimp held in ice. Fish Bull. 76: 73

Clark, B.S., & Berglund, R.M. 1965. Canning of marine sardine in Fish as Food Vol. 4. Edited by George Borgstrom (Academic Press).

Clark, D.S. & Takács, J. 1980. Gases as preservatives. In microbial Ecology of Foods. Vol. 1 International Commission on Microbiological Specifications of Foods. New York, Academic Press.

Coyne, F.P. 1933. The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish. Part II Gas storage of fresh fish. J. Soc. Chem. Ind. (London) 52, 19T - 24T.

Crosby, N.T. 1981. Food Packaging Materials aspects of analysis and migration of contaminants. Applied Science Publishers Ltd. London.

Cutting, C.L. 1965. Historical aspect of Fish, in Fish as Food Vol. 2 Edited by G. Borgstrom (Academic Press).

Driver, W.E. 1979. Plastics Chemistry & Technology. VanNostrand Reinhold Company.

Enfor, S.O., and Molin, G. 1981. The influence of temperature on the growth inhibitory effect of carbon dioxide on Pseudomas fragi and Bacillus cereus Can. J. Microbiol. 27: 15.

Enfors, S.O. and Molin, G. 1980. Effect of high concentrations of carbon dioxide on growth rate of Pseudomonas fragi, Bacillus cereus, and Streptoccus cremoris. J. Appl. Bacteriol. 48: 409.

Farber, L. 1965. Freshness Test, in Fish as Food Vol. 4 Edited by Georg Borgstrom. Academic Press (1965).

Finne, G. 1982. Modified & Controled - atmosphere storage of muscle foods. Food Technology 1982, 128 - 133.

Geiger, E., & Borgstrom G. 1962. Fish Protein - Nutritive aspects in Fish as Food Vol. 2, Edited by G. Borgstrom. Academic Press (1962). 29 - 114.

Gill and Tan (1980). Effect of carbon dioxide on growth of meat spoilage bacteria. Appl. Environ. Microbiol. 39: 317.

Gill, C.O., and Tan, K.H. 1979. Effect of carbon dioxide on growth of Pseudomonas fluorescens, Appl. Environ. Microbiol., 38, 327 - 340.

Griffin Jr., R.C. and Sacharow S. 1972. Packaging Material in Principles Packaging. AVI Publishing Co.

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. (London) 52: 137.

Hanlon, J.F. Packaging Engineering. 2nd Edition McGraw-Hill Book Company. 1984.

Hanson, N.A. and Duckworth, H.A.G. 1982. C A P = Preservation. Packaging, April: 23.

Hays, G.L., Burroughs, J.D. and Warner, R.C. 1959. Microbiological Aspects of pressure packaged foods II. The effect of various gases. Food Technol., 13, 567 - 570.

Hiltz, D.F., Lall, B.S., Lemon, D.W., and Dyer, W.J. 1976. Deteriorative changes during frozen storage in fillets and minced flesh of silver hake (Merluccius bilinearis) processed from round fish held in ice and refrigerated sea water. J. Fish Res. Bd. Can. 33: 2560.

Jason, A.C. 1962. Drying and dehydration in Fish as Food Vol 3. Edited by G. Borgstrom. Academic Press (1962).

Killeffer, D.H. 1930. Carbon dioxide preservation of meat and fish. Ind. Eng. Chem. 22, 140 - 143.

King, A.D., and Nagel, C.W. 1975. Influence of carbon dioxide upon the metabolism of Pseudomonas aeruginosa. J. Food Sci. 40, 362 - 366.

King, A.D. and Nagel, C.W. 1967. Growth inhibition of a Pseudomonas by carbon dioxide. J. Food Sci. 32: 575.

Lannelongue, M., Hanna, M.O., Finne, G., Nickelson II, R., and Vanderzant, C. 1982. Storage characteristics of finfish fillets (Archosargus probatocephalus) packaged in modified gas atmospheres containing carbon dioxide. Journal of Food Protection Vol 45, April 82, 440 - 444.

Lannelongue, M., Finne, G., Hanna, M.O., Nickelson, R., and Vanderzant, G. 1982A. Storage characteristics of brown shrimp (Paneus aztecus) stored in retail packages containing CO_2 - enriched atmospheres. Journal of Food Science Vo. 47: 911.

Lawrie, R.A. 1974. The storage and preservation of meat, in "Meat Science" p 196. Pergamon Press, New York.

Liston, J., Stansby, M.E. and Olcott, H.S. 1962. Bacteriological and chemical basis for deteriorative changes. In 'Industrial Fishery Technology', p. 345. Krieger Publishing Co. Inc., New York.

Littlewood, A.B. Gas Chromatography - Principles, Techniques, and Application. 2nd Edition. 1970. Academic Press New York and London.

Lueck, E. 1980. Antimicrobial Food Additives. Berlin, Springer - Verlag.

Lundstrom, R.C., Correia, F.F., and Wilheim, K.A. 1982. Dimethylamine production in fresh red hake (Urophycis chuss): The effect of packaging material oxygen permeability.

Lunstrom, Ronald C. and Racecot, L.D. 1983. Gas chromatographic determination of dimethylamine and trimethylamine in seafoods, in J. Assoc. Off Anal Chem 66(5), 1158 - 1163.

Mitsuda, H., Kawai, F., Yamamoto, A., and Nakajma, K. 1975. Carbon dioxide protein interaction in a gas-solid phase, in J. Nutr. Sci. Vitaminol. 21: 151.

NMFS. 1978. Current Fishery Statistics, No. 7500, Fisheries of the United States, 1977. Natl. Marine Fisheries Service. National Oceanic and Atmosphere Admin., U.S. Dept. of Commerce, Washington, D.C.

Ogrydziak, D.M., and Brown, W.D. 1982. Temperature effects in modified atmosphere storage of seafoods. Food Technology, May 1982, 86 - 96.

Ogilvy, W.S. and Ayres, J.C. 1951. Post-mortem changes in stored meats II. The effect of atmosphere containing carbon dioxide in prolonging the storage life of cut - up chickens. Food Technology 5, 97 - 102.

Ogilvy, W.S. and Ayres, J.C. 1953. Post-mortem changes in stored meats V. Effect of carbon dioxide on microbial growth on stored frankfurters and characteristics of some microorganisms isolated from them. Food Res., 18, 121 - 130.

Paine, F.A. and Paine H.Y. 1983. A handbook of Food Packaging. Leonard Hill.

Parkin, K.L., Wells, M.J. and Brown, W.D. 1982. Modified atmosphere storage of rockfish fillets. J. Food Sci. 47: 181.

Parkin, K. and Brown, W.D. 1983. Modified atmosphere storage of dungeness crab (Cancer magister) Journal of Food Science - Vol. 48, 1983, Pp 370 - 374.

Sainsbury, G.F. 1969. The potential of control atmosphere storage for minimizing oxidative deterioration in food products. In 'Proceedings of International Congress of Refrigeration,'. 3: 749.

Salle, A.J. 1973. Fundamental Principles of Bacteriology. 7th Edition. McGraw - Hill Book Company.

Sander, E.H. and Soo, H.M. 1978. Increasing shelf-life by carbon dioxide treatment and low temperature storage of bulk pack fresh chickens packaged in nylon/surlyn film. Food Sci. 43: 1519.

Seagram, H.L. 1958. Analysis of the protein constituents of drip thawed fish muscles. Food Research 23, 143 - 149.

Shewan, J.M. 1962. The microbiology of sea-waterfish. In Fish as Food, Vol. 1., Edited by George Borgstrom. Academic Press (1962).

Silliker, J.H., Woodruff, R.E., Lugg, J.R., Wolfe, S.K., and Brown, W.D. 1977. Preservation of refrigerated meats with controlled atmospheres. Treatment and post treatment effects of carbon dioxide on pork and beef. Meat Sci. 195.

Souder, J.M. 1980. "Microbiological characteristics of minced rockfish stored under modified atmosphere." M.S. thesis Univ. of California, Davis.

Stansby, M., and Griffiths, F.P. 1935. Carbon dioxide in handling fresh fish, Ind. Eng. Chem. 27, 1452 - 58.

Tarr, H.L.A. 1948. Control of rancidity in fish flesh. Physical and chemical methods. J. Fish Res. Bd. Canada 7:237.

Tomkins, R.G. 1932. The inhibition of the growth of meat attacking fungi by carbon dioxide. J. Soc. Chem. Ind. 51, 261T - 264T.

Veranth, M.F., and Robe, K. 1979. CO_2 - enriched atmosphere keeps fish fresh more than twice as long. Food Processing 40:76.

Vyncke, Wilfred. 1983. "Shelf - life of thawed cod (Gadus morhua) fillets kept in ice". Z. Lebensm-Unters - Forsch 177(1); 19 - 21. Ministry of Agric., Fisheries Research Station, Ankerstraat 1, B - 8400, Oostende, Belgium.

Wang, M.Y., and Brown, W.D. 1983. Effects of elevated carbon dioxide atmosphere on storage of fresh water crayfish (Pacifastacus leniusculus) Journal of Food Sci., 48: 158 - 162.

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

Windsor, M.L. and Thoma, T. 1974. Chemical preservation of industrial fish - New preservation mixtures. J. Sci. Food Agric. 25: 993.

Wolfe, S.K. 1980. Use of CO- and CO_2-Enriched atmospheres for meats, fish, and produce. Food Technology, March 1980, 55.

Woyewoda, A.D., Bligh, E.G., and Shaw, S.J. 1984. Controlled and Modified Atmosphere Storage of Cod Fillets. Canada Inst. Food Sci. Technol. J. Vol 17, No.1. pp 024 - 027.

Yokoseki, M., Uchiyama, H., and Amano I. 1956. Studies on the preservation of fish cakes IV. Factors involved in preservation of carbon dioxide. Bull. Jpn. Soc. Sci. Fish 22: 35 - 40.