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Use of Carbon Dioxide Dissolved in Refrigerated Brine for the Preservation of Pink Shrimp (*Pandalus* spp.)

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ABSTRACT—The need to travel farther to productive fishing grounds has made it increasingly difficult for Pacific Northwest and Alaska fishermen to land iced, whole pink shrimp of high quality. Comparative studies were conducted to determine the effectiveness of holding pink shrimp in ice, untreated refrigerated brine, and refrigerated brine treated with carbon dioxide as a means of increasing the keeping quality of the landed shrimp. Holding the shrimp in the brine-CO_2 mixture inhibited bacterial and enzymatic changes causing spoilage and extended the keeping quality of the shrimp from 3 to 4 days in ice to 6 days or more in the treated brine. Recommendations are made for additional studies prior to commercial applications.

INTRODUCTION

The pink shrimp (*Pandalus* spp.) fishery makes an important contribution to the volume and value of the fisheries of the Pacific coast and Alaska. Annual catches in recent years have exceeded 130 million pounds (Pacific Packers Report, 1977) and values in excess of $15 million ex-vessel.

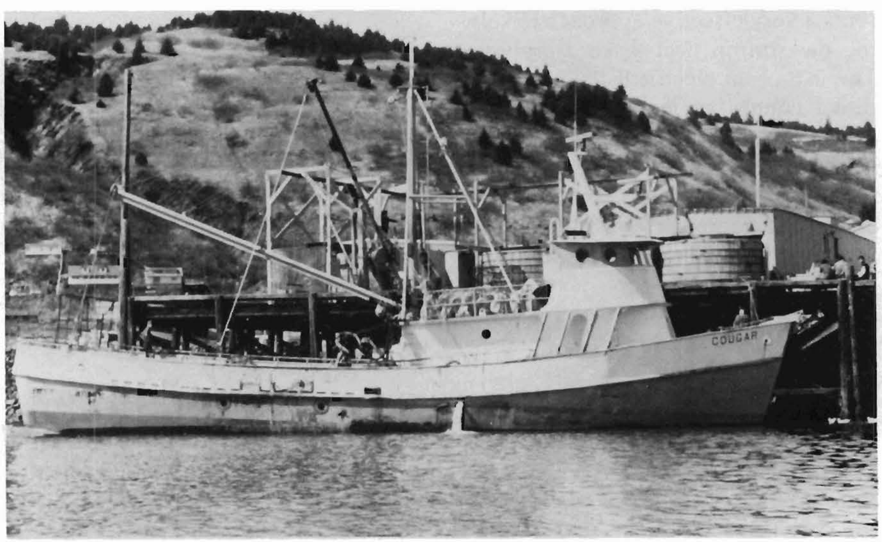
The north Pacific pink shrimp are small, from 80 to 120 whole shrimp per pound commonly, and are usually stored whole in ice aboard the fishing vessel (Fig. 1). Because they spoil easily, the shrimp must be delivered to the processing plant within 3-4 days after harvesting. However, because of the rapid growth in the shrimp industry since the late 1950's, fishing grounds located near processing ports are no longer as productive as they once were. As a result catch quotas have been established for many areas to prevent depletion of stocks, and vessels frequently travel farther to fish, making it increasingly more difficult to bring in full loads of acceptable quality shrimp. The time limit of 4 days in iced storage therefore becomes a controlling factor in expanding the fishing areas. Thus, there is a need for methods of preservation that permit the retention of quality during longer periods of storage on the vessel.

Existing information describing tests to preserve pink shrimp in refrigerated seawater (RSW) indicates that the technique gives good results (Roach and Harrison, 1954; Stern, 1958; Collins, 1960a; Seagran et al., 1960; and Idyll et al.[1]); nevertheless, it has never been consistently satisfactory on a commercial scale. Some failures can be attributed to poorly engineered and misused systems, but allowing for variation in commercial applications, RSW-held shrimp are usually no better in quality than iced shrimp held for the same period.

In studies to improve the RSW method (Barnett et al., 1971), we found

[1]Idyll, C. P., J. B. Higman, and J. B. Siebenaler. 1952. Experiments on the holding of fresh shrimp in refrigerated seawater. Processed Report, ML 2738, 23 p. Fla. State Board Conserv.

Figure 1. —A typical north Pacific shrimp boat.



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that CO_2 gas dissolved in chilled brine effectively retarded the growth of spoilage bacteria on whole fish. Carbon dioxide has been experimentally used before to preserve finfish and pink shrimp (Coyne, 1932, 1933; Hjorth-Hansen, 1933; Stansby and Griffiths, 1935; Makashev, 1954); however, except for a study (Anonymous, 1968) conducted by the Fiskerministeriets Forsøgabortorium, the research has been confined mainly to the effectiveness of storage in controlled gaseous atmosphere. Because the results of our tests in preserving fish in the CO_2-refrigerated brine were so promising, experiments were initiated to determine the effectiveness of the technique applied to pink shrimp. The study described here is concerned with the storage life, quality, and composition of pink shrimp held in refrigerated brine containing dissolved CO_2.

REFRIGERATED BRINE EQUIPMENT

Although the use of CO_2 in brine has favorable results with respect to increased storage life of both fish and the brine, the addition of CO_2 has undesirable side effects such as the acceleration of corrosion rates of metals coming into contact with the acidified brine. For example, NaCl brine saturated with CO_2 is moderately acid and highly corrosive (Barnett et al., 1971). To lessen the occurrence of corrosion in our shrimp experiments in the laboratory, we used the equipment described in the initial publication. This consisted of two fiberglass, insulated, 55-gallon, epoxy-coated drums and a brine chiller (Fig. 2). Polyethylene liners were suspended in the drums to prevent the shrimp from freezing to the sides of the drums. Rotary-type circulating pumps were used for recirculating chilled brine (a solution containing 3.0 percent NaCl and comparable to RSW)[2]. Carbon dioxide gas was fed through a ¼-inch Tygon[3] hose into the suction side of one of the pumps for maximum diffusion.

[2]Sodium chloride brine was used in lieu of natural seawater because clean seawater was not conveniently available to the laboratory. This technique had been used by others (Collins, 1960b; Seagran et al., 1960; Davis and Clark, 1944) and found to give good results. In comparative experiments, Roach and Harrison (1954) demonstrated that fish held in refrigerated brine were of equal quality to fish held in RSW. The term RSW is therefore frequently used to refer to either the seawater or 3.0 percent salt brine media.

Figure 2.—Brine chiller unit for shrimp-holding experiment. Refrigeration unit is at left. Epoxy-lined holding tanks with plastic cooling coils used to chill the tanks are being covered with insulation.

EXPERIMENTS AND OBSERVATIONS ON STORAGE LIFE AND QUALITY

The shrimp (*Pandalus jordani*) used for the experiment were caught in a trawl off the coast of Oregon. In preparing the samples, 375 pounds of shrimp caught about 24 hours before were divided into three equal lots. One lot was iced in a plastic bin and held in a refrigerated room at 34°F. The other two lots were placed in the polyethylene-lined drums at a shrimp-to-brine ratio as close to 1:1 (weight/weight) as possible.

One tank of brine had been treated with CO_2 gas before the shrimp were loaded into it. The gas was fed into the brine continuously throughout the experiment at a rate of 0.2 foot^3/hour. The second tank of brine was not treated with CO_2. The temperature of the brine in both tanks was maintained at 30 ±0.5°F.

Periodically, shrimp from three stored lots and a sample of brine from each of the brine storage experiments were removed for examination.

Criteria used for evaluation included sensory attributes, chemical and physical changes, and bacteria counts of shrimp held in RSW modified with CO_2, RSW, and in ice.

Bacteriological Measurements

Total bacterial plate counts were made on the shrimp by the methods described by Pelroy and Eklund (1966). Briefly, the method is as follows: 45 g of headed shrimp (shells on) were homogenized aseptically with 180 ml of sterile 0.1 percent peptone solution at 3.8°F. Serial dilutions in 0.1 percent peptone-water were prepared for pour plates from the homogenate.

Total plate counts were made by use of TPY solution (1.5 percent trypticase, 0.5 percent peptone, 0.5 percent yeast extract, 0.2 percent glucose, 0.5 percent

[3]Reference to trade names does not imply endorsement by the National Marine Fisheres Service, NOAA.

Table 1.—Microbiological and chemical changes occurring in ice, untreated refrigerated brine, CO_2-treated refrigerated brine, and in the flesh of shrimp held in these brines.

	Data on icing				Data on refrigerated brine without CO_2							Data on refrigerated brine with CO_2						
Storage time (Days)	CO_2 conc. flesh (ppm)	pH flesh	Total bacterial counts flesh (No./g)	Salt conc. flesh (%)	CO_2 conc. flesh (ppm)	CO_2 conc. brine (ppm)	pH flesh	pH brine	Total bacterial counts flesh (No./g)	Total bacterial counts brine (No./ml)	Salt conc. flesh (%)	CO_2 conc. flesh (ppm)	CO_2 conc. brine (ppm)	pH flesh	pH brine	Total bacterial counts flesh (No./g)	Total bacterial counts brine (brine)	Salt conc. flesh (%)
0	1,364	7.4	3.4×10^5	0.5	1,364	462	7.4	7.0	3.4×10^5	—	0.5	1,364	2,552	7.4	4.3	3.4×10^5	—	0.5
1								7.5	1.9×10^4	1.3×10^5					6.3	9.6×10^4	6.5×10^4	
2	—	8.0	1.6×10^6	0.4	482	—	8.3	7.7	5.5×10^5	—	2.1	2,900	5,300	7.2	6.4	8.0×10^5	—	2.0
3																		
4									2.3×10^5	1.8×10^5						2.1×10^5	1.1×10^5	
5	1,012	8.2	1.6×10^8	0	790	1,190	8.2	7.8	3.8×10^7	4.0×10^6	2.1	4,600	5,650			1.2×10^5	1.1×10^5	2.2
6																		
7														7.1	6.5	2.8×10^5	2.0×10^5	2.4

NaCl, and 1.5 percent agar). Counts were made on the brines by taking 1-ml samples, making appropriate serial dilutions in 0.1 percent peptone-water mixture, and plating out into the TPY medium. The plates were incubated at 22 °C for 5 days. Table 1 shows the results for total bacterial plate count analysis on the shrimp and brine samples.

Bacterial counts of the shrimp held in ice increased from 10^5 to 10^6 organisms/gram during the first 2 days of the experiment. A count of 10^6 organisms/gram usually indicates spoilage. At this time, the shrimp had been out of the water about 3 days and showed some loss of color and a slight off-odor. Although no bacterial counts were made between the second and fifth days of the test, the iced shrimp were judged subjectively to be spoiled on the fourth day of the experiment (Table 2). By the fifth day of storage, the iced shrimp were putrid. Total plate counts had risen to 1.6×10^8 organisms/gram.

Bacterial counts decreased in the shrimp held in the untreated brine (Table 1) during the initial stages of storage, probably due to a washing and diluting effect of the brine. The shrimp had an off-odor suggestive of incipient spoilage by the fourth day, although plate counts were below 10^6 organisms/gram at the time. The fewer numbers of bacteria found in both the shrimp and the brine compared with shrimp in ice was due in part to the lower storage temperature and probably also selective bacterial growth patterns that may occur in the nearly anaerobic environment of the brine (Shewan, 1964). A sharp increase in bacteria count was observed on the fifth day of the test corroborating the organoleptic findings that the shrimp were spoiled (Table 2).

Table 2.—Sensory evaluations of shrimp held in ice, untreated refrigerated brine, and CO_2-treated refrigerated brine.

Storage time (Days)[1]	Overall sensory scores on cooked shrimp samples: Ice	Brine	Brine + CO_2	General comments on raw and cooked shrimp samples: Ice	Brine	Brine + CO_2
0	[2]9.0	9.0	9.0	Excellent quality	Excellent quality	Excellent quality
2	7.5	7.8	8.3	Good color, odor but some loss of flavor	Good color, odor but some loss of flavor; slightly salty	Good color, odor, and flavor; slightly salty
3	7.0	6.7	7.5	Slight-moderate, loss of color, moderate off-odor, bland flavor soft texture	Slight loss of color, bland flavor, soft texture, slightly salty	Good color, odor, and flavor, slightly salty, firm texture
4	4.0	—	—	Unacceptable, moderate loss of color, strong spoilage odor, poor flavor, soft texture	Pale color, slight spoilage odor	Good color and odor
5	[3]	4.0	—	—	Unacceptable. Strong spoilage odor, poor flavor, soft texture	Good color and odor
6	[3]	[3]	6.7	—	—	Good color, odor, and flavor. Slightly tough, salty.

[1] Storage time given is the number of days held at the laboratory. The shrimp was held iced approximately 24 hours aboard the fishing vessel before delivery.
[2] A score of 10 denotes a product of highest quality; one of 5 denotes a product of borderline quality.
[3] Too spoiled to taste.

Bacterial counts of the shrimp held in the modified brine (Table 1) were also reduced initially by the dilution effect of the brine. There was an increase in bacteria in the shrimp after 2 days of storage but counts in both the shrimp and brine remained below the level considered to be indicative of spoilage for the remainder of the experiment.

Chemical Measurements

pH

A Beckman combination electrode was used to measure the pH of the shrimp flesh and brines. The shrimp was prepared for analysis by homogenizing a 2:1 mixture by weight of distilled water and raw shrimp flesh. The pH values of the brines are given in Table 1. Almost without exception, the pH of the flesh of the shrimp held in ice and in untreated brine increased during storage. These changes occurred simultaneously with increases in microbial populations in both flesh and brine samples. The pH increase in both the shrimp and the brine was probably due to ammonia from the growth of bacteria.

In the case of CO_2-treated brine, there was an initial and rapid increase in pH from 4.3 to 6.3. This is attributed to buffering by proteins and other soluble components from the shrimp. The initial pH measurement was made before the shrimp came into contact with the brine. The pH of the brine was reasonably constant at about 6.4 after that time. The pH of the flesh of the shrimp held in the modified brine dropped slightly during the experiment but remained within the normal pH range of good quality shrimp. Although tests for ammonia were not made, the good quality and the lower pH of the shrimp throughout the 7 days of storage indicated that little or no ammonia was formed.

CO_2 Concentration

Analyses for CO_2 content in the flesh and brines were carried out as previously described by Barnett et al. (1971). Test results showed (Table 1) that relatively high levels of CO_2 as bicarbonate existed in the meat of the fresh raw shrimp. The concentration of CO_2 in the flesh appeared to remain relatively constant in the flesh during iced storage. The shrimp held in the untreated brine showed a reduction in the concentration of CO_2 in the flesh during storage. A significant increase in CO_2 content of the brine accompanied the general decrease of CO_2 in the flesh. The change suggests that the increase in CO_2 was contributed by protein and metabolic products dissolved from the shrimp.

The flesh of the shrimp held in modified brine contained considerably more CO_2 than did the flesh of the iced and brine-held shrimp. The modified brine, of course, contained a high concentration of CO_2 throughout the holding period.

It is apparent that the high concentrations of CO_2 in the flesh and brine inhibited microbial growth sufficiently to extend the storage life of the shrimp. Similar observations were made by Makashev (1954) and King and Nagel (1967).

Salt Concentration

The concentration of sodium chloride was measured by the methods described in Horwitz (1960). Results are shown in Table 1. The salt content of the iced shrimp was 0.5 percent initially and decreased to zero during 5 days of storage. The maximum concentration of salt in the flesh of the shrimp held in untreated brine was 2.1 percent. This concentration was reached by the second day of storage, after which there was no change. The absorption of salt in the flesh of the shrimp held in the modified brine was closely comparable. The ultimate concentration of salt in the flesh of the CO_2-brine-held shrimp was 2.4 percent. This was reached after 7 days of storage. Similar findings were made by Collins (1960a, 1961) in detailed studies to determine the changes that occur in pink shrimp held in ice and in refrigerated brine storage. He also observed in the study that water, ash, and solids contents in the system changed as a function of time causing both the raw and cooked meats to lose considerable solids.

Sensory Evaluation

Samples of shrimp from each variable in the experiment were examined at intervals to evaluate sensory changes. Samples of each were evaluated both raw and cooked.

Raw Shrimp

During the first 3 days of storage, the raw shrimp from each of the storage experiments were considered to be of acceptable quality. During this time, however, significant changes were observed in the quality of both iced- and untreated-brine-held shrimp. These were in the form of loss of natural odors and pink color. No significant loss of color or development of off-odors was detected in the shrimp held in the CO_2-treated brine. By the fourth day of the experiment, iced shrimp displayed strong ammoniacal odors indicative of microbial spoilage. They were judged to be spoiled at this time. After 5 days of storage, a pronounced sour odor existed in the untreated brine and shrimp. By this time, the shrimp had lost all their natural color and were totally unacceptable. The brine was brown-black in color, the result of what appeared to be an enzymatic reaction.

After 6 days, the shrimp held in the CO_2-modified brine retained much of their natural pink color and were essentially odorless. There were no off-odors in the brine which was light pink in color. The high concentration of CO_2 clearly inhibited the enzymatic changes causing the discoloration of the shrimp and brine when no CO_2 was added.

Cooked Shrimp

The shrimp from each experimental storage condition were prepared for taste panel evaluation by cooking in boiling brine containing 3.0 percent NaCl by weight for 3 minutes. They were then cooled in running tap water. For the purpose of tasting, the brine-held shrimp were cooled slightly longer than the iced shrimp to remove excess salt. The cooked samples were evaluated for appearance, odor, flavor, texture, and overall quality using a 10-point numerical scale. Results are given in Table 2.

The data show that the iced shrimp declined quite rapidly in quality and were unacceptable on the fourth day of storage. Similarly, the shrimp held in the untreated brine declined in quality and were judged by the taste panel to be unacceptable by the fifth day. Although the overall quality of the shrimp held in the modified brine decreased during the experiment, the product was considered to be organoleptically acceptable after 6 days of storage. Except for a slightly tougher texture and somewhat salty flavor, the product was comparable to the quality of shrimp held in ice for 2 or 3 days.

SUMMARY AND RECOMMENDATIONS

Storing north Pacific pink shrimp at 30 °F in refrigerated brine treated with CO_2 doubled the storage life of the shrimp to 6 days or more compared with 3-4 days for the shrimp held in ice. The effect of the dissolved CO_2 was to slow the rate of growth of spoilage bacteria and was caused apparently both by the lower pH of the brine and a specific inhibition by the CO_2. A black discoloration was evident in the head and gut of shrimp after 2 or 3 days of iced storage and in both the brine and shrimp after 5 days when no CO_2 was added. This discoloration is attributed to an enzymatic reaction. The shrimp and CO_2-treated brine showed no discoloration after 6 days indicating effective inhibition of the enzymatic reaction by dissolved CO_2.

The modified seawater technique should be considered for commercial use; however, it is recommended that additional experimental investigations be conducted to determine what effect holding pink shrimp in modified brine has on machine peeling characteristics, yield, and the acceptability and quality of both the frozen and canned products.

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