Bacterial Growth Rate in Iced Fresh or Frozen-Thawed Atlantic Cod, *Gadus morhua*

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Introduction

During times or seasons of fish harvesting gluts, it may be the practice of some processors to freeze the surplus fillets and to defrost at times of scarcity and release for sale in competition with fresh-caught fish. The same situation may also exist with fish that may have been frozen at sea and thawed at port at the end of the trip. The bacterial spoilage pattern and storage life of iced fresh fish is well established (Reay and Shewan, 1949) and familiar to most retailers, but less is known about thawed fish.

Some retailers have expressed concern that thawed fish in ice may have a different spoilage pattern which could affect retail distribution. The prevailing opinion among many retailers, not based on a controlled study, is that the thawed fish would spoil faster. Therefore, it was considered of practical interest to investigate this situation with Atlantic cod, *Gadus morhua*, a species of commercial importance caught in great quantity in the U.S. northeast during certain seasons.

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ABSTRACT—Fillet portions of freshly landed Atlantic cod, Gadus morhua, *were either directly stored in ice or frozen for a brief period, thawed, and then stored in ice to determine and compare subsequent shelf lives so a retailer handling both products could be cognizant of the comparative perishability of both product treatments. The frozen-thawed portions were found to have as long or a slightly longer iced-storage life compared with nonfrozen portions.*

Materials and Methods

Sample Preparation

Eviscerated market-size Atlantic cod of 1-day post mortem age were obtained from a Gloucester processor. The fish were filleted at the NMFS Gloucester Laboratory of the Northeast Fisheries Center in accordance with standard commercial practice; the fillets were halved, and individual portions were sealed in polyethylene bags and coded. Some fish were stored in flake ice for various times prior to filleting, to determine the effect of post mortem age on freezing.

Samples to be frozen were quick frozen in a plate freezer to a center temperature of −18°C (monitored by thermocouple) and subsequently stored for up to 9 months in a walk-in freezer maintained at −18°C (0°F). When required, samples were allowed to thaw overnight by transferring to a refrigerator at 2°C. For iced storage studies, the packaged samples were buried in a container of flake ice and kept refrigerated at 2°C.

Microbiology

Microbiological analyses were made in duplicate on 50 g samples in accordance with methodology outlined in the Bacteriological Analytical Manual (FDA, 1984). Appropriate dilutions were spread-plated on the surface of pre-poured agar plates and colonies were counted after 5 days incubation at 20°C. For the aerobic plate count, standard methods agar was fortified with 5 g bacto peptone and 5 g NaCl per liter as recommended by Lee and Pfeifer (1974) for seafoods. For counting *Pseudomonas putrefaciens*, bacto peptone iron agar was used as recommended by Levin (1968).

Results and Discussion

The aerobic plate counts (APC) during iced storage of control (never frozen) or thawed samples of cod previously held frozen at −18°C (0°F) for 1 day, 1 month, or 6 months are shown in Figure 1. The initial count in thawed samples was reduced with increasing length of time held frozen. Bacteria in the vegetative state are sensitive to low temperatures and usually die, not always immediately, but may require long periods of frozen storage (Borgstrom 1955). She-

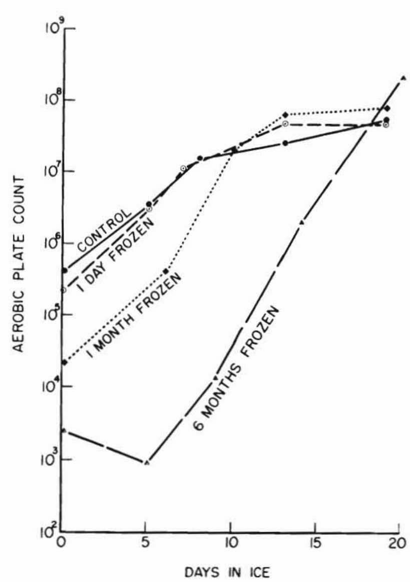

Figure 1.—Aerobic plate counts during iced storage of control or thawed samples of Atlantic cod previously held frozen at −18°C for various times.

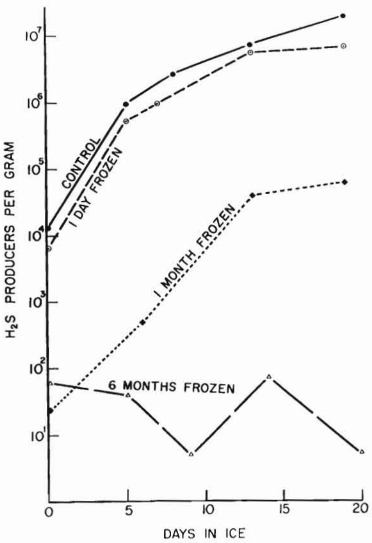

Figure 2.—Bacterial counts of hydrogen sulfide formers (P. putrefaciens) during iced storage of control or thawed samples of Atlantic cod previously held frozen at −18°C for various times.

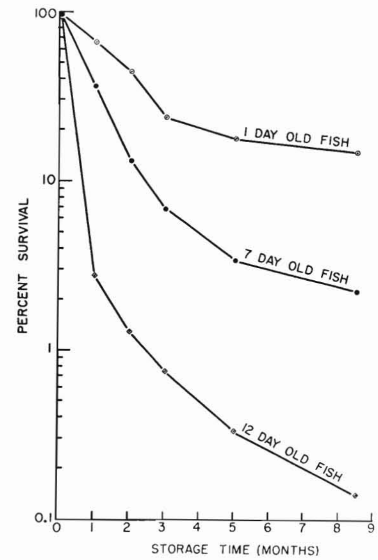

Figure 3.—Percent survival of aerobic bacteria during frozen storage (−18°C) of Atlantic cod at various post mortem ages.

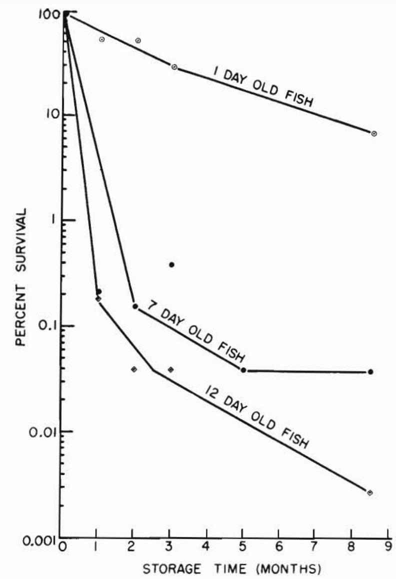

Figure 4.—Percent survival of P. putrefaciens during frozen storage (−18°C) of Atlantic cod at various post mortem ages.

wan (1961) also reported that freezing and cold storage reduced the bacterial load on fish, and based on this fact he surmised that defrosted fish should not spoil as quickly as fresh fish.

In this study it is not considered that there was any difference in growth rate between control samples and samples which had only been held frozen 1 day. With longer frozen storage, there was a slight early lag in bacterial growth, but eventually all treatments reached the same maximum count. We have usually observed that spoilage of iced cod fillets occurs when the APC reaches about 10^6/g. With this stipulation, the control and 1-day frozen samples would have the same iced shelf life, but samples held frozen longer would have a slightly extended iced shelf life. This conclusion was also supported by the observation that during iced storage, the fresh samples developed spoilage odors sooner than the defrosted samples. Other researchers working with Cape hake also found that due to a lowering in bacterial numbers during frozen storage, the frozen-thawed fish had a longer storage life at chill temperatures compared to fresh fish (Simmonds and Lamprecht, 1980).

The growth rates of *Pseudomonas putrefaciens* during iced storage of control or frozen-and-thawed samples can be seen in Figure 2. This particular bacterial species is the principal H_2S producer on fish flesh and is considered to be a major fish spoilage organism because of its proteolytic activity (ability to reduce trimethylamine oxide to trimethylamine, having a fishy odor) and production of hydrogen sulfide (Castell et al., 1949). This microorganism was very sensitive to long-term frozen storage; in thawed samples held frozen for 6 months, there was no growth during iced storage.

The effect of frozen storage time at −18°C on survival of the aerobic bacteria on cod is shown in Figure 3 for 1, 7, or 12 days post mortem age prior to freezing. The survival curves indicated that the older the fish at the time of freezing, the greater the reduction in bacterial numbers during frozen storage. This could be due to a quantitative change in the microbial flora during post mortem iced storage with a predominance of more freeze-sensitive cells in the older fish. There also was a greater rate of inactivation during the first few months of frozen storage. The more freeze-sensitive cells in the flora probably died off first. From these results one might surmise that keeping fish frozen for an extended time prior to thawing and storing in ice might be an effective way of reducing the bacterial load and thus increase subsequent iced storage life. However, Reay (1935) reported that the course of bacterial decomposition in iced fresh or thawed haddock (previously held frozen at −21°C) was the same, but physical-chemical changes (oxidative rancidity and textural toughening) during extended frozen storage impaired the quality of the thawed fish during subsequent iced storage.

The fate of *Pseudomonas putrefaciens* during frozen storage of cod of various post-mortem ages (Fig. 4) was similar to that of the aerobic plate count; that is, the older the fish at time of freezing, the greater the rate of inactivation. However, *Pseudomonas* was more readily inactivated during frozen storage. For example, after 2 months, the percent survival was about 0.1 for the *P. putrefaciens* and 4.0 for the total aerobes when comparing the averages of 7- and 12-day-old fish. Reduction in numbers of these major fish spoilage bacteria during frozen storage may have played a principal role in the extended iced shelf life of the thawed samples.

Conclusions

The aerobic bacterial count of cod fillet portions was reduced during frozen storage at $-18°C$, a greater reduction occurring with increasing storage time. The older (staler) the fish at time of freezing, the greater the reduction in bacterial numbers during frozen storage. *Pseudomonas putrefaciens*, a major fish spoilage bacteria, was readily inactivated during frozen storage. The iced shelf life of frozen-and-thawed cod compared with fresh cod was similar or extended depending on length of frozen storage.

Literature Cited

Borgstrom, G. 1955. Microbiological problems of frozen food products. *In* E. M. Mrak and G. F. Stewart (editors), Advances in food research. Vol. 6, p 163-228, Acad. Press, Inc., N.Y.

Castell, C. H., J. F. Richards, and I. Wilmot. 1949. *Psuedomonas putrefaciens* from cod fillets. J. Fish. Res. Board Can. 7(7):430-431.

FDA. 1984. Bacteriological analytical manual. 6th ed. AOAC Publ., Arlington, Va.

Lee, J. S., and D. K. Pfeifer. 1974. Influences of recovery media and incubation temperatures on the types of microorganisms isolated from seafoods. J. Milk Food Technol. 37:553.

Levin, R. E. 1968 Detection and incidence of specific species of spoilage bacteria on fish. Appl. Microbiol. 16(11):1734-1737.

Reay, G. A. 1935. The preservation of fresh and thawed fish in ice. J. Soc. Chem. Ind. (Mar. 1935):96-98.

_______ and J. M. Shewan. 1949. The spoilage of fish and its preservation by chilling. *In* E. M. Mrak and G. F. Stewart (editors), Advances in food research. Vol. 2, p. 343-398. Acad. Press Inc., N.Y.

Shewan, J. M. 1961. The microbiology of seawater fish. *In* G. Borgstrom (editor), Fish as food. Vol. 1, p. 487, Acad. Press Inc., N.Y.

Simmonds, C. K., and E. C. Lamprecht. 1980. Spoilage patterns of wet and thawed frozen Cape hake during chilled storage. *In* J. Cornell (editor), Advances in fish science and technology, p. 417-421. Fish. News Books, Ltd., Farnham, Surrey, Engl.