The Effect of Handling or Processing Treatments on Storage Characteristics of Fresh Spiny Dogfish, *Squalus acanthias*

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ABSTRACT—This investigation has determined the spoilage pattern and iced-storage life of spiny dogfish, Squalus acanthias, *and shows how its storage life might be affected by such handling practices as 1) gutting and/or bleeding the fish immediately following removal from the water or 2) holding these fish at other than the ideal iced temperature. Well-iced dogfish have a shelf life of about 11-12 days. If gutted immediately, the shelf life can be extended about 4 days. Despite their high urea content, ammonia formation does not seem to be a problem in well-iced dogfish. However, at elevated temperatures (46.4° and 57°F), ammonia formation is much more rapid and drastically shortens the species' shelf life. A self-contained kit for quick and simple field estimation of ammonia in dogfish flesh was found useful.*

Introduction

Spiny dogfish, *Squalus acanthias*, have been fished off the northeast coast of the United States since the late 1800's. Before the synthesis of vitamin A in 1947, the rich, oily dogfish livers provided a valuable source of that vitamin, with tens and hundreds of thousands of pounds of dogfish harvested annually from the Northwest Atlantic (Jensen, 1967). Thereafter, dogfish catches continued on a small scale, providing raw materials for other industrial processes.

However, a thriving dogfish industry never developed, and the underutilized U.S. stocks flourished and were viewed primarily as a nuisance by commercial and sport fishermen because of the damage they caused to both fishing gear and catches of more valuable fishes. Dogfish control methods even received serious consideration (Alverson and Stansby, 1963).

More recently, heavy demands on world fishery stocks have nearly depleted many higher-valued and more popular species, forcing examination of alternate species and technologies for preserving their quality. The present U.S. dogfish market is small but developing (Anonymous, 1982; Dean et al.[1]). Many European countries, on the other hand, consider this species an important food fish (Ronsivalli, 1978), thus providing U.S. fishermen with export opportunities.

Based on data collected during NMFS Northeast Fisheries Center bottom trawl research surveys, minimum biomass estimates of spiny dogfish off the northeast coast of the United States have increased sharply in the last several years (Anonymous, 1983). In 1982, biomass was estimated at 900,000 metric tons (t), 2.5 times greater than the 1968-80 average. Domestic landings peaked in 1981 at 7,000 t. Based on a long-term average biomass of 300,000 t, a potential yield of 65,000 t has been estimated (Anonymous, 1983), nine times the maximum domestic harvest to date.

Simply stated, the spiny dogfish off the northeastern U.S. coast represents a greatly underfished resource. Future development of this fishery will probably depend on the strength of the export market more than on expansion of the domestic dogfish market.

Unfortunately, many U.S. fishermen are not accustomed to handling this fish with the care needed to retain maximum acceptable quality for human consumption. The image of dogfish as a "trash fish" or as a "villain" of the sea seems to lend to the poor treatment it receives from some fishermen.

Spiny dogfish, like other sharks and elasmobranchs, have a large concentration of urea (1.7 percent) in their blood and flesh (Simidu, 1961). Urease, a bacterial enzyme, degrades this urea to ammonia and carbon dioxide, and this action proceeds rapidly if dogfish are improperly handled from the time of catch. And, as the concentration of ammonia builds up, acceptability of the flesh decreases. Vyncke (1970) showed that in spiny dogfish held 5 days, ammonia increased earlier and reached higher values when the dogfish were held for 15 hours at 15°C before being iced and kept at 0°C.

Low temperature is generally considered to be the most important factor in maintaining high quality in fish. Between storage temperatures of 25°C and 0°C, every 5°C decrease yields about a 70 percent increase in time before spoilage of dogfish (Anonymous, 1981). Vyncke (1968)

[1]Dean, L. M, D. R. Ward, and J. Axelson, 1982. A preliminary study to determine the feasibility of marketing spiny dogfish in Virginia. Final Rep. Grant "01-01-10000, Mid-Atl. Fish. Develop. Found., Annapolis, Md.

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found that ammonia increased very slowly during the first 8 days of storage in dogfish which were gutted and stored in ice at 1°C immediately after capture, but increased rather rapidly after that period to reach values of 75 mg % N after 14 days.

Foreign nations accustomed to high quality dogfish products will not accept less from an imported product. Belgium denies entry to dogfish if the ammonia nitrogen concentration exceeds 55 mg per 100 g of fish (55 mg % N) as determined by an accelerated microdiffusion method (Vyncke, 1968). France has set an upper limit of 100 mg % N (chemical test unspecified).

Since ammonia development is generally considered the most obvious indicator of dogfish spoilage, the USDC Fish Inspection Service monitors it as an indicator of flesh quality. More extensive knowledge, however, of spoilage patterns of refrigerated dogfish is needed to identify all problems associated with handling. In this four-part investigation, we have examined the spoilage patterns of iced and refrigerated dogfish and how they may be affected by handling techniques. Our primary objective was to offer evidence for recommended guidelines in dogfish handling. Secondary objectives were to establish the reliability of various chemical or physical tests for assessing sensory quality and to develop a rapid quantitative test for ammonia that could be used in the field.

Treatment of Fish

Study 1—Iced Storage Life of Spiny Dogfish

Part I

Fresh dogfish, averaging 10 pounds round weight, were procured in July 1980 from a Gloucester, Mass., day boat. Fish less than 24 hours postmortem were gutted, rinsed, and iced. They were stored in standard 125-pound capacity wooden fish boxes with three layers of fish per box and three fish per layer, and with generous icing between layers. Additional flake ice was added only to the top layer of fish during the storage period to replenish melted ice. The boxes of iced fish were held in a refrigerated room at an ambient temperature of 34-37°F (1-3°C). After intervals of 0, 2, 5, 9, 12, 15, 19, and 21 days, three fish were taken from storage for testing. One fillet from each fish was used for analytical tests, the other for organoleptic evaluation.

Part II

In December 1980, dogfish caught by a day boat were landed at Point Judith, R.I. They were held at the Pt. Judith Fish Co-op[2] in 125-pound capacity wooden fish boxes with ice until they were transported to our Gloucester Laboratory 2 days later, when they were headed and gutted (H&G), rinsed with tap water, and reiced. Storage of these fish was the same as in Part I. Fish which were 3, 5, 9, 12, 16, 18, and 23 days postmortem were removed from storage for testing. Belly flap and fillet portions of each of three fish were examined separately for each test period. One fillet and corresponding belly flap of each fish were used for analytical testing, and the other fillet and belly flap were used for sensory evaluation.

Study 2—Effect of Heading and Gutting, and Gutting Only

Arrangements were made with the skipper of a Gloucester day boat in September 1981 to supply us with three lots of 1-day postmortem dogfish which were 1) gutted, 2) H&G, and 3) whole. We considered that the H&G treatment would also serve to bleed the fish. We concede, however, that the most effective method of bleeding dogfish is by notching the tail vein or chopping off the caudal peduncle. This permits the heart, if the fish is still alive, to pump out most of the blood.

Upon arrival of these fish at the laboratory, initial Torrymeter readings were made and each fish was tagged for identification. Wood boxes containing two layers of four fish each, with generous amounts of flake ice, were stored in a refrigerated room at 35°F (1.6°C). Two fish from each lot were removed for analysis after being held in ice for 1, 4, 7, 10, 15, 18, 21, and 24 days. Fillet portions only were used for testing; belly flaps were discarded. With each fish, one fillet was used for analytical tests, the other for sensory evaluation.

Study 3—Effect of Storage Temperature

In October 1982, 1-day postmortem whole, well-iced dogfish, landed in Gloucester, Mass., were received at the laboratory. Initial Torrymeter readings were made and each fish was tagged. The fish were stored in wood boxes in a single layer without ice, four fish to a box. The fish were divided into two lots, half being stored at 57°F (14°C) and half at 46.4°F (8°C). For analysis, two fish were removed from storage at 46.4°F after 0, 1, 4, 7, and 11 days and at 57°F after 0, 1, 4, 5, and 6 days. One fillet from each fish was used for analytical tests and the other for sensory evaluation.

Study 4—Rapid Estimation of Ammonia

Excess dogfish flesh from Study 3 was stored in glass jars at −20°F (−28.9°C) until analyzed.

Analytical Tests

The analytical tests performed for Part I of Study 1 included moisture, pH, ammonia, and trimethylamine analyses. Tests for Part II of Study 1 included moisture, pH, thiobarbituric acid, ammonia, trimethylamine oxide, trimethylamine, and dimethylamine analyses, and aerobic plate counts.

For Studies 2 and 3, moisture, pH, thiobarbituric acid, ammonia,

[2]Mention of trade names or commercial products or firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

trimethylamine oxide, trimethylamine, dimethylamine, and Torrymeter tests were conducted, and aerobic plate counts were made. For Study 4, ammonia was analyzed. The entire fillet or belly flap to be analyzed was finely minced and thoroughly mixed. Test methods are given below.

Moisture

A 10-15 g sample of minced flesh, accurately weighed, was dried in an oven at 217.4°F (103°C) for 24 hours to constant weight.

pH

Twenty grams of minced flesh was blended with 40 ml distilled water for 1 minute, and the pH of the homogenate was measured with a Fisher Model 320 expanded scale pH meter.

DMA, TMA, TMAO

Dimethylamine (DMA) and trimethylamine (TMA) were determined by a modification of a gas chromatographic method described by Tokunaga et al. (1977). Modification by Lundstrom and Racicot (1983) included substitution of a 9-foot × 2 mm i.d. glass column packed with Chromosorb 103 which allowed a baseline separation of DMA and TMA, and use of a nitrogen-phosphorus specific flame ionization detector. For quantitation of the amines, N-propylamine was used as an internal standard. Trimethylamine oxide (TMAO) was determined as TMA after reduction by titanous chloride (Yamata et al., 1969).

TBA Number

For determining thiobarbituric acid (TBA) reactive substances, the method of Yu and Sinnhuber (1957) was modified by the addition of disodium ethylenediamine tetraacetate (EDTA) and propyl gallate to prevent oxidation during blending. TBA number was calculated by the procedure reported by Sinnhuber and Yu (1958).

Ammonia

Ammonia (NH_3) content of the flesh was determined by the microdiffusion method developed by Seligson and Seligson (1951) and later modified by Vyncke (1968). The Chemetrics Ammonia Nitrogen Test Kit Model AN-10 was also used when a comparison of methods was desired.

Total Volatile Bases

Total volatile bases (TVB) is a measure of the total volatile amine compounds present, and it collectively includes ammonia, monomethylamine, dimethylamine, and trimethylamine (Simidu, 1961). In this study, monomethylamine was not detected in measureable quantity, and TVB was therefore considered to consist of ammonia, di-, and trimethylamine. These latter compounds, individually or as TVB, have been employed as chemical indices of spoilage in fish. There are specific methods for determining TVB; however, we did not employ them. Instead, ammonia, TMA, and DMA were ascertained separately and the contents at any given time were summed to obtain TVB. The concentration was expressed as mg nitrogen per 100 g sample or mg % N.

Torrymeter

The Torrymeter (Jason and Lees, 1971; Jason and Richards, 1975) is an electronic instrument developed by the Torry Research Station, Aberdeen, Scotland, and designed to directly assess the relative quality of fresh (unfrozen) fish by measuring changes in the electrical properties of fish flesh during storage. In Study 1, readings were taken at three consecutive positions along the lateral line, starting at the first dorsal fin and proceeding to the caudal peduncle. Readings were generally constant in the region between the two dorsal fins, but were lower at the caudal peduncle; the average of the three readings was recorded.

For Study 2, five readings were taken in about the same location midway between the two dorsal fins and averaged. That is, when a reading was taken, the meter was removed and then repositioned in about the same spot and read again, etc. The average standard deviation for five such readings was 0.80. The magnitude of this standard deviation points out the uncertainty associated with a single reading.

Chemetrics Kit Comparison With Vyncke Method

Flesh and distilled water were blended together in a Waring blender in a 1:1 ratio for 1 minute. Then, 10 g of this homogenate was combined with 190 ml distilled water and blended for 2 minutes. The homogenate was filtered with suction, and portions of the filtrate were used for ammonia determination by both the Vyncke method and Chemetrics kit method. Further dilutions of the filtrate with distilled waters were made when necessary.

Glass ampoules in the Chemetrics kit hold a premeasured color-forming reagent (Nessler reagent) sealed under vacuum. To test the water sample, the ampoule tip is broken below the surface of the water sample with a special snapper device supplied with the kit. A vacuum draws the sample into the ampoule where it is mixed. A yellow-orange color develops immediately, allowing an instant comparison of the test sample with liquid color standards supplied in the kit. After mixing, the ampoule must stand for 1 minute and then be read immediately because the color intensifies with time. The presence of TMA offers no interference with the measurement of ammonia by this kit.

The Model AN-10 kit is supplied with two comparators which cover the ranges 0-1 ppm ammonia and 1-10 ppm ammonia. In dogfish flesh the concentration of ammonia will usually be within the range of 0-1,000 ppm or 0-100 mg N per 100 g (mg % N). Therefore, after extraction of the sample, a final hundredfold dilution will provide a sample with an ammonia content within the range suitable for analysis with this kit. The

comparator block contains ten color standards representing ammonia concentrations ranging from 1 to 10 mg % N. In most cases the color intensity of the unknown sample will lie between two adjacent standards and interpolation is required. Thus, a probable range of ammonia concentration will be obtained with the kit rather than an absolute value. However, an estimate of the absolute ammonia concentration can be obtained from the median value of the range. Therefore, if the actual range observed was 0-8 mg % N, the absolute concentration is estimated as 4 mg % N.

Fat Content

Total lipid was obtained by the methanol-chloroform extraction procedure of Bligh and Dyer (1959).

Aerobic Plate Count

Aerobic plate count (APC) was made from appropriate dilutions of the flesh onto pour plates of TPE agar (Standard Methods Agar reinforced with 0.5 percent Bacto-peptone and 0.5 percent NaCl) as recommended by Lee and Pfeiffer (1974) for seafoods. Duplicate plates were incubated at 68°F (20°C) and colony counts were made after 5 days.

The number of urease-positive bacteria was estimated by inoculating half of the colonies from a TPE agar plate containing about 30-100 colonies into individual tubes of Bacto-urea broth. Following an incubation period, if a positive reaction was observed, the number of positive cultures was then multiplied by two and also by the dilution factor of the agar plate from which they were taken to obtain the urease-positive count per gram.

All chemical, physical, or microbial analyses were made on duplicate samples and the average result is reported. Statistical calculations and analyses were performed on a Hewlett-Packard 97 programmable calculator.

Sensory Analysis

For organoleptic evaluation, samples were enclosed in aluminum foil and baked in an oven at 400°F (204°C) for 15-20 minutes for belly flaps, or for 20-30 minutes for fillets. The odor, flavor, and texture of cooked samples were rated on a scale of 9 (excellent) to 1 (inedible) by a six-member taste panel from the scientific laboratory staff. Limit of acceptability was reached when the average sensory rating for either odor, flavor, or texture dropped to a value of 6 (fair). In addition to the usual sensory attributes, panelists were instructed to concentrate on their awareness of ammonia and note its presence when detected.

Results and Discussion

Study 1—Iced Storage Life of Spiny Dogfish

To determine the iced storage life of spiny dogfish, our study was carried out in two parts. The first, using gutted fish, was preliminary, and provided information on spoilage patterns which might be encountered in dogfish and explored the reliability of some chemical and physical tests for quality assessment.

The results of the first part of this study are shown in Figure 1. Flavor and odor of the fillets changed little during the first 15 days of iced storage. Thereafter, quality deteriorated rapidly, and the samples were considered to have lost their shelf life after 16-17 days. Ammonia, TMA, TVB, and pH increased slightly during the first 15 days, but increased markedly thereafter. Thus, the decrease in quality coincided with the increase in volatile amines and pH.

This delayed development of ammonia was unanticipated. Since foreign nations such as Belgium and France concentrate their attention on the ammonia content of imported dogfish, we expected that an early production of ammonia would limit the shelf life. Instead, the ammonia content remained relatively low for the first 2 weeks, and the spoilage pattern seemed not much different from that which develops in iced gadoid fish.

There was a high correlation between ammonia content and either flavor score (r = 0.89) or odor score (r = 0.92). From linear regression analysis, an ammonia concentration of about 32 mg % N was estimated as being indicative of marginal quality dogfish. A high degree of correlation was also observed between flavor score and either pH (r = 0.94), TMA

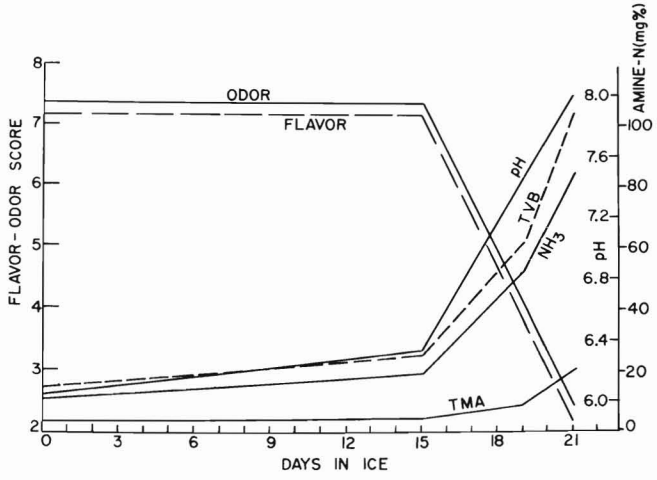

Figure 1.—Storage characteristics of gutted dogfish held in ice.

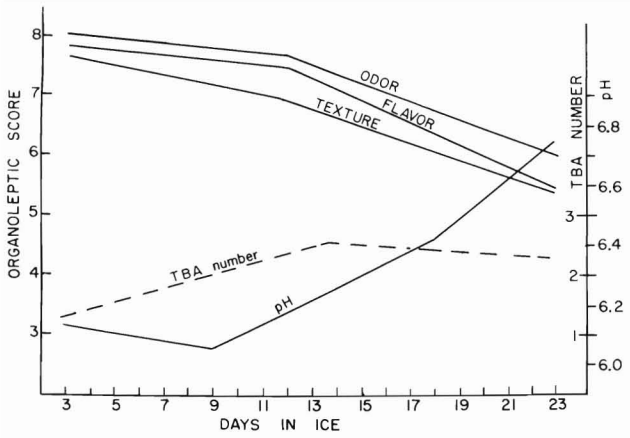

Figure 2.—Storage characteristics of H&G dogfish (fillets) held in ice.

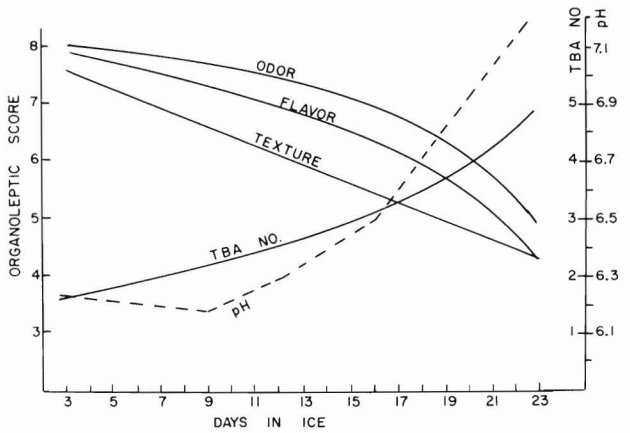

Figure 3.—Storage characteristics of H&G dogfish (belly flaps) held in ice.

content (r = 0.88), or TVB content (r = 0.91). Thus, from these preliminary results it appears that any of these biochemical tests might be useful for assessing spoilage in dogfish providing there is a sufficient degree of accuracy and reproducibility among different batches of fish.

Ammonia content also correlated very well with pH (r = 0.97). Thus, pH measurement also shows potential as a simple, rapid method for monitoring ammonia content.

In the first part of this study attention was focused on icing the fish thoroughly. However, toward the very end of the experiment all the ice beneath the bottom layer of fish had melted and the ventral side of the fish, though still well iced above, was in contact with the wood container. Therefore, Part II of the iced storage study was conducted in December 1980 with H&G fish to ascertain whether the storage life might be extended beyond 16-17 days under conditions which assured complete icing at all times.

In our preliminary study, a few taste test panelists detected rancidity rather than ammonia. And, owing to the dogfish's high fat content (Jhaveri and Constantinides, 1981; Bilinski et al., 1983) we thought it important to include this parameter in our follow-up tests. Because belly flaps have a higher fat content than fillets, the two sections were analyzed separately. We also felt that bacterial testing would be useful, considering the nature of ammonia formation in dogfish.

In Figure 2, the pH value, TBA number, and organoleptic scores for flavor, odor, and texture have been plotted as a function of iced storage time for fillet portions taken from the stored H&G fish. The same parameters are shown in Figure 3 for the belly-flap sections. During storage there was a progressive deterioration in odor, flavor, and texture (softening), and this was accompanied by an increase in pH (due to accumulation of basic amines) and TBA number. Spoilage occurred faster in belly flaps than in fillets. In fact, the fillets were only on the threshold of spoilage after 18 days, whereas the belly flaps were regarded as marginal at about 12-13 days. Rancidity, as measured by TBA number, was more evident in belly flaps; however, a few members of the taste panel reported rancid flavors in the fatty strip along the lateral line of the fillet as early as the 16th day, even though the lean muscle tissue was devoid of rancid flavor throughout the entire storage.

In general, fillet spoilage was due to textural softening and development of ammonia, whereas belly flap spoilage was caused by rancidity, ammonia, and textural breakdown. Thus, from the results of this first study we concluded that the iced shelf life of gutted dogfish, excluding belly flaps, was 16-18 days.

In the scientific literature there is much variation in the estimated storage life for iced dogfish. James and Olley (1971) cited a shelf life for shark (species unidentified) of 10 days at 32°F from a Japanese reference; most important, that shark had been stored in air at 32°F and not in melting ice at 32°F. Southcott et al. (1960) reported that less ammonia and TMA were produced in dogfish stored in ice than when stored in air at 32°F. Therefore, this implies that the iced storage life of the shark referred to by James and Olley (1971) would be greater than 10 days.

An iced storage life of 16-19 days was determined by Southcott et al. (1960) for H&G dogfish. The species was reported as Pacific coast dogfish, *Squalus suckleyi* (since established as the same species as the Atlantic coast spiny dogfish, *Squalus acanthias*). Stansby et al. (1968) concluded that the storage life of iced H&G Pacific coast dogfish was limited to the development of rancidity and shelf life was estimated as 14 days. In our study, storage lives were determined separately for the belly flap and fillet section with flap removed; we con-

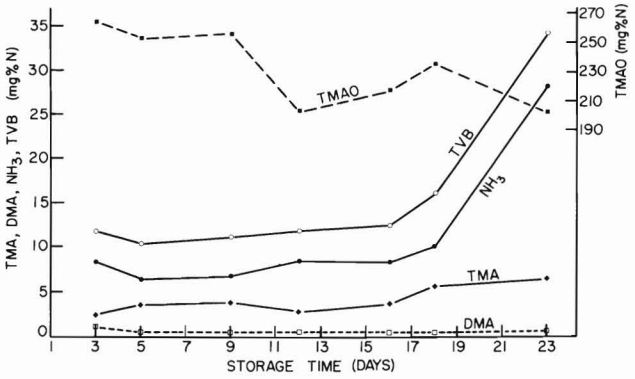

Figure 4. – Effect of storage time at 32°F (0°C) on the content of certain volatile amines in H&G dogfish (fillet portion).

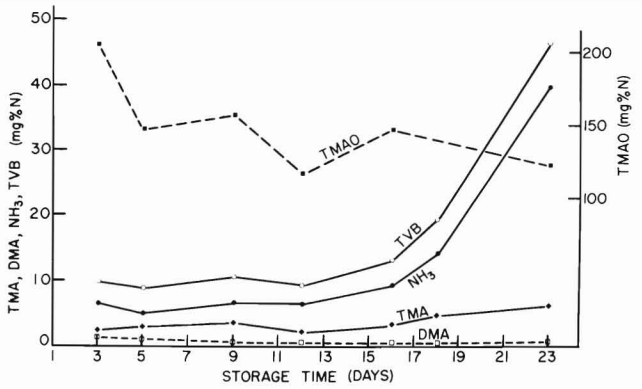

Figure 5. – Effect of storage time at 32°F (0°C) on the content of certain volatile amines in H&G dogfish (belly flaps).

ceded that rancidity was probably a significant factor involved in belly flap spoilage but not of the flapless fillet. Stansby et al. (1968) may have been testing the entire fillet with belly flap attached. Even so, from the cited shelf lives it would appear that the iced shelf life of H&G dogfish would be comparable to that for cod and haddock.

Most recently Bilinski et al. (1983) found no significant increase in ammonia during 20 days of storage of iced gutted dogfish; however, quality loss during iced storage was detected earlier due to the development of off odors and off flavors, softening of the flesh, and autolysis of the abdominal walls. They concluded that these changes limited the useful iced storage time to 8-10 days.

The shortest life reported for iced H&G dogfish was 7 days, based on ammonia detection at that time (Jhaveri and Consantinides, 1981). In our study, the ammonia content (and TVB) were relatively stable during the first 16 days of storage, but increased thereafter (Fig. 4, 5). Stansby et al. (1968) similarly found that the TVB was low (< 12 mg % N) during the first 14 days of storage of iced dogfish. Moyer et al. (1959) reported that the TVB remained at a level of about 10 mg % N throughout the first 12 days of iced dogfish storage, and after 3 weeks had only increased to 18 mg % N with no ammonia detected by sensory analysis.

Elliot (1952) also noted a slight trace of ammonia after just 6 days in the minced dogfish stored in jars at 32°F. However, minced fish is certainly more prone to microbial degradation than intact muscle because of the increased surface area, rupture of cells releasing enzymes, nutrients, etc., and dogfish spoils faster in air at 32°F than in ice at 32°F. Therefore, these factors could account for the early ammonia production observed by Elliot (1952). Also, Elliot's generally high values of ammonia are likely due in part to his method of analysis which utilized dogfish muscle extracts without the removal of protein.

Vyncke (1968) reported slight increases in ammonia in dogfish during only the first 8 days of storage in ice and a rapid increase after that period to reach values of 75 mg % N flesh after 14 days. His analyses were carried out on protein-free extracts; however, the postmortem age of his samples was not indicated. In another study, Vyncke (1970) used 5-day postmortem fish with an initial TVB concentration of 30 mg % N and reported a slow rise to about 35 mg % N until 9 days postmortem, a sharper rise to about 55 mg % N until 12 days postmortem, and finally a very rapid increase to more than 90 mg % N until 15 days postmortem. Ammonia accounts for the major portion of the TVB production since, as Elliot (1952) reported, the high concentrations of trimethylamine occurring in dogfish flesh and other elasmobranchs are reduced only in small amounts to trimethylamine. The results of our study are most compatible with those of Moyer et al. (1959) and Stansby et al. (1968).

In the second part of our study an ammonia content of about 20 mg % N was predicted at the estimated spoilage time. A value of about 30 was noted in the first part of this study. These spoilage levels are much less than the value of 60 mg % N recommended by Vyncke (1968) as the cutoff value for acceptable/unacceptable imported dogfish.

DMA concentration remained relatively constant and insignificant (< 1 mg % N) throughout storage of both fillets and belly flaps (Fig. 4, 5). Hence our results indicate that a spoilage test based on DMA content would not reliably measure quality of iced dogfish.

The TMA content increased gradually over the 23-day storage period, attaining a final maximum value of about 6 mg % N. Usually at spoilage, at least for cod, a TMA con-

centration of about 15 mg % N is obtained (Dyer and Dyer, 1949). In their study with iced dogfish, Moyer et al. (1959) reported that the TMA content was low throughout 21 days of iced storage, and had only reached a maximum value of 5 mg % N. Similar results were obtained by Southcott et al. (1960). These investigators found that the TMA content of iced dogfish remained constant for about the first 14 days and reached an estimated value of 10 mg % N after 20 days. Elliot (1952) likewise encountered a relatively low final TMA concentration in his studies with minced dogfish muscle stored at 32°F.

Trimethylamine is formed by the action of a bacterial enzyme, triamine oxidase, on the precursor substance, trimethylamine oxide (TMAO), found in marine species. The optimum pH for activity of this enzyme was reported to be 7.2-7.4 in cod (Castell and Snow, 1949). This optimum range was also confirmed in dogfish by Elliot (1952), who speculated that the reason for only a small amount of TMA produced in dogfish (compared with gadoid species) during spoilage is that the urease-positive bacteria become active sooner than the TMA-producing bacteria, thus forming ammonia which soon elevates the pH beyond the optimum for triamine oxidase activity.

More TMAO was degraded (reduced) than could be accounted for by the production of end products such as TMA or DMA. For example, the TMAO content of fillets decreased by about 58 mg % N over the 23-day period, yet TMA only reached a maximum value of about 6 mg % N. Dyer et al. (1946) studied iced gutted cod and observed a disappearance of TMAO which they attributed to leaching by the melting ice. There was a higher initial concentration of TMAO in the fillet portion (263 mg % N compared with the belly flaps, 204 mg % N), and this could be related to the compositional differences between these two anatomical sections.

Among sharks, dogfish must have a unique fat content. Sidwell et al. (1974) reported a fat content of 0.5 ± 0.2 percent for mixed shark species. And in 19 out of 21 shark species compared, the fat content was less than 2 percent (Gordievskaya, 1971). The two exceptions were sevengill shark (13 percent fat) and Greenland shark (10 percent fat).

We found the dogfish fat content (mean ± 1 S.D.) averaged 11.2 ± 2.2 percent for fillets and 22.6 ± 3.7 percent for belly flaps. A seasonal variation was reported in the lipid content of dogfish with a mean value and standard deviation of 14.5 ± 2.2 percent, and highest fat content occurring during the winter months (Jhaveri and Constantinides, 1981). However, these researchers analyzed a composite sample taken from dorsal and ventral areas, and therefore our results cannot be compared directly with theirs.

Bilinski et al. (1983) reported a muscle fat content of 16.51 percent, SE = 0.62. Again, our direct comparison with their results is impossible because their determinations were made on homogenates of three steaks (25 mm thick and devoid of skin and bone) cut from the head, middle, and tail regions of each fish and containing both the dorsal and the belly portion of the muscle.

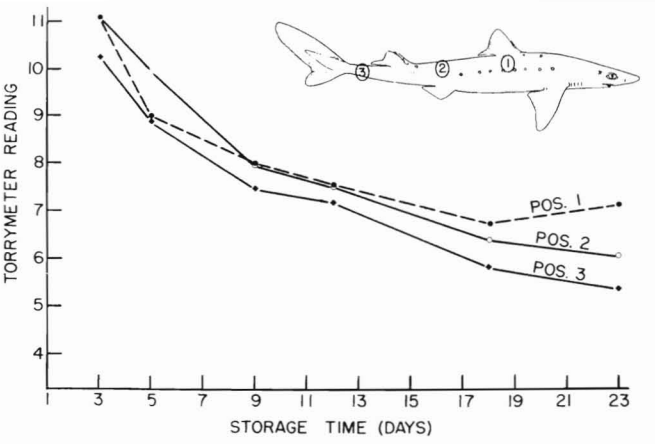

Figure 6.—Effect of storage time at 32°F (0°C) on Torrymeter readings taken at three different positions on dogfish.

Thus, while all sharks are subject to spoilage from ammonia formation, a few species—including dogfish—are additionally susceptible to rancidity because of their high fat content.

Moisture content of 24 spiny dogfish caught in September 1981 ranged between 67.96 and 76.20 percent, with an average moisture content and standard deviation of 71.56 ± 1.94 percent. For 21 fish caught in December 1981, fillets and belly flaps were analyzed separately. Moisture content of the fillets ranged between 67.68 and 74.65 percent, with an average moisture content and standard deviation of 71.35 ± 1.99 percent; the belly flaps ranged between 60.0-68.70 percent with an average moisture content and S.D. of 64.55 ± 2.35 percent. Very good correlation (r = 0.93) was observed between fat content and moisture content. The regression equation was $Y = 114.7 - 1.45X$. Thus, a quick estimate of the fat content of dogfish muscle could probably be obtained using a rapid moisture determination method.

Quality loss during iced storage was also monitored with the Torrymeter. Readings were taken at three consecutive positions along the lateral line (Fig. 6) between the first dorsal fin and the caudal peduncle. Readings were generally constant in the region

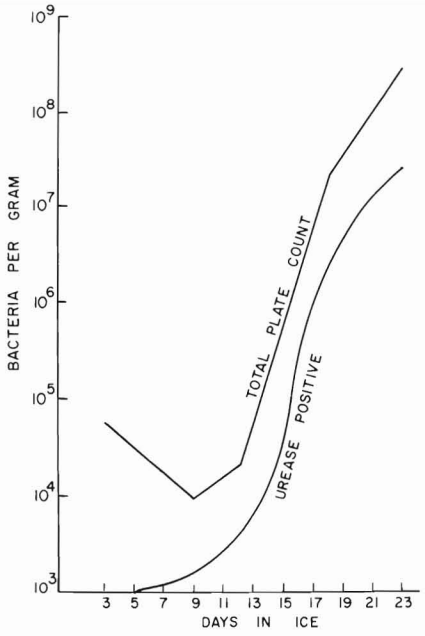

Figure 7.—Bacterial content (total plate count or urease-positive bacteria) of H&G dogfish held on ice.

Table 1.—Regression data for flavor score of either dogfish fillets or belly flaps as a function of various spoilage indicators.

Item	Regression	Correlation coefficient	Intercept	Slope	n
Fillet	Flavor score vs. TBA number	0.42	8.15	−0.54	21
Belly flap		0.77	8.79	−0.79	20
Fillet	Flavor score vs. TMA content	0.77	8.61	−0.43	21
Belly flap		0.84	9.34	−0.75	21
Fillet	Flavor score vs. ammonia content	0.79	8.01	−0.09	21
Belly flap		0.91	7.75	−0.09	21
Fillet	Flavor score vs. TVB content	0.81	8.22	−0.08	21
Belly flap		0.91	8.01	−0.08	21
Fillet	Flavor score vs. pH	0.84	24.46	−2.77	21
Belly flap		0.92	26.94	−3.14	21
Fillet	Flavor score vs. log APC	0.93	9.72	−0.49	21
Belly flap		0.98	9.82	−0.48	21
Fillet	Ammonia content vs. pH	0.84	−143.4	24.57	21
Belly flap		0.90	−206.4	33.87	16
Fillet	Flavor score vs. Torrymeter reading	0.76	3.89	0.39	

between the two dorsal fins, but were lower at the caudal peduncle. The average Torrymeter readings taken at the three different positions are plotted in Figure 6 as a function of storage time.

The readings dropped steadily from an initial (3 days postmortem) average reading of 11 to a final reading of about 6-7 at the end of shelf life. A Torreymeter reading of about 6-7 is the usual value we have observed in previous studies of marginal quality fish with cod and haddock. Thus, the Torrymeter could have possible application for measuring quality of iced dogfish if the limitations of the instrument are understood and if the instrument is consistent from one lot of fish to another.

Postmortem ammonia in most fish arises from the enzymatic deamination of proteins, amino acids, and some other basic nitrogen compounds in the flesh. However, in elasmobranchs, which have an unusually high level of urea in the flesh and blood, most postmortem ammonia is formed by enzymatic degradation of urea. Shewan (1951) reported such levels up to 2 percent in dogfish muscle. The enzyme responsible for this activity is urease which is present in certain microorganisms. During our 23-day iced-storage study, the total number of aerobic bacteria (APC) and the numbers of microorganisms capable of splitting urea into ammonia (urease-positive) were monitored. The APC decreased slightly during the first 12 days, then rose sharply during the following 11 days to a final average count of about 300 million per gram (Fig. 7). Moyer et al. (1959) reported an APC of about 74 million per gram at the time iced dogfish was considered of marginal quality.

In our study, the APC at the threshold of spoilage was estimated as 15 million per gram. Throughout storage, the percentage of urease positive bacteria remained relatively constant, ranging from 3 to 20 percent of the total microbial population; however, as the total bacterial count increased, so did the number of urease-positive bacteria which at spoilage was estimated at 2 million per gram. Southcott et al. (1960) monitored the APC and urease-positive bacterial count of iced H&G dogfish, concluding that an obligate urea-utilizing population was not established to the exclusion of other types. Our result is consistent with their conclusion.

Linear regression data for flavor score of either fillets or belly flaps are presented as a function of various chemical or physical spoilage indicators for fish in Table 1. In general there was good correlation between flavor score with either TBA number, TMA, ammonia, TVB, pH, Torrymeter reading, and (log) APC. The only exception was the low correlation between flavor score of fillets and TBA number. Fillet spoilage was more associated with bacterial activity than with lipid oxidation. Although some of these chemical/physical parameters correlated well with flavor score, they would only be useful as spoilage indicators and not freshness indicators.

The ammonia nitrogen content at spoilage was estimated from the regression equation to be 20-23 mg % N. This is well below the specified limit for acceptable exported dogfish and corroborates the results of the first part of this study.

There also was good correlation between ammonia content and pH, which confirms the results of the first part of this study; however, the regression lines computed from the two parts of this study $Y = 33.9X - 194.1$ (Part I) and $Y = 24.6X - 143.4$ (Part II) were sufficiently diverse as to render this predictive parameter (pH) questionable at this time.

Flavor score correlated well with pH ($r = 0.89$) and the relationship is

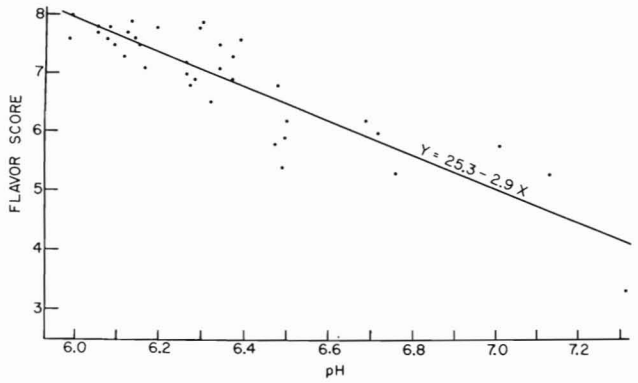

Figure 8. – Flavor score of iced H&G dogfish as a function of pH.

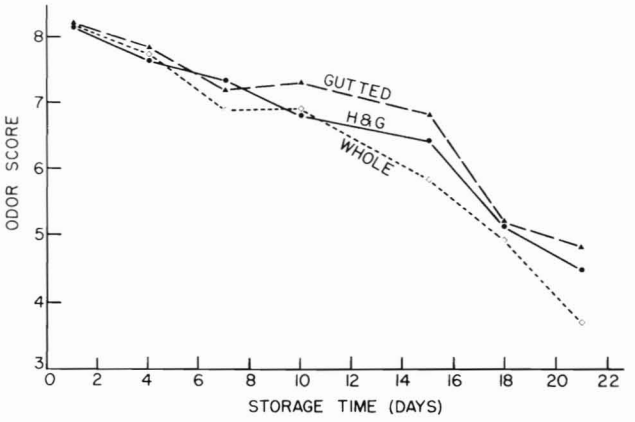

Figure 9. – Effect of storage time on the odor score of cooked fillets cut from dogfish dressed in various ways and held in ice.

shown in Figure 8. Although the degree of scatter of the data points precludes the use of pH for accurately assessing flavor score, this parameter might possibly be employed as a rapid screening method to delineate acceptable from nonacceptable fish by establishing some arbitrary pH, such as pH 6.7, as the cutoff value. A rapid measurement on a fresh fillet or skinned fish could be made with a surface electrode.

Study 2 – Effect of Gutting, and Heading and Gutting

This study was made to determine the effect of heading and gutting, gutting alone, and bleeding on the quality of dogfish during storage in ice. Dogfish blood contains a high amount of urea, which decomposes to ammonia, as well as a high concentration of TMAO, the precursor of TMA, a compound absent from the blood of teleosts (Benoit and Norris, 1945). Viscera are also a source for spoilage, particularly when the fish have been feeding, and the products of bacterial metabolism can diffuse through the stomach and intestinal walls and permeate the flesh. Therefore, it is important to determine the role of these potential spoilage factors on the shelf life of iced dogfish.

Bleeding is recommended to preserve flavor and prevent darkening of the flesh due to oxidation of the blood pigments hemoglobin and myoglobin. However, in the European market a red color of the flesh is considered an indicator of high quality in fresh dogfish. Therefore, bleeding the fish would appear to be counterproductive since this treatment would tend to produce a white or light-colored flesh.

During the first 10 days of iced storage there was not much difference in the odor score of cooked fillets cut from fish stored in one of the three different ways. Beyond that time the whole fish (fillets) were scored slightly lower than the fillets from either gutted or H&G fish which were rated about the same (Fig. 9). The graphs of flavor score as a function of storage time followed a similar pattern (Fig. 10). On the 15th day some panelists reported a slight rancid flavor in the fillets from the gutted fish and a slight flavor of decomposition in fillets from the whole fish. No ammonia flavor was detected at this time but after 18 days some comments on an ammonia flavor were noted. After 21 days all samples were rancid and, in addition, fillets from the whole fish had a strong decomposed odor/flavor. The odor of rancidity and putrefaction overpowered any ammonia present. Texture scores during storage have been plotted in Figure 11 for the three treatments. No textural differences were observed during about the first 8 days but beyond that the whole fish (fillets) appeared to be rated slightly lower compared with the other two treatments owing to a softer texture. The approximate storage times at which the fish were considered to be of marginal quality due to either an unacceptable odor, flavor, or texture were determined by linear regression to be as follows:

Storage method	Iced shelf life (days)		
	Odor	Flavor	Texture
Whole	14.0	11.5	13.0
Gutted	16.5	16.0	17.0
H&G	16.0	16.0	15.0

Thus, it appears that flavor deterioration or off-flavor development was the limiting factor for product acceptability. The advantage of gutting in extending the shelf life is also apparent.

Prolonged storage of spiny dogfish in the round could seriously diminish the shelf life if the belly flaps were to be utilized. After 4 days of iced storage, an undesirable yellow-green discoloration was observed on the belly flaps of some of the whole fish. We believe that contact with certain visceral organs was responsible for this.

Moisture content did not change significantly throughout the 24-day iced-storage period although there was some variability among in-

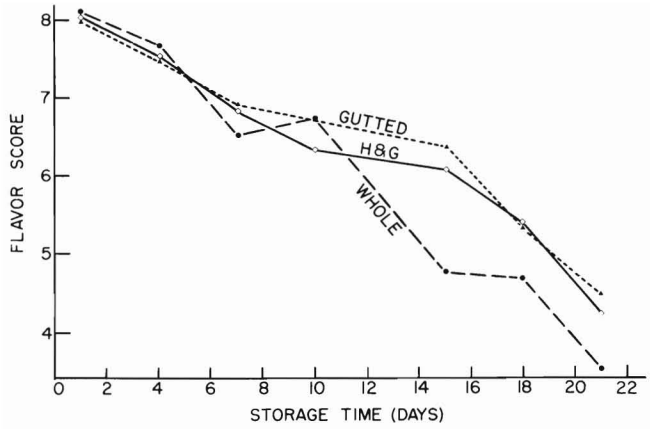

Figure 10.—Effect of storage time on the flavor score of cooked fillets cut from dogfish dressed in various ways and held in ice.

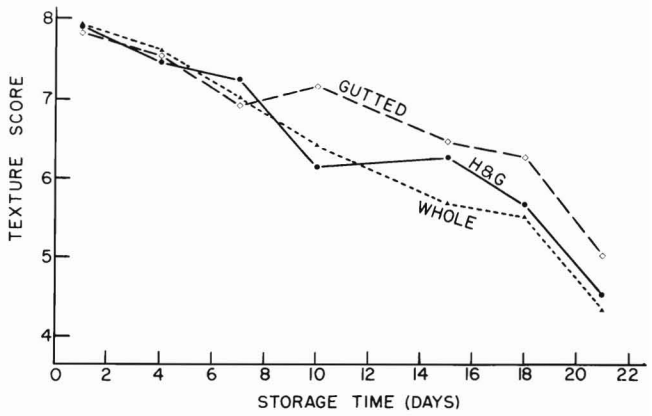

Figure 11.—Effect of storage time on the texture score of cooked fillets cut from dogfish dressed in various ways and held in ice.

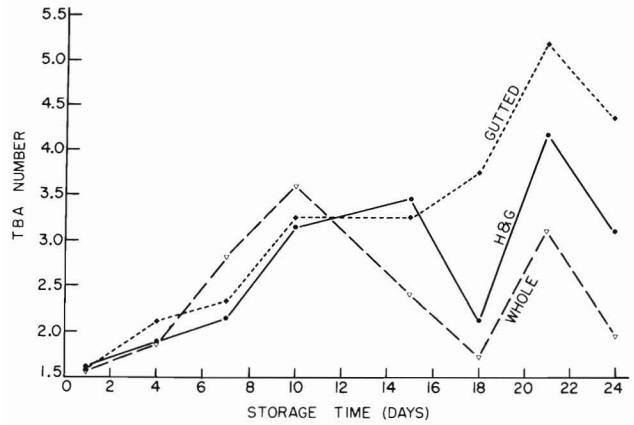

Figure 12.—Effect of storage time on TBA number of fillets cut from dogfish dressed in various ways and held in ice.

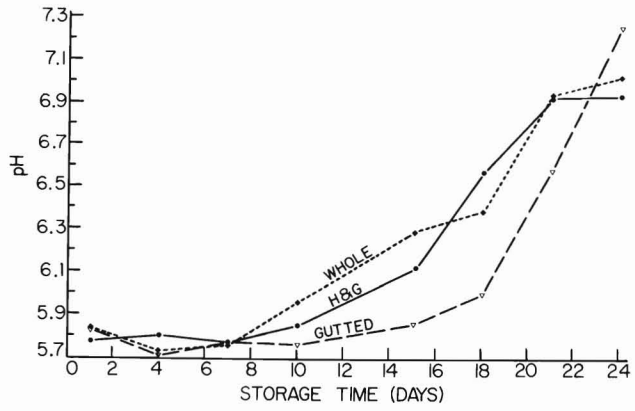

Figure 13.—Effect of storage time on the pH of fillets cut from dogfish dressed in various ways and held in ice.

dividual fish. Based on 16 fish per treatment, the average moisture content (±1 S.D.) for the three treatments during storage was: Whole, 70.9 ± 3.0; gutted, 69.7 ± 1.9; and H&G, 69.7 ± 2.0.

Dogfish stored whole had about a 1 percent higher moisture content compared with gutted fish. However, this slightly higher moisture content was not associated with the slightly softer texture of the whole fish since correlation between texture score and moisture content was found to be low. Enzymes in the gut were probably responsible for the softer texture of the whole fish.

The development of oxidative rancidity (indicated by TBA number) is shown for the three different storage treatments in Figure 12. From the general trend of the data points, it appears that over the 24-day storage period rancidity developed to a greater degree in the gutted or H&G fish than in the whole fish. This is reasonable since gutting the fish exposes the visceral cavity to air which is responsible for oxidation. Stansby et al. (1968) and Bilinski et al. (1983) also reported less rancidity (based on peroxide value) in the oil extracted from iced dogfish stored in the round compared with H&G or gutted dogfish. The rate of increase in TBA number per day (mg malonaldehyde/kg/day) was determined to be 0.14 for gutted fish, 0.08 for H&G fish, and 0.01 for whole fish. The pH remained relatively constant during the first 10 days and began to increase thereafter, fastest in the whole fish and slowest in the gutted fish (Fig. 13).

Trimethylamine nitrogen content remained relatively stable at an average value of about 3 mg % N for all treatments over 18 days and then started to rise. This agrees with the results of Bilinski et al. (1983) who found that TMA contents of whole, bled, or gutted dogfish did not exceed

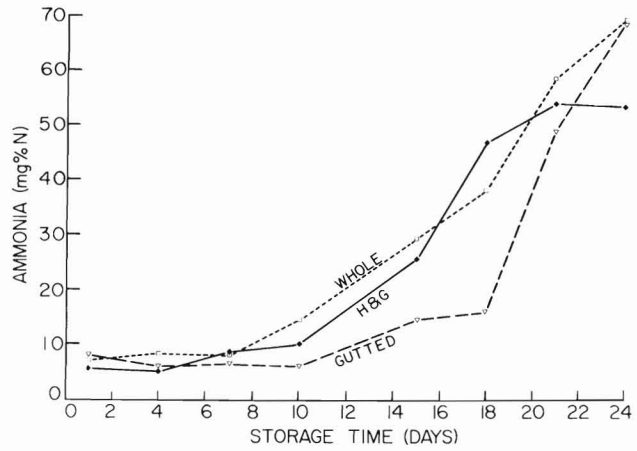

Figure 14.—Effect of storage time on ammonia nitrogen content of dogfish (fillets) dressed in various ways and held in ice.

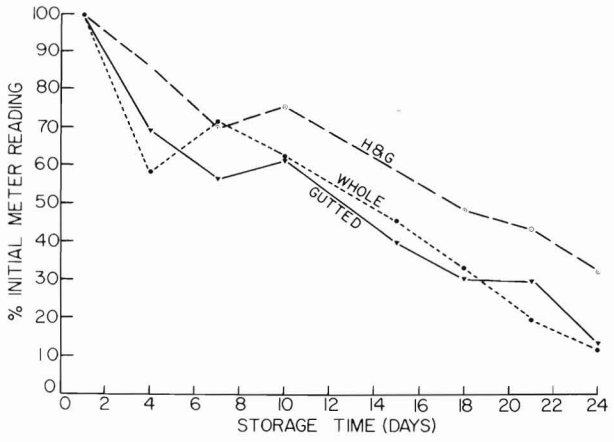

Figure 15.—Effect of storage time on the percent change in Torrymeter reading taken on dogfish dressed in various ways and held in ice.

1 mg % N during storage on ice for 15 days. Only afterward did the TMA of whole fish show a slight increase, though by 20 days it still remained under 3 mg % N. However, after 18 days, our fish were either spoiled or on the verge of spoilage. Therefore, we concluded that TMA production did not play a major role in quality deterioration other than contributing to the TVB content.

There was no change in DMA nitrogen content over the entire 24-day storage period for any treatment. The average contents (and S.D.) were as follows: Whole fish, 0.30 mg % N (0.16); gutted fish, 0.23 mg % N (0.11); H&G fish, 0.22 mg % N (0.10). There was no significant difference between these average values and we concluded that DMA content did not affect the spoilage process.

Over the 24-day storage, the TMAO-N content decreased linearly from an average initial value for the three treatments of 275 mg % N to a final average value of 100 mg % N. The rate of TMAO-N decrease was 9.5 mg % N per day for whole fish compared with 5.8 mg % N per day for gutted fish or 5.3 mg % N per day for H&G fish. During this period we found only about 6-7 mg % N from TMA formed in any of the fish. From these data it is difficult to attribute the unaccountable decline in TMAO content to leaching from melting ice, as has been suggested, since there was a greater rate of loss in whole fish compared with gutted fish which had more leachable surface exposed.

Ammonia content remained unchanged for about the first 10 days of storage and then began to increase, most rapidly in the whole fish and least rapidly in the gutted fish (Fig. 14). Bilinski et al. (1983) found little increase in ammonia in any of the whole, gutted, or bled dogfish during the first 11-12 days of storage on ice; hence, little difference between the treatments. After 14 days a slightly greater concentration of ammonia was apparent in the unbled fish than in those bled, whereas after 20 days there was considerably more ammonia in the iced/ungutted fish ($\approx$ 67 mg % N) than in the gutted fish ($\approx$ 13 mg % N fish). The amount of ammonia found in our iced and gutted samples far exceeded the amount found by Bilinski et al. (1983), and after 20 days was only slightly less than in the iced/ungutted fish. Unfortunately, those investigators did not continue their bled vs. unbled study beyond 14 days during the period of greater ammonia production. Even so, an exact comparison of our results would not have been possible since our methods of bleeding differed.

The course of ammonia production for the three different treatments seemed to coincide with pH change. The estimated time at which the ammonia nitrogen content reached a value of 55 mg % N—the limit mandated by Belgium for acceptability—was 20-22 days; yet, the taste panel regarded the useful storage life to have terminated after 11-16 days because of quality deterioration other than an objectionable ammonia content.

The accumulation of TVB followed the same general pattern as ammonia production. This was expected since, as stated, TVB is predominantly ammonia. In the industry, a value of 30 mg % N for TBV is considered to be indicative of spoilage in fish (Farber, 1965). This value is within the range we found at the end of dogfish shelf life.

The percent decrease in Torrymeter reading during storage is shown in Figure 15. These percentages were computed by comparing the reading at a particular storage time with the reading initially recorded for that tagged fish. The highest readings, indicative of best quality, were consistently obtained with H&G fish. The rates of percent decrease in Torrymeter reading per day were determined for the different treatments by linear regression to be: Whole fish, 3.3; gutted fish, 3.1; H&G fish, 2.7.

The average initial reading based on 48 fish was 11.3 ($\pm$1.5 S.D.). By

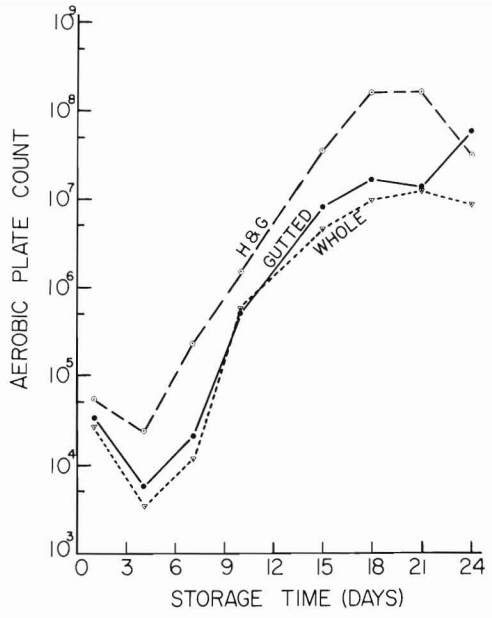

Figure 16.—Effect of storage time on the bacterial content of fillets cut from dogfish dressed in various ways and held in ice.

Table 2.—Regression data for flavor score of dogfish fillets as a function of various spoilage indicators.

Treatment	Regression	Correlation coefficient	Intercept	Slope	n
H&G	Flavor score vs. ammonia content	0.92	7.62	−0.06	14
Gutted		0.85	7.61	−0.07	14
Whole		0.84	7.58	−0.06	14
H&G	Flavor score vs. TMA content	0.64	8.50	−0.55	14
Gutted		0.33	7.76	−0.35	14
Whole		0.10	6.71	−0.19	14
H&G	Flavor score vs. TVB content	0.92	7.79	−0.05	14
Gutted		0.82	7.76	−0.06	14
Whole		0.79	7.76	−0.06	14
H&G	Flavor score vs. TBA number	0.69	8.71	−0.88	14
Gutted		0.81	8.81	−0.74	14
Whole		0.22	7.08	−0.40	14
H&G	Flavor score vs. pH	0.89	21.44	−2.46	14
Gutted		0.77	23.92	−2.93	14
Whole		0.79	20.44	−2.33	14
H&G	Flavor score vs. log APC	0.91	10.92	−0.71	12
Gutted		0.81	10.39	−0.70	12
Whole		0.84	10.78	−0.92	12
H&G	Flavor score vs. Torrymeter reading	0.88	2.91	−0.43	14
Gutted		0.77	4.73	−0.30	14
Whole		0.86	0.42	−0.42	14
H&G	Ammonia content vs. pH	0.97	−234.3	41.91	16
Gutted		0.96	−238.7	42.78	16
Whole		0.96	−219.3	39.57	16
H&G	Texture score vs. moisture content	0.28	17.83	−0.16	14
Gutted		0.02	5.99	0.01	14
Whole		0.41	10.03	−0.18	14

linear regression analysis we determined that at spoilage, the Torrymeter reading had decreased to 40-55 percent of the initial value. This would indicate a meter reading at end of shelf life of 5-6 which is comparable to the reading of 6 that was determined in our previous study.

Bacterial content (APC) during storage in ice is plotted in Figure 16. The APC at any time was greatest in H&G fish and least in the whole fish. The flesh of freshly caught fish is sterile, but bacteria are on the skin, gills, and in the intestines. When fish are gutted, the visceral cavity is exposed to contamination, particularly if the fish have not been properly gutted. Improper gutting, (i.e., rupture of intestines or stomach) can cause heavy bacterial contamination of the gut cavity which can shorten the shelf life of gutted fish. Bacteria in the belly cavity of gutted fish will have access to more air than bacteria growing in the belly cavity of ungutted fish, and may proliferate at a faster rate. Bacteria growing where air is restricted, as in the digestive organs of whole fish, may alter their metabolism (become anaerobic) and produce malodorous compounds (i.e., hydrogen sulfide) typical of "bilgy" fish (McLean and Castell, 1956). The tough hide of dogfish would seem to provide a good barrier against bacterial penetration; therefore, bacterial invasion of the flesh probably originates inside the belly wall.

Removing the head creates another cut surface for potential bacterial contamination and a foci for invasion of tissues such as through the vascular system. By not eviscerating, intestinal bacteria are contained within that system and do not invade the flesh; however, as stated, bacterial growth within the intestines will eventually produce undesirable end products which may diffuse through the intestinal wall, permeate the flesh, and cause spoilage even though the flesh may contain a relatively low bacteria count. This appears to be the scene depicted by the curves in Figure 16.

Regression data for flavor scores and other parameters as a function of various spoilage indicator tests are given in Table 2. There was good correlation between flavor scores and either ammonia content, TVB, pH, bacterial content, or Torrymeter reading. Flavor scores correlated fairly well with TBA number in gutted or H&G fish, but poorly in whole fish. There also was a low correlation between flavor scores and TMA content. The following spoilage criteria, predicted by the regression lines for the various parameters, signalled the end of useful shelf life.

Ammonia N content	24-29 mg% N
TVB	28-34 mg% N
pH	6.2-6.3
Torrymeter reading	5-7
TBA number	3-3.8

There was a high degree of correlation between ammonia content and pH. The regression value for pH corresponding to an ammonia nitrogen content of 55 mg % N was about 6.9.

These results seem to indicate that there would be some advantage to the immediate gutting of fish, especially if high-quality belly flaps are desired. However, if fishing trips are short (1-2 days), the fishermen's time would be best spent on chilling the fish as quickly as possible and keeping them well-iced until processed.

Study 3—Effect of Storage Temperature

This study was made to determine the effects of temperature abuse on dogfish quality from mishandling, either aboard the fishing vessel or in the processing plant. During storage,

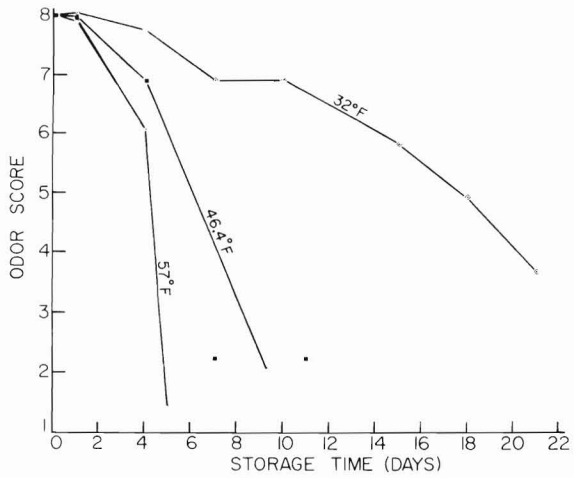

Figure 17.—Effect of storage time on odor score of cooked fillets from dogfish stored whole at various temperatures.

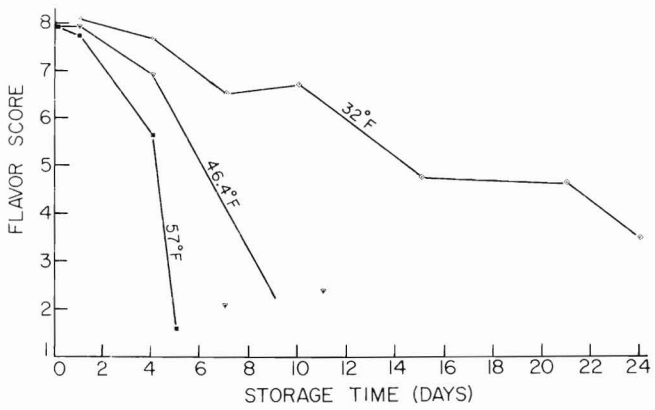

Figure 18.—Effect of storage time on flavor score of fillets from dogfish stored whole at various temperatures.

the rate of decrease in odor, flavor, and texture on scores of fillets removed from whole fish was commensurate with the storage temperature (Fig. 17, 18, 19). During the first 2 days of storage, quality differences due to temperature were not as great as anticipated. After the first day a slight bitter flavor was detected in fillets from fish held at either 46.4° or 57°F (8° or 14°C), but no ammonia or decomposition odors were noted. We believe that off-flavor may have reflected the initial stages of oxidative rancidity. After 4 days some stale flavor was noted, and ammonia was slightly detectable in the cooked samples; however, a strong ammonia odor prevailed after 5 days at 57°F (14°C) or after 7 days at 46.4°F (8°C). Shelf life, from the curves in Figures 17, 18, and 19, was estimated as follows:

Storage temperature	Shelf life (days) based on: Odor	Flavor	Texture
32°F (ice 0°C)	14	12	13
46.4°F (8°C)	5	5	5
57°F (14°C)	4	3.5	4

As in Study 2, flavor change was the most important sensory factor governing shelf life. These shelf lives are somewhat longer than others (Anonymous, 1981) reported: 6, 3.5, 2, and 1.5 days at 5, 10, 15, and 20°C (41, 50, 59, and 68°F), respectively. Vyncke (1967) reported ammonia concentrations of about 70 mg % N in skinned and headed dogfish held 2 days at 20°C (68°F) and in others held 5 days at 15°C (59°F). Ammonia in the fish held at 15°C (59°F) reached about 37 mg % N after 2 days. All those studies were begun 5 days postmortem with ammonia concentrations of about 26 mg % N.

One might reason beforehand that at the two elevated storage temperatures the fish would lose some moisture which could affect texture. During the latter part of our storage period, the fish held at either 46.4°F (8°C) or 57°F (14°C) did have a dry skin and a slightly shrivelled appearance, but the flesh moisture content did not decrease. Thus, dogfish skin appears to be a good protector of flesh moisture.

In Figure 20, shelf life (days) based on flavor deterioration has been plotted as a function of storage temperature over a range from 32° to 57°F (0°-14°C). The relationship appears to be a first order reaction. The sharp slope change over the 32°-46°F (0°-14°C) span compared with the 46°-57°F (8°-14°C) span shows why the product should be stored as close to 32°F (0°C) as possible. The reciprocals of these shelf lives were considered to represent the spoilage rates for the reaction at the corresponding temperatures, and from these data an Arrhenius plot was constructed (Fig. 21). From the slope b of the regression line, the activation energy, E_A, for the reaction was determined:

$$\text{Slope } b = \frac{-E_A}{(2.303)(R)},$$

where R = molar gas constant,
= 1.987.

The activation energy was computed to be 14,700 cal/mole and this is in accord with the activation energy values of 15,000-18,000 cal/mole reported by Torry Research Station scientists for various spoilage tests on wet fish at temperatures from 34° to 59°F (1°-15°C) (cited by James and Olley, 1971). A slightly higher activation energy of 17,000 cal/mole was calculated by James and Olley (1971) for spoilage of uniced shark (species not named) based on the time required for the ammonia nitrogen content to reach 30 mg % N. Their raw data were taken from various published studies—perhaps even from studies on mixed shark species. In general, the spoilage times cited by James and Olley (1971) were lower by a factor of about 0.75 than ours.

There was no significant increase in TBA number at any of the storage temperatures to signify a major development of oxidative rancidity. In Study 2 rancidity was not a problem in iced whole fish, and Study 3 in-

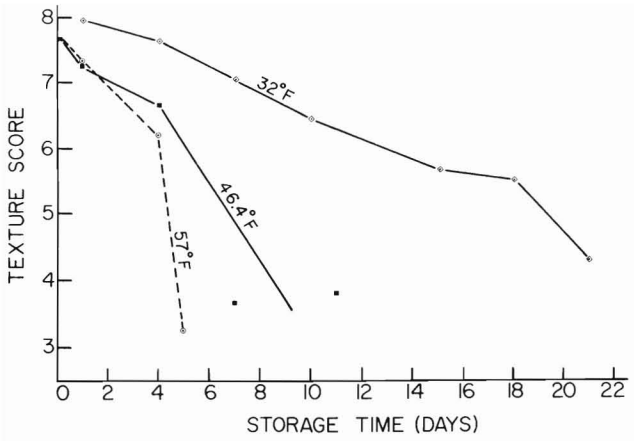

Figure 19.—Effect of storage time on texture score of fillets from dogfish stored whole at various temperatures.

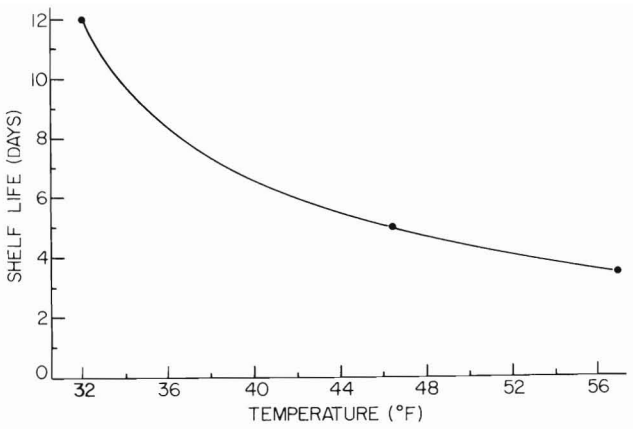

Figure 20.—Shelf life of whole dogfish as a function of storage temperature.

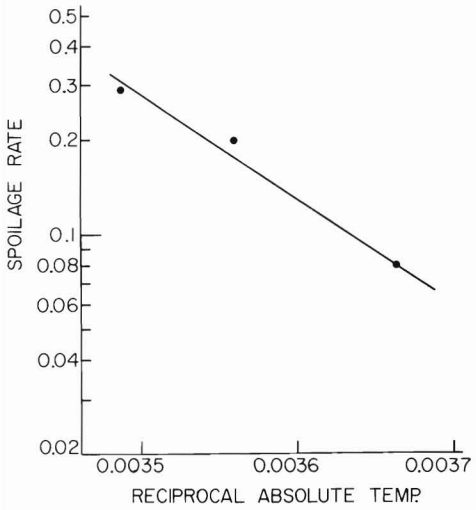

Figure 21.—Spoilage rate of whole dogfish as a function of temperature.

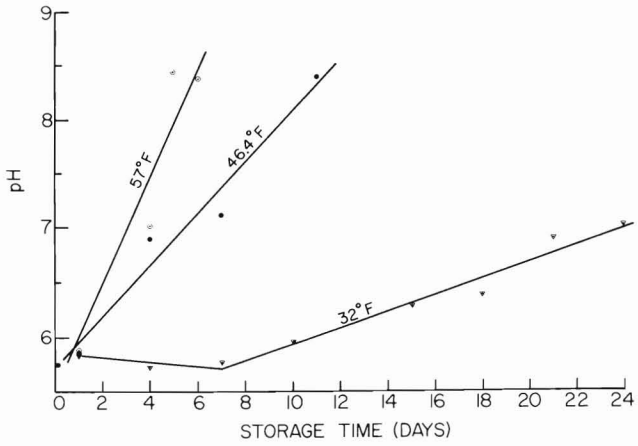

Figure 22.—Effect of storage time on pH of fillets from dogfish stored whole at various temperatures.

dicates no serious problem even at temperatures up to 57°F (14°C) although some rancidity did eventually develop. Bilinski et al. (1983) also reported low TBA values in dogfish stored at elevated temperatures, 41° and 50°F (5° and 10°C).

The rate of increase in pH during storage was also temperature dependent (Fig. 22). The initial pH of the fish in both Studies 2 and 3 was about 5.8. The ultimate pH for most teleosts at the end of rigor has been stated to be 6.2-6.5. Gordievskaya (1971) reported that, compared with other fish, shark meat is rather acidic. One would expect this acidic nature of dogfish flesh to enhance its shelf life.

The effect of storage temperature on production of the basic volatile amines—ammonia, TMA, DMA—is shown in Figures 23, 24, and 25. While in the previous studies we found no significant increase in TMA or DMA during dogfish storage on ice, we found a substantial increase in these amine compounds during uniced storage at 46.4°F or 57°F (8°C or 14°C) similar to that reported by Bilinski et al. (1983) at 41° and 50°F (5° and 10°C). Since these amines are produced as a result of microbial activity, one would expect the bacterial growth to reflect a similar temperature dependency. That they did can be seen in Figure 26; however, there is a notable lag in bacterial increase during the first 8 days at 32°F (0°C) which contributed to the extended shelf life at this lower temperature.

The decrease in dogfish Torrymeter readings during storage is shown in

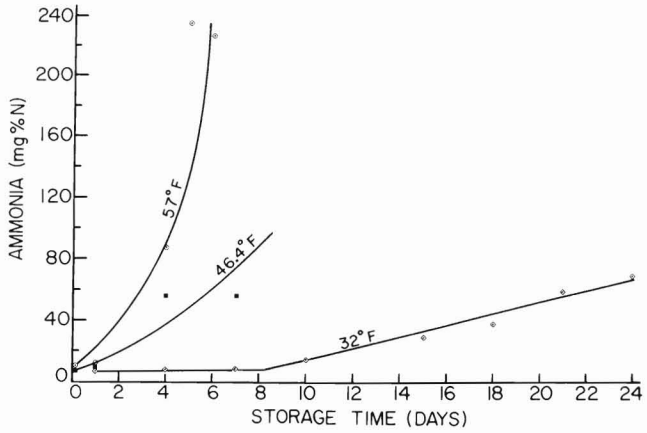

Figure 23.—Effect of storage time on ammonia nitrogen content of fillets from dogfish stored whole at various temperatures.

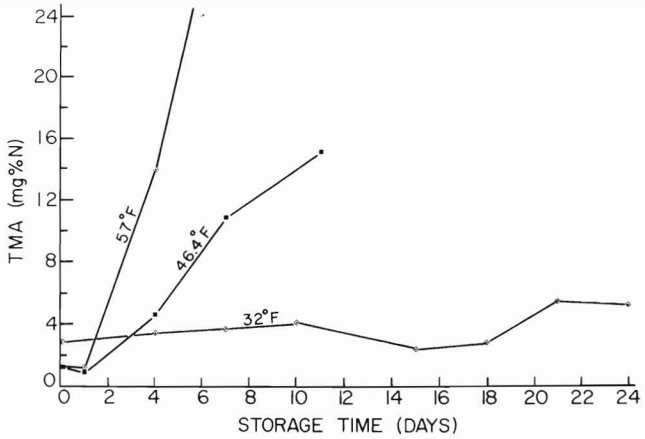

Figure 24.—Effect of storage time on trimethylamine nitrogen content of fillets from dogfish stored whole at various temperatures.

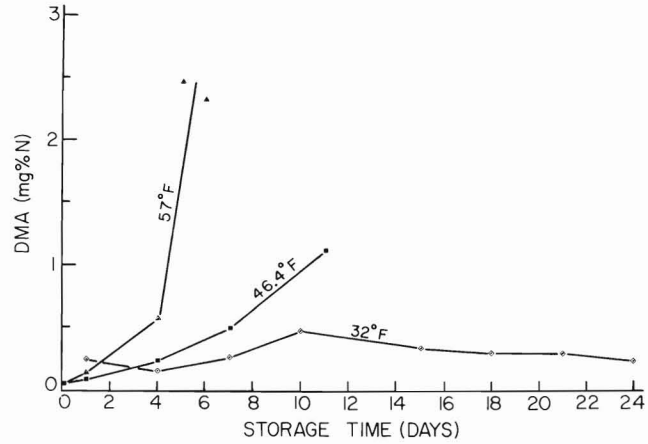

Figure 25.—Effect of storage time on dimethylamine nitrogen content of fillets from dogfish stored whole at various temperatures.

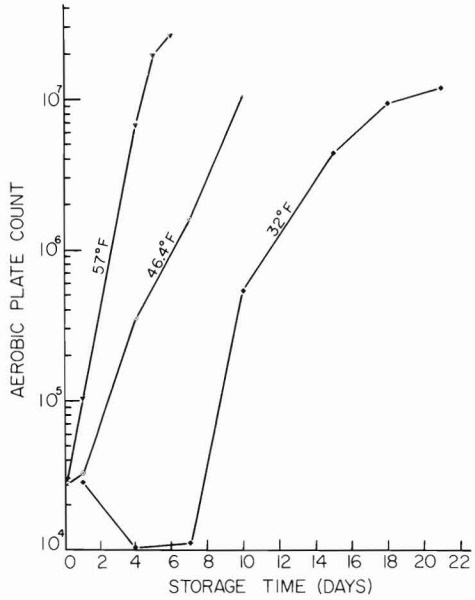

Figure 26.—Bacterial content of dogfish stored whole at various temperatures.

Figure 27. By interpolation we determined that a meter reading of 4-6 would indicate the end of shelf life, based on shelf lives of 12 days at 32°F (0°C), 5 days at 46.4°F (8°C), and 3.5 days at 57°F (14°C), as estimated from sensory evaluation. During storage at the two higher temperatures, the skin began to dry and the Torrymeter would not register a readout unless the skin was slightly moistened.

Rates of TMA, DMA, ammonia production, and bacterial growth are plotted as a function of temperature in Figure 28. These rates are the regression slopes of the curves shown in Figures 24-27, and illustrate the rapid increase in rate of either bacterial growth or volatile amine production at storage temperatures above about 50°F (10°C). Therefore, it behooves the fisherman or processor to maintain low storage temperatures for dogfish to retard the production of the volatile amines and other bacterial decomposition products strongly associated with spoilage. Thus, with proper iced storage, dogfish can have keeping quality comparable to similarly stored cod and haddock.

Some regression data for flavor scores of dogfish, stored whole at either 32°, 46.4°, or 57°F, (0°, 8°, or

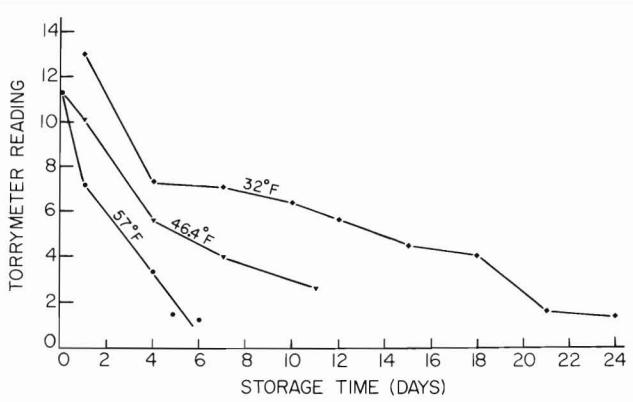

Figure 27.—Effect of storage time on Torrymeter reading on dogfish stored whole at various temperatures.

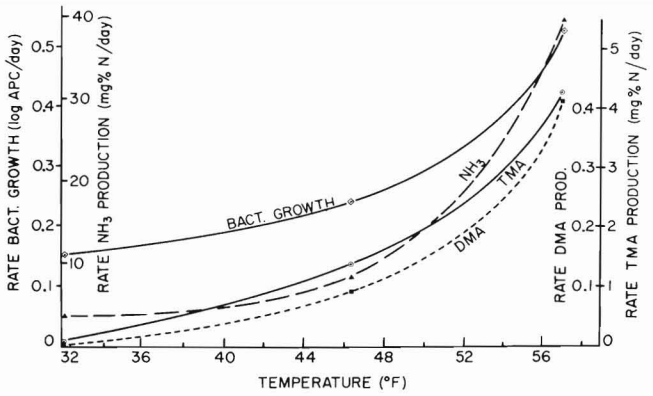

Figure 28.—Rate of bacterial growth and trimethylamine, dimethylamine, and ammonia production in dogfish stored whole as a function of temperature.

Table 3.—Regression data for flavor scores of dogfish stored whole at various temperatures as a function of certain spoilage indicators.

Storage temp. (°F)	Regression	Correlation coefficient	Intercept	Slope	n
32.0°	Flavor score vs. pH	0.81	27.12	−3.45	14
46.4		0.77	24.57	−2.88	8
57		0.97	21.30	−2.28	8
32	Flavor score vs. ammonia content	0.79	7.54	−0.06	14
46.4		0.61	7.77	−0.05	8
57		0.98	8.24	−0.03	8
32	Flavor score vs. TMA content	0.10	6.69	−0.18	14
46.4		0.96	8.32	−0.42	9
57		0.93	8.38	−0.24	8
32	Flavor score vs. log APC	0.80	10.80	−0.89	14
46.4		0.89	18.18	−2.33	10
57		0.80	15.83	−1.71	8
32	Flavor score vs. Torrymeter reading	0.86	3.41	0.42	14
46.4		0.86	1.11	0.64	10
57		0.79	2.85	0.53	8
32	Ammonia content vs. pH	0.95	−237.6	42.59	15
46.4		0.95	−246.9	43.66	8
57		0.98	−482.9	84.22	10

14°C), as a function of some chemical or physical spoilage indicators, are given in Table 3. There was good correlation between flavor and pH at each of the three storage temperatures; however, the regression lines predicted slightly different pH values at end of shelf life. These were pH 6.2 (32°F), pH 6.5 (46.4°F), and pH 6.7 (57°F). When regression analysis was performed on the combined data from the three temperatures, a pH value of 6.4 was determined at end of useful shelf life (flavor score = 6). In general, this pH value is lower than the value reached in teleosts when shelf life has expired.

Similarly, there was fairly good correlation between the flavor score and ammonia content at each of the storage temperatures; however, at the expiration of shelf life the ammonia nitrogen content varied from 26 mg % N at 32°F storage to 74 mg % N at 57°F storage.

Our main interest was to ascertain the time that dogfish can be stored under different conditions and still be acceptable after cooking. Since ammonia is volatilized during cooking, spoilage based on odor of the raw fish would probably have been judged to occur earlier than the values we are reporting. Nevertheless, it is difficult to accept raw fish with an ammonia N content of 74 mg % N as representative of marginal quality, and there is no rational explanation as to why the taste panel did not react more strongly to this high level of ammonia if it were indeed present.

Flavor score and TMA content correlated poorly with fish held at 32°F (0°C), but very well with fish stored at 46.4° or 57°F (8°C or 14°C). At 32°F (0°C), TMA content did not begin to show any significant increase until the product was on the verge of spoilage, whereas at the other two temperatures TMA production increased rapidly after a 1-day lag. There was good correlation between flavor score and bacterial count at each of the three temperatures and the average count at the end of shelf life was shown to be about 2 million per gram. We caution, however, about the risks in using absolute bacterial numbers to predict shelf life, since fish spoilage is associated more closely with the numbers of certain spoilage types of bacteria rather than with the total number of bacteria.

The principal spoilage bacteria in marine fish are the pseudomonads, and these normally constitute just a small percentage of the microflora of freshly caught fish. However, depending upon the sanitary conditions in the fishing boat or in the processing plant, their concentration on fish can be markedly increased through contact with contaminated surfaces, and

this would affect the shelf life and also the total number of bacteria (APC) at spoilage. It would be conservative to state that at the threshold of spoilage the APC would be in the millions, but one cannot state with certainty that it would be 2, 10, or 50 million.

Flavor score correlated fairly well ($r = 0.79$-0.86) with Torrymeter readings of dogfish stored at the three different temperatures. The reading at end of shelf life was predicted to be 6-7 by the regression lines.

Study 3 clearly illustrates the importance of immediate and continued icing of dogfish by fishermen while at sea. The elevated temperatures used in this study should not be considered exaggerated, but are typical of those found in dogfish landed during warm summer months and allowed to lie on deck under the sun unattended or to be stored in the ship's hold without ice.

Study 4—Rapid Estimation of Ammonia

Although the development of off-flavors, off-odors, and texture deterioration all precede the development of high concentrations of ammonia in well-iced spiny dogfish and thus limit the shelf life of this species, delayed icing and prolonged holding at elevated temperatures result in more rapid formation of ammonia. Since the dogfish caught by U.S. fishermen are primarily for export, quality losses during storage on ice are not so much a problem as is the loss of quality due to abusive handling which may well be accompanied by elevated ammonia concentrations. As stated, French and Belgian dogfish imports measure ammonia concentration of the flesh to gauge dogfish quality. Several methods are used to determine ammonia concentration, but all require either considerable time, scientific expertise, or reasonably sophisticated equipment.

Government inspectors mainly determine whether or not the quality of dogfish is acceptable for export. Certainly these inspectors are able to perform these chemical tests; however, the time and equipment required may present problems. Although precise chemical tests will probably always be necessary for accurate measurement of ammonia, we felt that a fast and simple ammonia estimation technique which inspectors might use in the plants on questionable samples might serve to screen samples and thereby reduce the number which would have to be returned to the laboratory for accurate chemical analysis. Processors, too, might find such a technique useful.

The use of ion-selective electrodes is relatively fast and easy and requires inexpensive equipment. Two basic units are needed besides the ion-selective electrode: A reference electrode and a pH/mV meter, or concentration measurements can be read directly on specific ion meters specially designed for this purpose. In Study 4 we investigated the use of the ammonia specific electrode with the Fisher Model 320 expanded scale pH meter. However, because of interfering substances, especially TMA, it soon became clear that although this electrode had the potential to measure quality (with respect to flesh TVB), it could not be used to measure the ammonia in dogfish specifically. Attempts to remove the TMA bias by coupling a TMA electrode (Chang et al., 1976) or the modified version (Brown et al., 1980) with the ammonia electrode to measure TMA and TMA plus ammonia independently were unsuccessful. On successive days, shifts in the ammonia electrode potential were common and the electrode routinely showed poor response and considerable drift.

A special time-response graph paper developed by Orion Research, Inc., was used in an attempt to clarify the data; however, this step converted the method from a field test to a laboratory test. In this procedure, periodic readings taken over about 10 minutes are plotted and the line drawn through the points is extrapolated to infinite time to obtain the desired reading.

The electrode manufacturer was contacted for assistance or suggestions and their recommendations was to soak the electrode in acid daily to remove the build up of scale which apparently was causing the problem. However, this step changes the baseline reading of the electrode and requires a standard curve to be made each day the electrode is to be used. We decided that this electrode technique would not provide processors or inspectors with a simple method for measuring the ammonia content of dogfish.

Currently, there are many kits available for the examination of contaminants or dissolved gases in aqueous samples. These kits are inexpensive, simple, rapid, and completely self-contained. One such kit, available from Chemetrics, Inc., Warrenton, Va., for the measurement of dissolved ammonia in water samples, was examined. The concentration of ammonia in portions of the same water extraction mixtures of dogfish (from Study 3) was determined using both the method of Vyncke (1968) and the Chemetrics kit.

There was excellent correlation ($r = 0.99$) between the Vyncke ammonia content and the Chemetric median values. The regression line and 95 percent confidence limits are shown in Figure 29. The slope ($b = 1.04$) of the regression line was found by a t test to not be significantly different at the 1 percent level from the slope of the hypothetical line ($B = 1.00$) denoting perfect correlation between the two results. The parametric equation for the t test was:

$$t = \frac{b - B}{S_b}$$

$$\text{where } S_b = \frac{S}{\sqrt{\Sigma n_i (X_i - \bar{X})^2}}$$

(Hald, 1957).

Although the results with this kit lack the precision of a more involved chemical analysis such as Vyncke's method, it should provide a close estimate of the ammonia content of a fish extract. It certainly should enable a processor or inspector to judge whether or not a sample meets the specification for ammonia content.

The Sigma Chemical Company also offers a chemical kit for measuring ammonia, and this device has been applied for assaying ammonia in

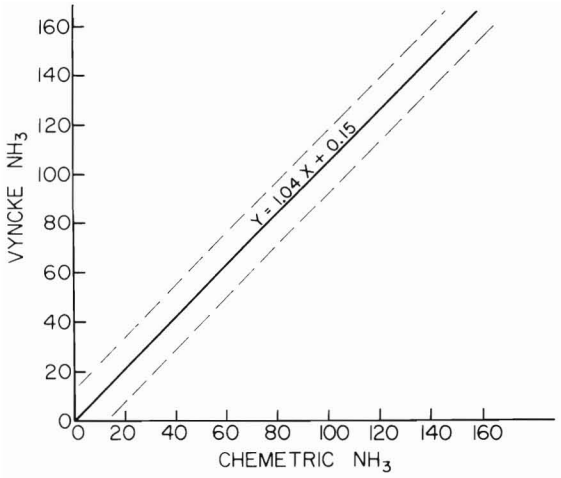

Figure 29. – Regression line and 95 percent confidence limits for Vyncke ammonia content as a function of Chemetric ammonia content.

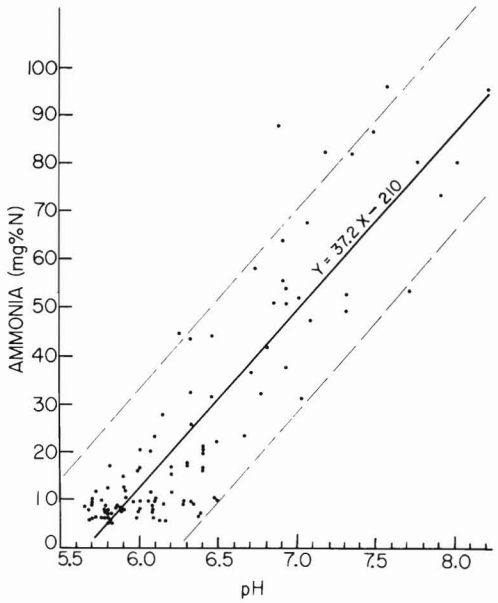

Figure 30. – Ammonia content of dogfish as a function of pH. (Regression line and 95 percent confidence limits.)

clinical samples, waste matter (Robbins and Weber, 1977), and in shrimp (Knight and Toom, 1980). Recently, Smith et al. (1980) reported on the application of this test for measuring ammonia in dogfish. The test is relatively simple and is based on the enzymatic (glutamate dehydrogenase) conversion of alphaketoglutarate to glutamate in the presence of ammonia. A special narrow bandwidth spectrophotometer is required in this text and we believe that this requirement precludes this method from the field-test category.

We found ammonia and pH to correlate well in each of our independent studies, but we did not attempt to determine the reliability of pH in predicting ammonia content from one lot of stored fish to another. All the ammonia/pH data from our experiments, with the exception of those data in which the pH exceeded 8.2, have been plotted in Figure 30. These latter data were excluded because they did not bear an apparent linear relation with the other data points. The regression line and 95 percent confidence limits ($\pm t_{0.95} \times$ S.E.) have been constructed on the figure. On the basis of these combined experiments there was very good correlation (r = 0.89, n = 112) between ammonia nitrogen content and pH; however, there also was considerable scatter among the data points. From the regression line, a pH of about 7.1 is seen to predict an ammonia nitrogen content of 55 mg % N. However, 95 percent of the time, the ammonia nitrogen content at that pH will range from about 33 to 75 mg % N.

A processor could employ a pH value of 6.6 as the cut-off point to ensure that no fish with an ammonia nitrogen content in excess of 55 mg % N would be exported. However, this plan would also reject some perfectly good fish. Although it does not appear that pH measurement can be used as a precise method for monitoring ammonia content in dogfish, there still is some potential utility in this parameter for rapid screening of fish quality on the basis of either acceptable, questionable, or unacceptable. By increasing the number of replicate samples taken for pH analysis, the average variability may be reduced and the reliability of the predicted ammonia concentration may be increased. Waller (1978) suggested using pH measurement, either obtained with surface electrode or pH paper, to assess shark quality due to ammoniation, and he designated pH ranges that would correspond to the postmortem age of iced fish.

However, Waller also pointed out that at any given time there can be a variation in ammonia content either along the length of the fish or through a cross section of the body. In dressed fish, ammonia content was higher at the anterior because of the exposed (cut) surfaces; it was also high in the vent area because the folds of the belly flaps created insulated air pockets with a slightly higher temperature than other areas of the fish, thus promoting faster bacterial growth.

Another source of ammonia variation is related to sample thickness. Ammonia formation occurs mainly on the exposed surface of fish, and therefore there will be a difference in ammonia content between two samples taken for analysis, both having the same ammoniated surface area but of different thicknesses. This variability in ammonia concentration may account for some of the scatter of the data points in Figure 30.

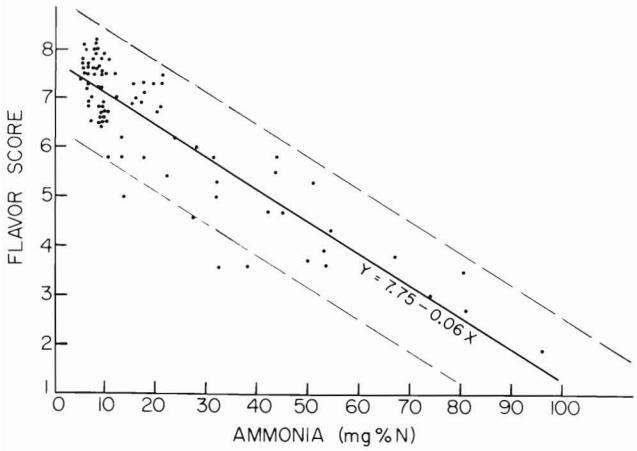

Figure 31.—Regression line and 95 percent confidence limits for flavor score of dogfish as a function of ammonia content.

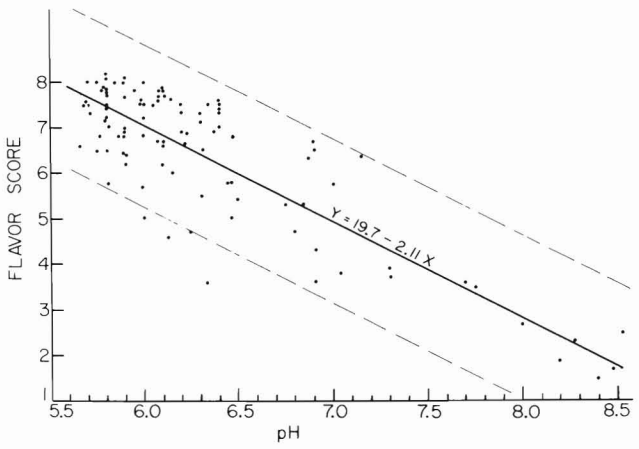

Figure 32.—Regression line and 95 percent confidence limits for flavor score of dogfish as a function of pH.

Regression analysis on flavor score as a function of ammonia nitrogen content was performed on the composite data (n = 87) of Studies 1 and 2. The correlation coefficient was found to be high (r = 0.89). The regression line and 95 percent confidence limits are presented in Figure 31. Because of the considerable scatter about the regression line, ammonia nitrogen content cannot accurately assess dogfish quality. End of useful shelf life would be predicted by an ammonia nitrogen content of about 27 mg % N. However, at this concentration the flavor rating could conceivably range from slightly poor to good 95 percent of the time. It should be remembered that flavor deterioration can be caused by the presence of other compounds in addition to ammonia.

The regression of flavor score on pH is shown in Figure 32 for the combined data (n = 102) of Studies 1, 2, and 3. There was good correlation (r = 0.84) between flavor score and pH as would be expected since flavor score, ammonia content, and pH are all interdependent. End of shelf life was predicted by a pH of 6.5. However, there will be a certain degree of unreliability with this predictor value as evidenced from the confidence limits for the regression line. Waller (1978) pointed out that physical mishandling of shark after capture can lower the quality of the fillets and this will not be reflected by the pH reading. However, he believed that a system of quality assessment could be established based on both surface pH measurement and physical appearance.

Conclusions

The shelf life of properly handled and iced spiny dogfish is comparable in length to that of properly iced gadoid fishes. Despite the high urea content of dogfish, ammonia formation does not account for quality losses during the first 2 weeks of iced storage. The process of bleeding and/or gutting seems to help extend the shelf life of iced dogfish. Abusive handling practices which allow this species to remain at elevated temperature (>32°F or 0°C) hasten ammonia formation. For processors to assess ammonia levels in dogfish intended for export, a rapid and simple field test method is needed and a self-contained kit similar to the one we tested may prove useful.

Acknowledgment

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