Kurt A. Wilhelm is with the Gloucester Laboratory, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Emerson Avenue, Gloucester, MA 01930.

Extended Fresh Storage of Fishery Products With Modified Atmospheres: A Survey

KURT A. WILHELM

ABSTRACT—Alterations in the pressure or gaseous composition of storage conditions can be applied to reduce bacterial and oxidative spoilage of fresh fishery products. These methods, vacuum packaging, hypobaric storage, hyperbaric storage, and the use of such gases as carbon dioxide and ozone, are reviewed in this paper. While oxygen depletion is effective in retarding the growth of the typical spoilage bacteria, there is a possibility that if the product is temperature abused, it may become toxic (i.e., from Clostridium botulinum*) before spoilage is apparent. Under strict temperature control, atmospheric modification can be shown to extend the shelf life of fresh fish. CO_2-enriched atmospheres have thus far received the greatest commercial usage for the bulk shipment of fresh fish.*

Introduction

Fish is one of the most perishable of foods. In unfrozen seafood, bacterial activity is responsible for the most pronounced offensive changes in odor and flavor. Lipid oxidation in fish with a high fat content results in rancidity. Fish such as herring, *Clupea* spp., and trout, *Salmo* spp., can become rancid before microbial spoilage is evident (Hansen, 1963). Endogenous proteolytic enzymes adversely affect the texture of fish, but their activity is relatively insignificant during commercial storage periods (Liston, 1965).

Refrigeration has for many years been successfully used to retard spoilage of fresh fish. However, at 0°C the shelf life of a lean fish such as Atlantic cod, *Gadus morhua,* is about 14 days and at 5°C the shelf life is only about 6 days (Ronsivalli and Charm, 1975). After a short storage period the product must be discarded. From an economic viewpoint, the ability to preserve the quality and nutritional value of nonfrozen fish for extended periods of time could be greatly rewarding in terms of reduced waste, increased value of the product, and increased sales (Gorga et al., 1979).

As a supplement to refrigeration, variations in the pressure and gaseous composition of storage conditions have been proposed as methods of extending the fresh storage life of fishery products. This paper reviews those methods.

In the literature, the term "modified atmosphere" is usually limited to those storage conditions where the atmospheric gas concentrations are altered before storage. In "controlled atmosphere" systems, the selected atmospheric concentrations of gases are actively maintained throughout storage. However, in a general sense, atmospheric modification may include any deviation from normal atmospheric pressure or composition. The pressure is reduced under hypobaric storage and increased under hyperbaric storage conditions. Modifications of atmospheric composition include storage under levels of carbon dioxide, oxygen, nitrogen, and other gases which differ from normal air. If, for example, carbon dioxide is added to the storage container, the environment is referred to as "CO_2-enriched." Atmospheric modification may be within an individual package or within a bulk container.

Vacuum Packaging

Vacuum packaging represents a static form of hypobaric storage which is widely applied in the food industry due to its effectiveness in reducing oxidative reactions in the product at relatively low cost. This technique is applied to frozen and heat-treated products. Although it would also be effective in reducing the growth of the typical spoilage bacteria, its use is not recommended for refrigerated fish. Until proven otherwise, it must be assumed that the conditions exist for the growth of such toxin producing organisms as *Clostridium botulinum.* Botulism can occur in unsterilized, nonacid products held in anaerobic conditions at temperatures above 3°C.

There are, however, several reasons why vacuum packed fish may not become toxic to a consumer. *Clostridium botulinum* requires a reduced substrate on which to grow. Eliminating oxygen from the environment can create this condition, but Johannsen (1965) notes that there are many other naturally available oxidizing agents which can counteract the effect of low oxygen levels. Secondly, competing microorganisms may have an inhibitory effect on clostridia. Vacuum packaging favors the growth of lactobacilli. Johannsen (1965) mentions that the lactobacilli form peroxides and acids which inhibit the growth and toxin formation of *C. botulinum.* Also, the fish flesh may contain antimicrobial agents. Zak (1970) has found that haddock, *Melanogrammus aeglefinus,* contains an antimicrobial polypeptide which inhibits the growth of *C. botulinum.* It may also be present in other lean fish species.

A further factor is the initial number of bacteria present. A small bacterial population is more readily inhibited than a large population. It is also possible that the product will not be stored at temperatures above 3°C. Finally, Licciardello et al. (1967a) have reported that even if botulinum toxin is formed, normal cooking procedures will inactivate it.

Johannsen (1965) summarizes these effects by asserting that it is the condition of the product itself and not vacuum packaging per se which determines whether or not botulinum toxin will form.

It should be noted that the inhibitory effects may not be sufficient to prevent toxin formation and that the effectiveness of vacuum packaging in retarding growth of the typical spoilage bacteria may lead to a product which has become toxic before spoilage is apparent. Both vacuum- and nonvacuum-packaged smoked fish have been implicated with botulism outbreaks. Temperature abuse of the product was indicated in these cases. (Cann et al., 1965).

Huss (1972) compared the quality of plaice, *Pleuronectes platessa,* and haddock held in ice without packaging with fish packed in polyethylene without vacuum, vacuum-sealed polyethylene, and vacuum-sealed polyamide (Nylon 11[1]) pouches held in ice. During the 20-day storage period the plaice packed in evacuated bags had the lowest oxygen content, the lowest bacterial count, and the highest quality score. Compared to the unwrapped plaice, a 6-day extension of shelf life was obtained. Unexpectedly, the haddock did not show an extension of shelf life for the evacuated polyamide pouch. This result was not explained. Packaging without vacuum was judged as not advantageous for either plaice or haddock.

Licciardello et al. (1967b) identified the spoilage bacteria of irradiated (150 krd) and nonirradiated, vacuum-packed (74 mm Hg) haddock fillets after storage at 2°C in metal containers. With the nonirradiated fillets, the spoilage bacteria after 13 days storage was predominantly proteolytic pseudomonads. At that time the fillets had only a slight fishy odor, but 6 days later the odor became definitely stronger, indicating the breakdown of trimethylamine oxide (TMAO) to trimethylamine (TMA). With the irradiated fillets the spoilage bacteria were chiefly lactobacilli. The odor of the fillets at 48 days storage was slightly fishy. Air-packed control fillets were putrid within 13 days. Spoilage of vacuum-packed petrale sole, *Eopsetta jordani,* fillets by pseudomonads has also been reported by Pelroy and Eklund (1966).

Hansen (1972) found that Atlantic herring, *Clupea harengus,* and trout, *Salmo irideus,* stored directly in ice became rancid in 6 days. Fish stored in evacuated polyamide bags did not become rancid during 20 days of storage, but they did develop an objectionable odor and flavor due to bacterial activity. Jorgensen and Hansen (1966) reported that by irradiating (50, 100, and 200 krd) herring and trout before vacuum packaging, the storage life could be extended to 4 weeks.

Use of the bacteriostat ethylenediaminetetraacetic acid (EDTA) with vacuum-packed petrale sole and ocean perch, *Sebastes alutus,* fillets was investigated by Pelroy and Seman (1969). Nonvacuum-packaged ocean perch spoiled in 5 days, and vacuum-packaged fillets spoiled in 6 days due to TMA production by pseudomonads. Application of disodium EDTA resulted in a 9-day extension of shelf life for the vacuum-packaged fillets. Vacuum-packaged petrale sole fillets spoiled after 7 days. Treatment with EDTA extended the shelf life at least 2 days. At that time the predominant flora consisted of coliforms and lactic acid bacteria. Air-packaged petrale sole fillets treated with EDTA had a shelf life of 6 days.

Vacuum packaging appears to have a moderate effect on the spoilage rate. High initial bacterial loads and insufficient vacuum contribute to its lack of effectiveness. In many of the studies the amount of vacuum is not reported, indicating a possible lack of pressure control. Reduction of the bacterial population with supplemental treatment further extends the shelf life of fish products.

Vacuum packaging of individual products can present a problem to the retailer in that although the product may appear marketable, all of the volatiles from decomposition are sealed within the package, and the product may actually have spoiled. Also, there is no clear evidence at this time that if the product is temperature abused, i.e., held at temperatures above 3°C, it will not become toxic.

Hypobaric Storage

The term "hypobaric storage" is usually applied to a dynamic low pressure system. This type of storage has recently been applied commercially for the preservation of fruits, flowers, and vegetables (Burg, 1976). In vacuum packaging, the container is evacuated and sealed. The practice in hypobaric storage is to use a rigid, temperature-controlled container under continuous evacuation. Because this treatment also removes moisture from the product, the container is continuously resupplied with water vapor. This dynamic evacuation accelerates the escape of such volatiles as ammonia and TMA from the product. The pressure in the chamber is maintained to within 2 mm Hg (Jamieson, 1980). The oxygen content is therefore regulated to 0.05 percent of the desired value.

As noted previously, the oxygen content is a critical factor in spoilage due to bacteria and rancidity. The effective oxygen content is related to pressure. A reduction in pressure from normal atmospheric pressure (760 mm Hg) containing 21 percent oxygen to one-tenth normal results in an effective oxygen content of 2.1 percent. The total pressure equals the sum of the partial pressures of the individual gases including water vapor. The partial pressure of water vapor is a function of temperature and water availability. Under evacuation, the water in the product is vaporized to maintain the partial pressure of the water vapor in the chamber. At 0°C the partial pressure due to water vapor is 4.6 mm Hg. So at low pressures a greater proportion of the total pressure is due to water vapor and the other gases are accordingly further reduced. Thus, the oxygen content is controlled.

Haard et al. (1979) investigated the use of hypobaric storage with Atlantic herring, *Clupea harengus,* and Atlantic cod held at 2°-4°C. Eviscerated cod held at normal pressure either in air or 0.2 percent oxygen were considered unacceptable after 6 days of storage. Cod held hypobarically at 13.7 mm Hg remained acceptable in appearance, odor, and microbiological counts during

[1]Mention of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

13 days of storage. Eviscerated herring stored under normal pressure were judged unacceptable after 8 days storage, while those held at 20 mm Hg remained acceptable for 16 days. Hypobaric storage of whole herring did not delay deterioration due to endogenous proteolytic enzymes. Bellyburn was more frequent and much more severe in herring held at 20 mm Hg than in those stored at normal atmosphere.

Varga et al. (1980) reported that the storage life of cod; mackerel, *Scomber scombrus*; and herring fillets could be extended when stored under a pressure of 10 mm Hg. Treatment with EDTA further extended the storage life of the cod fillets. Mermelstein (1979) noted that the storage life of shrimp and freshwater prawn can also be doubled when stored at 10 mm Hg, $-1°C$, and a relative humidity of 95 percent.

Since refrigeration is part of the bulk storage system, as long as the equipment is operating properly the product is considered to be safe. Hypobaric storage appears more effective than vacuum packaging in prolonging the shelf life of fishery products, but its commercial feasibility has yet to be demonstrated.

Hyperbaric Storage

Hyperbaric storage refers to the use of high pressure systems. High pressure can stop microbial growth and reduce enzymatic activity. Charm et al. (1977) reported that at high pressure, the refrigerated storage life of Atlantic cod was greatly extended as determined by bacterial counts and sensory evaluation. After 30 days, fish held in a nonfrozen state at $-3°C$ and 238 atmospheres (1.8×10^4 mm Hg) were not significantly different from frozen controls held at atmospheric pressure and $-25°C$.

Because of the technical difficulties in building a commercially feasible high pressure storage unit, this preservation method has not been pursued.

CO_2-Enriched Atmospheric Storage

Of the methods for modification of atmospheric composition, the use of carbon dioxide has been investigated the most thoroughly. Research on the effect of CO_2 on the storage life of fish began in the 1930's. Killeffer (1930) briefly noted that fish stored in 100 percent CO_2 kept fresh from two to three times longer than controls in air at the same temperature. Coyne (1933) followed up on his preliminary studies (Coyne, 1932), which showed the growth of the spoilage bacteria *Achromobacter, Flavobacterium, Pseudomonas, Micrococcus,* and *Bacillus* could be effectively inhibited with high concentrations of CO_2. He reported that cod; lemon sole, *Microstomus kitt*; European plaice, *Pleuronectes platessa*; and whiting, *Merlangius merlangus,* could be kept at 0°C twice as long in a CO_2 atmosphere than in air. Optimal results were reported for CO_2 concentrations of 40-60 percent. At 10°C, 100 percent CO_2 was found to be the most effective. Whole fish and fillets were judged on the basis of appearance, odor, and texture. Stansby and Griffiths (1935) reported that the iced storage life of haddock could be doubled with the use of CO_2.

Recent studies (Banks et al., 1980; Brown et al., 1980) on vermilion rockfish, *Sebastes miniatus*; coho salmon, *Oncorhynchus kisutch*; Gulf trout, *Cynoscion nebulosus*; and croaker, *Micropogon undulatus,* have shown that while CO_2 is effective in inhibiting the growth of gram-negative bacteria (i.e., *Pseudomonas*) which produce trimethylamine and ammonia, growth of gram-negative bacteria such as *Lactobacillus* is stimulated. These bacteria produce acid, causing souring of fish during storage.

Pretreatment before storage in carbon dioxide has also been investigated to further improve the shelf life of fishery products. Mitsuda et al. (1980) noted an improvement in texture and color when fillets of *Seriola aurevittata* were dipped in 5 percent NaCl before gas storage. A propionic acid dip with either EDTA or ascorbic acid is reported to double the shelf life of sprat compared with CO_2 used alone (Windsor and Thoma, 1974). Fey (1980) reported that a combination of 1 percent potassium sorbate ice and modified atmospheres containing 60 percent CO_2 resulted in a shelf life of at least 4 weeks for red hake, *Urophycis chuss,* and 3½ weeks for chinook salmon, *Oncorhynchus tshawytscha.*

For voyages of up to 4 days, refrigerated seawater and chilled seawater holding tanks offer advantages in the quality and handling of fish aboard fishing vessels (Peters et al., 1965; Hulme and Baker, 1977). Further research has demonstrated that CO_2 bubbled through the brine reduced the bacteriological load and improved the shelf life of pink shrimp, *Pandalus borealis* (Bullard and Collins, 1978; Barnett et al., 1978); black rockfish, *Sebastes melanops* (Collins et al., 1980); chum salmon, *Oncorhynchus keta* (Barnett et al., 1971); silver hake, *Merluccius bilinearis* (Hiltz et al., 1976); and ocean perch, *Sebastes marinus* (Longard and Regier, 1974). There are many technological difficulties in using CO_2 with refrigerated seawater. These problems are due to the corrosive nature of CO_2, and are discussed by Nelson and Barnett (1971).

Carbon dioxide-enriched atmospheres are currently in use for the bulk shipment of Pacific salmon, *Oncorhynchus* spp. (Bell, 1980; Veranth and Robe, 1979). It has been shown that the shelf life of fresh fish can be doubled with CO_2. However, in view of the lack of toxicological studies to determine the botulism potential, the use of CO_2 in retail packages is discouraged.

Other Gases

Other inhibitory gaseous mixtures have also been studied for use in food preservation. Coyne (1932) showed that even at levels approaching 100 percent, nitrogen had no inhibitory effect on the typical spoilage bacteria. Ozone was found to have a preservative effect on fish (Haraguchi et al., 1969), and ammonia was reported to effectively preserve fish for 2 months at ambient temperatures (Subrahmanyan et al., 1965). Although the fish became soft, it was reported suitable for fish flour. Ethylene oxide, nitrous oxide, and other bactericidal or bacteriostatic gases have been investigated, but they are generally not considered practical for the preservation of fresh fish because of their inherently toxic properties.

Summary

The storage life of fishery products can be extended with modifications of the storage pressure and atmospheric composition. For bulk shipment of fishery products, the use of CO_2 has been accepted in commercial practice for its effectiveness in retarding spoilage at a reasonable cost. But there remains the unresolved threat of the potential for botulism that is theoretically associated with packaging under low partial pressures of oxygen. Until safety from botulism can be demonstrated, the use of low oxygen storage conditions cannot be recommended for retail packages. Above all, for prolonged fresh storage, strict temperature control is necessary under any atmospheric condition in storage to ensure quality and safety.

Literature Cited

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

Barnett, H. J., R. W. Nelson, P. J. Hunter, S. Bauer, and H. Groninger. 1971. Studies on use of carbon dioxide dissolved in refrigerated brine for the preservation of whole fish. Fish. Bull., U.S. 69:433-442.

Barnett, H. J., R. W. Nelson, P. J. Hunter, and H. Groninger. 1978. Use of carbon dioxide dissolved in refrigerated brine for the preservation of pink shrimp (*Pandalus* spp.) Mar. Fish. Rev. 40(9):24-28.

Bell, L. 1980. Gas in trucks, containers helps packages keep foods fresh. Package Eng. 25(3):72-75.

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

Bullard, F. A., and J. Collins. 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., U.S. 76:73-78.

Burg, S. P. 1976. Hypobaric storage and transportation of fresh fruits and vegetables. *In* N. F. Haard and D. K. Salunkhe (editors), Post-harvest biology and handling of fruits and vegetables, p. 172-188. AVI Publ. Co., Westport, Conn.

Cann, D. C., B. B. Wilson, G. Hobbs, and J. M. Shewan. 1965. The growth and toxin production of *Clostridium botulinum* type E in certain vacuum-packed fish. J. Appl. Bacteriol. 28:431-436.

Charm, S. E., H. E. Longmaid III, and J. Carver. 1977. A simple system for extending refrigerated, nonfrozen preservation of biological material using pressure. Cryobiology 14: 625-636.

Collins, J., K. D. Reppond, and F. A. Bullard. 1980. Black rockfish, *Sebastes melanops*: Changes in physical, chemical, and sensory properties when held in ice and in carbon dioxide modified refrigerated seawater. Fish. Bull., U.S. 77:865-870.

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

________. 1933. The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish. Part 2. Gas storage of fresh fish. J. Soc. Chem. Ind. (Lond.) 52: 19T-24T.

Fey, M. S. 1980. Extending the shelf life of fresh fish by potassium sorbate and modified atmosphere at 0-1° Celsius. Ph.D. Thesis, Cornell Univ.

Gorga, C., J. D. Kaylor, J. H. Carver, J. M. Mendelsohn, and L. J. Ronsivalli. 1979. The economic feasibility of assuring U.S. Grade A quality of fresh seafoods to the consumer. Mar. Fish. Rev. 41(7):20-27.

Haard, N. F., I. Martins, R. Newbury, and R. Botta. 1979. Hypobaric storage of Atlantic herring and cod. Can. Inst. Food Sci. Technol. J. 12:84-87.

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

________. 1972. Storage life of prepackaged wet fish at 0°C. II.—Trout and Herring. J. Food Technol. 7:21-26.

Haraguchi, T., U. Simidu, and K. Aiso. 1969. Preserving effect of ozone on fish. Bull. Jpn. Soc. Sci. Fish. 35:915-919.

Hiltz, D. F., B. S. Lall, D. W. Lemon, and W. J. Dyer. 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. Board Can. 33: 2560-2567.

Hulme, S. E., and D. W. Baker. 1977. Chilled seawater system for bulkholding sea herring. Mar. Fish. Rev. 39(3):4-9.

Huss, H. H. 1972. Storage life of repacked wet fish at 0°C. I. Plaice and haddock. J. Food Technol. 7:13-19.

Jamieson, W. 1980. Use of hypobaric conditions for refrigerated storage of meats, fruits, and vegetables. Food Technol. 34(3):64-71.

Johannsen, A. 1965. Public health aspects of prepackaging fish for retail. *In* Fish handling and preservation, p. 271-278. OECD, Paris.

Jorgensen, B. V., and P. Hansen. 1966. Storage life of vacuum-packed iced trout. II. Influence of radiation pasteurisation. J. Sci. Food Agric. 17:140-141.

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

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

________, L. J. Ronsivalli, and J. W. Slavin. 1967b. Effect of oxygen tension on the spoilage microflora of irradiated and non-irradiated haddock (*Melanogrammus aeglefinus*) fillets. J. Appl. Bacteriol. 30:239-245.

Liston, J. 1965. Bacteriological enzymes and their role in the deteriorative changes in fish. *In* R. Kreuzer (editor), The technology of fish utilization, p. 53-57. Fishing News (Books) Ltd., Lond.

Longard, A. A., and L. W. Regier. 1974. Color and some composition changes in ocean perch (*Sebastes marinus*) held in refrigerated sea water with and without carbon dioxide. J. Fish Res. Board Can. 31:456-460.

Mermelstein, N. H. 1979. Hypobaric transport and storage of fresh meats and produce earns 1979 IFT Food Technology Industrial Achievement Award. Food Technol. 33(7): 32-40.

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

Nelson, R. W., and J. H. Barnett. 1973. Fish preservation in refrigerated sea water modified with carbon dioxide. Proc. XIII th Int. Cong. Refrig. 3:57-64.

Pelroy, G. A., and M. W. Eklund. 1966. Changes in the microflora of vacuum-packed irradiated petrale sole (*Eopsetta jordani*) fillets stored at 0.5C. J. Appl. Microbiol. 14:921-927.

________, and J. P. Seman, Jr. 1969. Effect of EDTA treatment on spoilage characteristics of petrale sole and ocean perch fillets. J. Fish. Res. Board Can. 26:2651-2657.

Peters, J. A., C. J. Carlson, and D. W. Baker. 1965. Refrigerated seawater as a storage medium for fish. ASHRAE J. 7(4):64-67.

Ronsivalli, L. J., and S. E. Charm. 1975. Spoilage and shelf life prediction of refrigerated fish. Mar. Fish. Rev. 37(4):32-34.

Stansby, M. E., and F. P. Griffiths. 1935. Carbon dioxide in handling fresh fish—haddock. Ind. Eng. Chem. 27:1452-1458.

Subrahmanyan, V., N. L. Lahiry, M. N. Moorjani, R. Balakrishnan Nair, and M. A. Krishnaswamy. 1965. Possible use of ammonia for preserving fish. *In* R. Kreuzer (editor), The technology of fish utilization, p. 43-44. Fishing News (Books) Ltd., Lond.

Varga, S., R. A. Keith, P. Michalik, G. G. Sims, and L. W. Regier. 1980. Stability of lean and fatty fish fillets in hypobaric storage. J. Food. Sci. 45:1487-1491.

Veranth, M. F., and K. Robe. 1979. CO_2-enriched atmosphere keeps fish fresh more than twice as long. Food Process. 40(4):76-79.

Windsor, M. L., and T. Thoma. 1974. Chemical preservation of industrial fish: New preservative mixtures. J. Sci. Food Agric. 25: 993-1005.

Zak, J. M. 1970. Factors affecting the outgrowth of *Clostridium botulinum* type E. Ph.D. Thesis, Mass. Inst. Technol., Cambridge.