A Study in the Use of a High Concentration of CO_2 in a Modified Atmosphere to Preserve Fresh Salmon

HAROLD J. BARNETT, FREDERICK E. STONE, GLENN C. ROBERTS, PATRICK J. HUNTER, RICHARD W. NELSON, and JOSEPHINE KWOK

Introduction

The preservative effects of carbon dioxide (CO_2) on protein foods has been known for many years and is used to preserve perishables, such as meats and poultry. Early research by Coyne (1932), Stansby and Griffiths (1935), and others showed that CO_2 atmospheres have a beneficial preservative effect on certain species of bottomfish.

More recently, the National Marine Fisheries Service (NMFS) in cooperation with Whirlpool Corporation[1] (Nelson and Tretsven, 1975) made a series of laboratory experiments to determine the preservative effects of controlled atmospheres (CA) on fresh Pacific salmon, *Oncorhynchus* spp. Results from these studies showed that the shelf life of refrigerated salmon could be significantly extended by storage in a CA containing 11.5 percent CO_2, 87 percent nitrogen (N_2), and 1.5 percent oxygen (O_2). Until recently, however, commercial use of controlled atmosphere or modified atmosphere (MA) to preserve fish was not seriously considered for use by the industry because of economic and technical considerations.

Then in 1977, a salmon processor demonstrated interest in the techniques by making a few exploratory shipments of fresh salmon in special refrigerated vans from Alaska to the Pacific Northwest. The interest, of course, was created by the increased value of salmon and changing markets. The shipments, made in MA containing high concentrations of CO_2 and O_2, demonstrated the potential for improving the keeping quality of fresh salmon shipped in large containers or vans.

Problems appeared in the early tests, e.g., undesirable changes in quality in the salmon were found in several shipments. Changes in quality were related to inadequate cooling of the fish after catching and during handling and processing as well as to problems in adapting packaging for shipment. The latter consisted of using Wet-Lok-type containers, which were perforated to allow for removal of drip and free passage of the MA. Drying, caused by the vans' forced-air refrigeration system, resulted in product shrinkage and loss of quality through oxidation and discoloration. The high concentration of oxygen used in the MA gas mixture was thought to be a contributing factor to the oxidation and discoloration problem. The problem of drying was partly controlled by top icing the boxed fish for shipment.

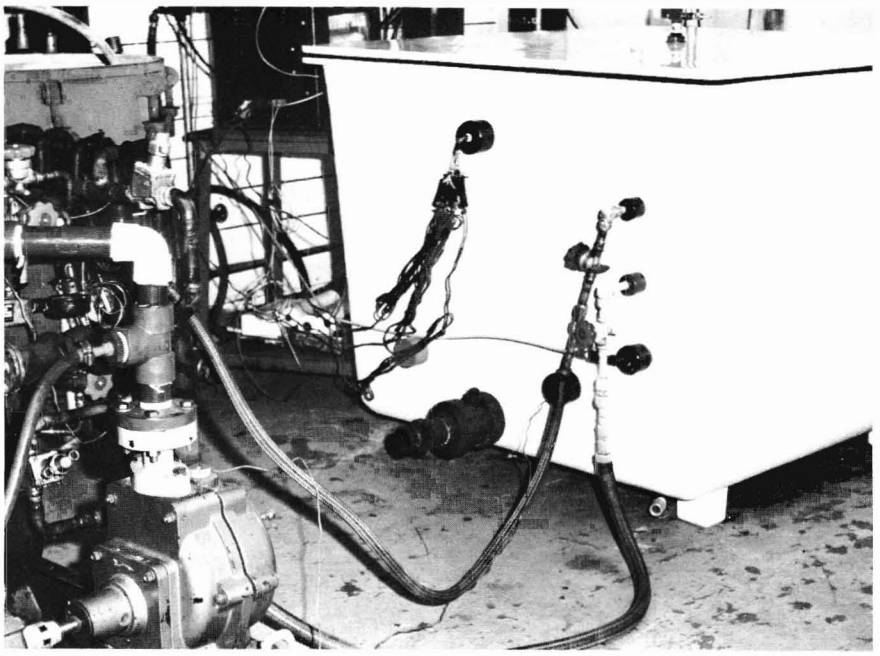
Because of the need for more information, we initiated studies to investigate the variables of low temperature and high concentration of CO_2 gas as an effective mechanism to control the quality of fresh Pacific salmon held in MA.

In recent studies by Williams et al. (1978), it was found that at 5°C (41°F),

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Harold J. Barnett, Research Chemist; Frederick E. Stone, Chemist; Glenn C. Roberts, Chemist; Patrick J. Hunter, Engineering Technician; and Richard W. Nelson, Supervisory Chemical Engineer, are with the Utilization Research Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Boulevard East, Seattle, WA 98112. Josephine Kwok, Food Technologist, was with the New England Fish Company, Pier 89, Seattle, WA 98119.

ABSTRACT—Commercial use of modified atmospheres to preserve fish during shipment has been limited because of economic and technical reasons. Recent interest in the use of the technique to ship Pacific salmon, Oncorhynchus *spp., out of Alaska has demonstrated the need for additional information to improve handling and shipping procedures. Studies were conducted to determine the effect of low storage temperature combined with the use of a high concentration of CO_2 gas as an effective combination for controlling the quality of fresh Pacific salmon held in modified atmospheres. Salmon were held in acceptable condition for 21 days in a modified atmosphere containing 90 percent CO_2. The effect of the CO_2 was to slow the growth of spoilage bacteria extending the fresh shelf life of the salmon from about 12 days on ice to 21 days in the modified atmosphere. Carbon dioxide absorbed in the flesh of the salmon causes some swelling in the canned product but the condition can be controlled. The modified atmosphere method described can be considered for commercial use. Recommendations are made for handling the salmon for shipping and for additional research on the preservative effects of CO_2 and other atmospheres with respect to different fish species.*



Figures 1.—Modified atmosphere container showing refrigeration flex lines, stainless steel "pop-off" valve on lid, and thermocouples connected to a multipoint recorder (shown to the left rear of container).

growth of bacteria, predominantly associated with the spoilage of fish, was almost totally inhibited by a 100 percent CO_2 atmosphere. In tests made by the Northwest and Alaska Fisheries Center's Utilization Research Division, Seattle, Wash., in cooperation with a local fish-processing company, walleye pollock and Pacific cod stored at 0°C (32°F) in a MA containing 90 percent CO_2 and 10 percent air were judged superior in quality to walleye pollock and Pacific cod stored at 0°C (32°F) in atmospheres containing 20 percent and 55 percent CO_2. Based on this knowledge, it was decided to investigate the use of the higher concentrations of CO_2 than now commercially used for shipping salmon in MA.

In this experiment, fresh, fall-caught butchered Pacific salmon were held at 0°C (32°F) in the MA for 21 days. This report describes the rationale and results of the experiment. In view of the continuing interest of the salmon canning industry in methods of extending the keeping quality of salmon prior to canning, a limited study was made of the potential use of MA-stored salmon for canning.

The Modified Atmosphere System

Laboratory equipment for this experiment consisted of a portable freon-12 refrigeration unit modified with a hot-gas, bypass system to maintain a partial load, at temperature, to prevent periodic cycling of the compressor. The system was designed to maintain uniform product temperatures within ±1°F (0.6°C).

The container (Fig. 1) used to store the fish was of a special design. Portable and constructed of fiberglass, it had a 1,700-pound fish-holding capacity and was insulated with 4 inches of urethane foam. Stainless steel chiller plates (coils) mounted on the inner walls were connected via special bulkhead fittings to the refrigeration unit by standard refrigeration flex lines. The false floor of the tank was perforated to allow excess melted ice, water, and drip to drain from the fish during storage. The top of the container was gasketed to facilitate making the container airtight. One-quarter-inch, stainless steel needle valves, located near the bottom of one side and on top of the lid, provided the necessary filling and venting ports for gassing the storage container. A stainless steel "pop-off" valve located on the lid prevented pressurizing the container. A multipoint recorder, equipped with stainless steel, sabretype thermocouples, was used to measure and record the internal temperatures of the storage container and fish.

The equipment described here was used only to simulate commercial conditions. Less elaborate, light-weight, multipurpose, plastic fish totes that can be made airtight for use with gases and shipped in refrigerated vans or by air are now being designed for commercial use.

Besides eliminating some of the previously described drawbacks of using cardboard containers, airtight bulk containers could expedite handling and packing of salmon as well as reduce costs. Such containers could also increase the potential for using the MA method in remote areas, such as Alaska's Bristol Bay. The containers could be multipurpose and made in a variety of sizes for shipping on vessels, by air, and in refrigerated vans or used to bulk store fish in processing plants. This could be especially useful during peak operating times.

Materials and Methods

Sample Preparation and Procedures

The 310 pounds of salmon used in this experiment consisted of mature coho, *Oncorhynchus kisutch*, and chum, *O. keta*, salmon that had been gillnetted in Puget Sound about 24 hours prior to beginning the experiment. They were headed and gutted and well iced shortly after being caught. The internal temperature of the fish when loaded in the container was about 32°F (0°C). Many fish showed signs of water marking and loss of brightness of color but were otherwise of very good quality. In the laboratory, the salmon were removed from the ice and placed randomly in the storage container. Thermocouples were inserted into two of the fish, one located on the bottom of the load and one on the

very top. Other probes were placed inside the holding tank to measure the internal ambient temperature which was maintained at 32°F (0°C) during the experiment. The storage container was then sealed and purged with CO_2 gas to obtain an atmosphere mixture containing 90 percent CO_2 and 10 percent air. The exact concentration of the gas was determined with a Burrell gas analyzer. The CO_2 gassing procedure was repeated each time the container was opened to examine and sample the fish.

The salmon were periodically sampled and evaluated for quality during the 21-day experiment. Criteria included sensory attributes, chemical and physical changes, and total aerobic bacterial plate counts. At each examination period, two fish were randomly sampled from storage, split along the long axis with one-half of each fish retained for bacteriological examination. Each of the remaining sides was evaluated chemically and organoleptically. Chemical analyses were made in duplicate of each fish.

Two additional fish were selected at each examination period, washed, frozen, glazed, vacuum sealed in a polyethylene bag, and stored at −18°C (−20°F) for future chemical and sensory examination. In addition, two fish were randomly selected at intervals from MA storage, washed, trimmed, sectioned, and packed in ½-pound cans and thermally processed. Cans were air-cooled and stored at ambient room temperature.

Analytical Methods

Total aerobic plate counts were made by the method described by Pelroy and Eklund (1966). Briefly, the method is as follows: 45 g of excised fish flesh is homogenized aseptically with 180 ml of sterile 0.1 percent peptone solution at 3.0°C (38°F). Serial dilutions in 0.1 percent peptone water were prepared for pour plates from the homogenate. Plate counts were made using TPY solution (1.5 percent trypticase, 0.5 percent peptone, 0.5 percent yeast extract, 0.2 percent glucose, 0.5 percent NaCl, and 1.5 percent agar). Plates were incubated at 22°C (72°F) for 5 days.

The pH of the flesh of the salmon was measured by homogenizing at 4:1 mixture by weight of distilled water and fish flesh. A Corning combination electrode was used to make the measurement.

Analysis of CO_2 content in the flesh of the salmon was made by the following method: A sample of flesh was blended in an alkaline (pH 10.4) Tris buffer (0.05 M), and then treated with an acid buffer to liberate CO_2. Concentrations were read from a calibration curve prepared from millivolt (Mv) readings obtained with an Orion carbon dioxide electrode connected to an Orion model 801 pH meter.

The salmon were chemically tested for oxidative rancidity (TBA number) using the method described by Lemon (1975). Essentially, the procedure calls for blending tissue in an extracting solution (trichloroacetic acid, propyl gallate, and EDTA) and filtering. The filtrate is reacted with TBA reagent and boiled. The cooled sample is read spectrophotometrically.

Sensory Tests

At each sampling, the raw salmon were judged for appearance, texture (as determined by finger pressure), odor, and color of the flesh. The salmon were prepared for sensory evaluation by baking in covered aluminum containers for 15 minutes at 350°F. The samples were evaluated by a five-member taste panel for flavor, texture, and rancidity using a 5-point numerical scale. A score of 2 or below for the sensory criteria indicates a product of unacceptable quality. Canned salmon was evaluated for fill weight, vacuum, color, and general appearance and acceptability.

Results and Discussions

Bacteriological Measurements

Results of the bacteriological examinations are shown in Table 1. Total bacterial counts made on the fish held in the MA remained low for the full 21 days of the test. The results show that the counts never exceeded 10^4 organisms/g, which is normal for very fresh fish. A total bacterial plate count of 10^6 organisms/g is generally considered indicative of incipient spoilage. In a similar study, Parkin (1979) reported essentially no growth of aerobic bacteria on rockfish fillets stored refrigerated in an atmosphere containing 80 percent CO_2/20 percent O_2 for 14 days. Similar observations were made by Barnett et al. (1971) on whole yellowtail rockfish and chum salmon, *Oncorhynchus keta*, held in refrigerated seawater modified with CO_2. King and Nagel (1975) in studies on *Pseudomonas aeruginosa* suggested inhibition of microbial growth by CO_2 is "in part due to a mass action effect on certain decarboxylating enzymes" which interferes with metabolic pathways essential for normal growth. *Pseudomonas* spp. bacteria are generally associated with spoilage of fresh fish and are controlled by the presence of as little as 5 percent CO_2 in the CA (Coyne, 1933).

Table 1.—Microbiological and chemical changes occurring in the flesh from butchered salmon held in a refrigerated, controlled atmosphere.

Storage time (days)[1]	Chemical Data			Total bacterial counts (no./g)
	pH	CO_2 Conc (ppm)	TBA (μ M/100g)	
0	6.22	247	0.25	2.8×10^4
6	6.23	760	0.23	4.2×10^3
10	6.23	687	0.67	1.0×10^4
15	6.11	817	0.44	1.3×10^4
20	6.20	1,118		3.4×10^4
21	6.22	913	0.64	2.0×10^4

[1]Storage time given is the number of days held at the laboratory.

Chemical Measurements

pH

The pH values of the salmon (Table 1) did not change significantly from their normal (initial) physiological pH during the test. Small differences are attributed more to biological variation among the fish than to the effects of the CO_2. However, Makashev (1959) observed a twofold increase in the acidity (pH) of fish muscle stored in a CO_2 environment. The pH of rockfish fillets (Parkin, 1979) decreased from about 6.7 to 6.3 after 1 week in a MA containing 80 percent CO_2. It is assumed that the pH of the salmon in this experiment did not become more acid because of the strong buffering capacity of the flesh or other physiological conditions existing during the experiment. The pH of the flesh of the fish would be expected to increase (become alkaline) if normal bacterial spoilage had occurred.

CO_2 Concentration

As shown in Table 1, the concentration of CO_2 found in the muscle of the MA-stored fish increased by a factor of about 5 during the experiment. The largest increase occurred between the first and fifth days of storage. A similar phenomenon was observed by Barnett et al. (1971, 1978) in experiments with use of CO_2 in refrigerated brine for preservation of fish and shellfish. The increase is partly due to a complexing of the CO_2 with muscle protein and/or combining with physiological electrolytes.

TBA Measurements

As indicated by the relatively low TBA values (Table 1), oxidation of lipids in the flesh of the salmon, because of exposure to a high concentration of CO_2, was not a significant factor in this experiment. This is attributed to the relatively low concentration of O_2, in the MA environment and also to the low-storage temperature. CO_2 is known to retard oxidation of fats (Makashev, 1959) at low concentrations (20-30 percent) and is apparently more effective at higher concentrations (Tarr, 1948).

Table 2.—Sensory evaluations of butchered salmon held in a refrigerated CO_2-modified atmosphere.

Storage time (days)	Average sensory scores[1] on baked salmon samples			General comments on raw salmon
	Flavor	Texture	Rancidity	
0	4.2 ± 0.8	4.6 ± 0.5	5.0 ± 0	Fish are mature but of excellent quality
6	4.0 ± 0.7	4.2 ± 0.4	4.6 ± 0.5	Good color, odor, and texture
10	4.3 ± 0.8	4.4 ± 0.9	4.5 ± 0.4	Very good quality
15	4.2 ± 1 6	4 4 ± 0.8	4 6 ± 0.7	Normal color, odor, firm texture
19	3.0 ± 1.1	4.4 ± 0.5·	4 6 ± 0.6	Slight oily odor, good color firm texture
21	3.7 ± 1.6	4 7 ± 0.5	4.4 ± 1.6	Slight oily odor, good color, and texture; fish are of acceptable quailty

[1]Scores are based on a 5-point numerical scale· Score of 2 denotes a product of borderline quailty.

Table 3.—Physical and sensory evaluations of canned salmon prepared from salmon held in a refrigerated, CO_2-modified atmosphere.

Days of storage	Code[1]	Net Weight (oz.)		Vacuum (in.)		Color	Remarks
		Range	Avg.	Range	Avg.		
0	(MDS)	7.6-8.5	7.9	1.0- 7.0	5.2	Avg.	Very slight oily flavor, slight curd, and normal texture
6	(BC)	7.7-8.4	8.0	0 - 5.0	3.5	Avg.(·)	Normal flavor, odor, and texture
	(DS)	8.0-8.4	8.3	1 0-10.0	6.5	Avg.( )	Normal flavor, muddy odor, slight soft texture and water marked (acceptable)
10	(BC)	7.5-8.4	7.9	0 -14 0	8.0	Avg.	Normal flavor, odor, and texture, moderate curd
	(DS)	8.0	8.0	8.0	8.0	Avg.( )	Typical flavor and odor for water-marked fish; slightly soft with moderate curd (acceptable)
14	(DC)	7.6-8.2	7.9	8.0-10.0	9.0	Avg	Normal odor, flavor, and texture for water-marked fish; moderate curd (acceptable)
	(DS)	7.5-7.7	7.6	8.0-10.0	9.0	Avg.	Normal odor, flavor; slight soft texture; moderate curd
19	(BS)	7.3-8.1	7.8	6.0- 8.0	7.0	Avg.	Normal odor; moderate, oily flavor and normal texture; moderate curd
	(DC)	7.2-8.1	7.7	6.0-12.0	8.5	Avg	Normal odor and flavor for water-marked fish; slight, soft texture; moderate curd (acceptable)
21	(DS)	7.6-8.0	7.7	8.0- 9.0	8.5	Avg.( )	Slight oily odor and flavor; normal texture, moderate curd
	(BC)	7.3-8.2	7.8	7.0- 8.0	7.7	Avg.	Normal flavor and odor; normal texture; moderate curd

[1]MDS = medium dark silver
BC = bright chum.
DS = dark silver
BS = bright silver

Sensory Evaluations

The salmon from the MA storage were examined periodically for sensory changes. Samples were evaluated raw, cooked, and canned.

Raw Salmon

No serious deterioration of overall quality was observed in the fish during the experiment (Table 2). In studies using CO_2 to preserve fresh sole and cod, Coyne (1933) found that the texture of the fish was slightly softer after prolonged storage in a high concentration (100 percent) of CO_2 than fish exposed to CO_2 concentrations of 40-60 percent. We did not observe subjectively any significant softening of texture in this experiment.

Cooked Salmon

Flavor scores generally indicate that the MA-held fish, irrespective of species, remained of acceptable quality for the duration of the test (Table 2). No significant differences (P 0.05) were found in the flavor and texture between the initial, untreated fish, and those held in the MA for 21 days. There was no significant difference in the organoleptic rancidity scores between the untreated fish and the fish held in MA. The sensory scores also indicate that the texture of the salmon was not adversely affected by exposure to the MA. Sensory examinations for rancidity correlated well with chemical tests for rancidity.

Canned Salmon

Canned salmon were evaluated for fill weight, vacuum, color, and general appearance and acceptability (Table 3). Net weights varied as a result of difficulty

in filling each can by hand using estimated in-fill weights. The occasional loss of vacuum in the cans was caused by overfilling with product. None of the canned product examined was considered spoiled or was rejected on the basis of decomposition. Low marks were mainly given on the basis of the maturity of the fish, i.e., water marked, muddy odor, texture and curd defects, and oily flavor.

Some swelling of the air-cooled cans was noted; but after several days in storage, the swelling subsided. The swelling of the cans was caused by excess (free) CO_2 liberated from the flesh during the cook, which resulted in occasional buckling of cans. Increasing the vacuum (24 inches) cycle of the can seamer by about 15 seconds prior to closing and a slow release of pressure from the retort after the cook helped to control the problem. Icing and holding the fish overnight prior to canning had no effect on resolving the excess CO_2 and the buckling problem.

Refrigerated Shelf-Life Test

After 2 weeks in the MA environment, 6 butchered salmon were individually packaged in polyethylene bags and stored in a 2°C (35°F) refrigerated room until organoleptically spoiled. The fish were periodically examined and evaluated for odor, color, and general appearance. They retained most of their good quality up to the fourth day of the test when a slight off-odor not associated with spoilage was detected. The odor was not detected after the fish were washed. No other significant changes were noted in the fish samples until the seventh day of storage. At this time, the remaining samples had a distinct sour odor on the skin, in the flesh, and in the poke (belly cavity). Significant discoloration of the peritoneal lining (not the underlying flesh) of the belly cavity was also observed. The samples were considered organoleptically unacceptable. At this time, the fish had been out of water about 23 days.

Summary and Recommendations

The results of the experiment described here show that fresh, butchered salmon can be bulk-held in acceptable condition for up to 21 days at 0°C (32°F) in a MA containing 90 percent CO_2. Butchered salmon usually spoil within 12 days on ice.

The effect of the CO_2 was to slow the growth of spoilage bacteria apparently by an inhibition of the CO_2 on specific metabolic pathways involved in the normal growth of the bacteria. It also appeared that the combination of low O_2 and high CO_2 concentration in the preservative gas protected the salmon from the effects of oxidative rancidity.

Retention of excess CO_2 in the flesh of the salmon caused some swelling in the canned product. This condition was the result of "free" CO_2 liberated during the cook. After cooling, swelling of the cans was not evident. Sensory evaluation of the canned product showed it to be normal in appearance, odor, color, flavor, and texture for canned mature, net-caught salmon. For commercial application in canning salmon of different quality or species, additional tests should be conducted.

Salmon removed after 2 weeks in MA storage and held on ice in a refrigerated room remained of acceptable quality for almost 1 week.

The MA preservation method described here can be considered for commercial use. It is recommended, however, that each user initiate shipments on a small scale to gain familiarity and experience with the technique and its special handling requirements. Above all, salmon to be shipped and marketed as fresh via this method should be of the highest quality. Furthermore, they should be thoroughly washed and prechilled prior to containerization. The temperature of the fish should be maintained at 0° ± 0.5°C (32° ± 1°F) at all times when in transit.

Because of the preliminary nature of the work described here, it is recommended that additional research be conducted to further evaluate the preservative effects of CO_2 and other atmospheres with respect to different fish species. The public health significance is important in using processes in which the normal microbiological spoilage pattern is modified as in the MA environment. The possible presence of *Clostridium botulinum* and its ability to grow and develop toxin in the CO_2 MA at higher (abuse) temperatures require that future research include studies and recommendations for insuring product safety during distribution and marketing.

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