Attenuation of Microbial Growth on Modified Atmosphere-Packaged Fish

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ABSTRACT

Four species of fish from Atlantic waters, Meronia americanus (perch), Cynoscion regalis (seatrout), Micropogon undulatus (croaker) and Pomatomus saltatrix (bluefish), were processed (gutted or filleted), packaged under carbon dioxide and refrigerated. Stability of the fish under the modified atmosphere preservation (MAP) system was compared to that of fish stored conventionally. Use of the MAP system resulted in a 45 to 55% increase in stability, primarily due to an extension in the lag phase of psychrotrophic organisms and to their reduced growth rate in the logarithmic phase. By the 10th day of storage, the conventionally packed fish always exhibited a 100-fold higher psychrotroph count than the CO2-packed fish. Levels of Vibrio parahaemolyticus were negligible in this MAP system and no Salmonella spp. or Staphylococcus aureus was detected, even at an abuse temperature (10°C) of storage. Positive evidence for performed Clostridium botulinum toxin was lacking.

The inhibitory effect of carbon dioxide on microbial growth on fish has been known for over fifty years (4,5,7). In recent years, this concept has been applied to increase the shelf-life of fresh fish, since the major cause of fish spoilage is microbial growth and its accompanying metabolic products.

One means of adapting this principle is modified atmosphere packaging (MAP). This involves evacuation of air from an oxygen-impermeable container, backflush with a gas (usually CO2) and resealing. The effectiveness of a MAP system in the preservation of fish lies in the combined inhibitory effect of low temperatures and CO2-storage atmosphere on microbial activity. MAP is currently being employed by some packing operations in the seafood industry (2,9,15), and a number of publications have dealt with its efficacy for a variety of fish species (1,3,6,8,10,12).

The basis for the effectiveness of the system is the bacterial selective action of CO2, first reported by Coyne in 1932 (4). While typical spoilage bacteria may be killed or inhibited by CO2 (4), bacteria such as Lactobacillus are not affected (11). Banks et al. (1) found in their MAP study of fish from the Gulf of Mexico, that while common gram-negative spoilage bacteria, such as Pseudomonas, are inhibited by a CO2 atmosphere, gram-positive bacteria (notably lactobacilli) proliferate.

The present investigation was designed to assess the effectiveness and safety of the MAP system in attenuating microbial growth on fresh fish from Atlantic waters. Its effectiveness on fish from colder waters is pertinent, as tropical fish usually respond more favorably to preservation techniques involving low temperatures. In addition, increased stability should not only permit the retail marketing of an acceptable product over a longer period of time, but an expansion of the market place should also accrue.

MATERIALS AND METHODS

Phase 1

Perch (Meronia americanus), seatrout (Cynoscion regalis), croaker (Micropogon undulatus) and blue fish (Pomatomus saltatrix) were caught in the Delaware Bay (day 0) and processed within 24 h. Samples were prepared as fillets or whole fish which were gutted, filleted and rinsed. The MAP fish were CO2-packed in nylon/surlyn bags (CO2 transmission at 0°C of 0.6 cc/ml/100 in.2/h atm at 0% R.H.) and heat sealed. The fish receiving no CO2 treatment were bagged without sealing and termed "ice-packed". Packaging under a CO2 atmosphere was achieved using a commercial flexible packaging machine (CVP Systems, Chicago, IL) to first evacuate the bag and then to backflush with CO2 at 15 in. (37.5 cm) Hg of CO2/24 h/atm and heat sealed. Fish receiving no CO2 treatment were bagged without sealing and termed "ice-packed". Packaging under a CO2 atmosphere was achieved using a commercial flexible packaging machine (CVP Systems, Chicago, IL) to first evacuate the bag and then to backflush with CO2 at 15 in. (37.5 cm) Hg of CO2/24 h/atm and heat sealed.

Initial Phase 1 microbial counts were taken at 0, 10, 20, and 30 d, with perch to estimate stability at 1.1°C. Data collected after these preliminary experiments were at day 1 and at more frequent intervals between days 10 and 25, the time interval when CO2-packed fish became commercially unacceptable.

Fillets were ascetically weighed and then homogenized in cold sterile 0.1% peptone water. Whole fish were sampled by swabbing a 25-cm2 area on the lateral line directly behind the pectoral fin. Appropriate dilutions were made with 0.1% peptone water and plated on trypticase soy agar (TSA, BBL). The mesophilic and psychrotrophic flora were enumerated after incubation of duplicate plates for 2 d at 37°C and 14 d at 4°C, respectively.

Assays for Salmonella, Staphylococcus aureus, Vibrio parahaemolyticus and preformed Clostridium botulinum toxin were done according to the procedures outlined in the Compendium of Methods for the Microbiological Examination of Foods (14).
**Phase II**

Seatout were packaged with and without CO₂ treatment and either transported by normal distribution channels (in the form of retail units in a large master pack) or sent directly to the University laboratory.

Fish were processed 4 d after harvest on receipt at the plant. This consisted of scaling, heading, gutting and spraywashing. The prepared fish were conveyed to a brine tank and held for varying lengths of time in this tank before superficial drying (to remove free water) and packaging. The fish were placed on expanded polystyrene meat trays and overwrapped with a gas permeable film in the form of retail units. Soaker pads were included in retail packs to avoid the problem of free liquid accumulation observed in Phase I. The trays of fish were placed in nylon/surlyn bags and, with the exception of control packs, a vacuum was pulled and the packs were backflushed with CO₂. The fish were handled under five different conditions: (a) packaged under CO₂ at a 20:1 ratio (in.7 CO₂/lb product), at a 10:1 ratio, or without gas treatment and transported via normal distribution channels; (b) packaged without gas treatment or under CO₂ at a 20:1 ratio with dock abuse at the wholesale level, i.e., held 6 h without refrigeration before transportation by normal channels; (c) packaged without gas treatment or under CO₂ at a 20:1 ratio and shipped directly to the University laboratory; (d) packaged without gas treatment or under CO₂ at a 20:1 ratio, held for 4 d at the processing plant and then transported by normal distribution channels; and (e) packaged without gas treatment or under CO₂ at a 20:1 ratio, held 4 d at the processing plant and then transported directly to the University.

The development of bacteria on fish was monitored before and after packaging, after distribution and at 1, 3, 5 and 7 d of retail storage. Samples sent directly to the laboratory were also monitored at the above time intervals.

All fish were sampled using the surface-swab method as described in Phase I. Appropriate dilutions were made in 0.1% peptone water, surface plated in duplicate on plate count agar (Difco), and incubated at 7°C for 10 d.

![Figure 1. Effect of atmosphere composition on microbial populations of gutted, whole perch (Meronia americanus) stored at 1.1°C. • CO₂-packed mesophilic population; ○, ice-packed mesophilic population; ▲, CO₂-packed psychrotrophic population; ▼, ice-packed psychrotrophic population.](http://meridian.allenpress.com/jfp/article-pdf/46/7/610/1656037/0362-028x-46_7_610.pdf)

**RESULTS AND DISCUSSION**

**Phase I**

Comparison of the mesophilic and psychrotrophic populations on whole perch held at 1.1°C for the MAP and ice-packed products are shown in Fig. 1. A marginal difference in mesophilic microbial numbers was observed for the MAP vs. ice-packed samples, particularly at the day 20 sampling, and yet at this point of storage the ice-packed samples were spoiled, whereas the MAP samples retained apparent acceptability. This same relationship was found with seatrout (data not shown). Little difference in mesophile counts, MAP vs. ice-packed, was evident at a stage when substantial differences in product stability became obvious.

In contrast to the mesophilic data, psychrotrophic counts displayed a greater differential between the MAP and ice-packed fish by day 10, and a differential of 1 log₁₀ persisted at day 20 (Fig. 1). If the time for psychrotroph numbers to reach 10⁷/cm² is compared in ice-pack vs. MAP fish, this level was reached by day 10 in ice-packed fish, whereas it was not reached until day 20 in MAP fish. Similarly, a differential was found in the psychrotrophic population of perch fillets (data not shown). Such data represent attenuation of microbial growth on these MAP fish, whether in the whole or filleted state, and thus psychrotrophic counts could be used as an indicator of stability in a MAP system.

Examination of other fish species demonstrated that the CO₂ effect was species independent. Similar attenuation of growth of psychrotrophic bacteria on croaker, bluefish and seatrout within the MAP system was observed. Data in Fig. 2 depict the delay in growth of microorganisms on CO₂-treated fish fillets vs. the ice-packed samples. There was a negligible difference in psychrotrophic counts between ice-packed and CO₂-packed fish after 1 d of storage; the CO₂ treatment did not exert an immediate lethal effect on psychrotrophic bacteria of the fish. Sampling after 10 d of storage always resulted in a substantial differential, and a lower psychrotroph count was always obtained for the CO₂-treated samples.

Not surprisingly, accelerated spoilage was recorded in fish samples stored at 10°C. Bacterial numbers were 3 log₁₀ higher by day 10 for CO₂-packed fish stored at 10°C as compared to those stored at 1.1°C. The effectiveness of the MAP system is clearly enhanced with use of temperatures close to 0°C.

When bags of CO₂-packed fish were opened, an initial acid-type odor was detected which dissipated rapidly. The resulting fish had either no odor or a mild to strong fishy odor depending on the length of storage. No acid odor was detected if bags were opened immediately after packaging under CO₂. The odor detected later may be the direct result of outgrowth of lactic acid bacteria in the MAP system. Such a change in the composition of the microbial flora during MAP storage was reported by Banks et al. (1) and would explain the difference in condition of the MAP product even when bacterial numbers approach similar levels in the later stages of storage.
CO₂-packed fish were examined for the presence of salmonellae, V. parahaemolyticus and S. aureus. Neither salmonellae nor S. aureus was detected with MAP samples regardless of storage temperature (1.1 or 10°C). The levels of V. parahaemolyticus were negligible, i.e., maximum number detected was 23 (MPN/cm²) on croaker and bluefish, and only approached this level in spoiled product.

Samples of both filleted and whole seatrout were stored at 1.1 and 10°C and examined for preformed C. botulinum toxin at 1, 3, 6 and 9 d. No toxin was detected in seatrout stored at 1.1°C using the mouse toxin neutralization test. This is in agreement with prior studies where no toxin was detected in CO₂-packaged fish stored at 7°C and lower temperatures (2). Schmidt et al. (13) reported 3.3°C as the minimum temperature for growth and toxin production by C. botulinum type E.

Sporadic positives for C. botulinum have been reported previously in vacuum- and CO₂-packaged fish stored at temperatures of 7°C and higher (2), but the fish were always “spoiled beyond any hope of human consumption”. In this investigation, no mice died displaying clinical symptoms of botulism after i.p. injection. Inoculation studies, however, should be done and any mouse deaths verified using protected mice. This would not only determine if growth and toxin production can occur, but whether they may be enhanced by MAP.

Phase II

A consistent 10-fold decrease in psychrotrophic populations was obtained after the spraywash, brine tank and drying steps. Before heading and gutting, the psychrotrophic population on seatrout was 1.6 × 10⁶/cm²; after heading and gutting it was 1.9 × 10⁵/cm²; and after the spraywash, brine tank, drying and overwrap it was 1.9 × 10⁴/cm².

Again, the MAP psychrotrophic population on seatrout was lower than that packed without CO₂. In all cases of this phase of the study, the growth rate of the psychrotrophic population was much less than under CO₂ (20:1 in.³CO₂/lb product) vs. the overwrap and no CO₂ treatment. Lanelongue et al. (8) found 100% CO₂ to be one of the most effective modified gas atmospheres for limiting bacterial growth.

During the critical 5 to 11 d into the storage period, the generation time for the psychrotrophic population under CO₂ (20:1 in.³CO₂/lb product) was approximately 24 h vs. 14 h for the untreated population (Fig. 3). This reduced growth rate is reflected in more than a 10-fold difference in bacterial numbers up to 13 d after catch. Figure 3 also shows a lack of effect in using CO₂ at the lower concentration of 10:1 in.³CO₂/lb product. Some reduction in growth rate and total bacteria was observed in comparison to fish with no gas treatment, but increased deterioration in fish quality was evident when compared to fish given the 20:1 CO₂ treatment.

Abuse of samples on the dock (6 h) at the wholesale level had little or no effect on either growth rate or maximum levels of psychrotrophs attained over the 17-d storage period (data not shown). Data for the dock-abused samples with CO₂ treatment or just overwrap were almost superimposable on those for the corresponding non-abused fish of Fig. 3. Such a similarity was also found in comparing microbiological data from seatrout held an additional 4 d before shipping from the processing plant vs. normal shipment procedures. Again the CO₂ treatment markedly curtailed the growth of psychrotrophic microorganisms and delayed the attainment of equal numbers vs. no CO₂ treatment for approximately 6 d. This delay was exhibited both in samples distributed by normal distribution channels and
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Figure 3. Psychrotrophic counts on seatrout distributed through normal channels to the supermarket level. ●, normally processed, 20:1 in. \(^3\)CO\(_2\)/lb product; +, normally processed, 10:1 in. \(^3\)CO\(_2\)/lb product; and ○, normally processed, overwrite only.

direct result of higher quality product reaching the market
place, provided that only quality product is packaged.

in those samples shipped directly to the laboratory. It was found that fish processed at 20:1 in. \(^3\)CO\(_2\)/lb product, whether abused, laboratory-held and/or held an additional 4 d before shipment, displayed superior stability at the critical storage period of 11 to 17 d after catch.

A comparison of fish quality and the psychrotrophic bacterial population revealed that bacterial numbers had to be at a high level (\(10^7\) to \(10^8\) cells/cm\(^2\)) for at least 2 d before a marked decrease in stability was noted. The lag between achievement of high bacterial levels and lower quality was undoubtedly related to the products of metabolic activity of the high bacterial numbers.

In summary, while numbers of psychrotrophs on fish increase over the storage period, the CO\(_2\)-MAP treatment caused a marked lag in the initiation of growth and a reduced growth rate of the spoilage flora, particularly in the initial stage of storage. Thus, CO\(_2\)-MAP provides for consistently improved stability of fresh fish. It should have the

Figure 3. Psychrotrophic counts on seatrout distributed through normal channels to the supermarket level. ●, normally processed, 20:1 in. \(^3\)CO\(_2\)/lb product; +, normally processed, 10:1 in. \(^3\)CO\(_2\)/lb product; and ○, normally processed, overwrite only.

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REFERENCES