FISH SPOILAGE

I - Determination of Bacterial Metabolites by Gas Chromatography

INTRODUCTION

The formation in fish of bacterial metabolites (chemical compounds) that have unpleasant odors and flavors is called spoilage. One of the principal approaches to the study of such spoilage has been through the identification and estimation of the metabolites formed. There is need, however, not only for identification and quantitative determination of metabolites, but also for a study of their spoilage pattern; that is, for a study of the sequence in which the metabolites are formed under controlled spoilage conditions.

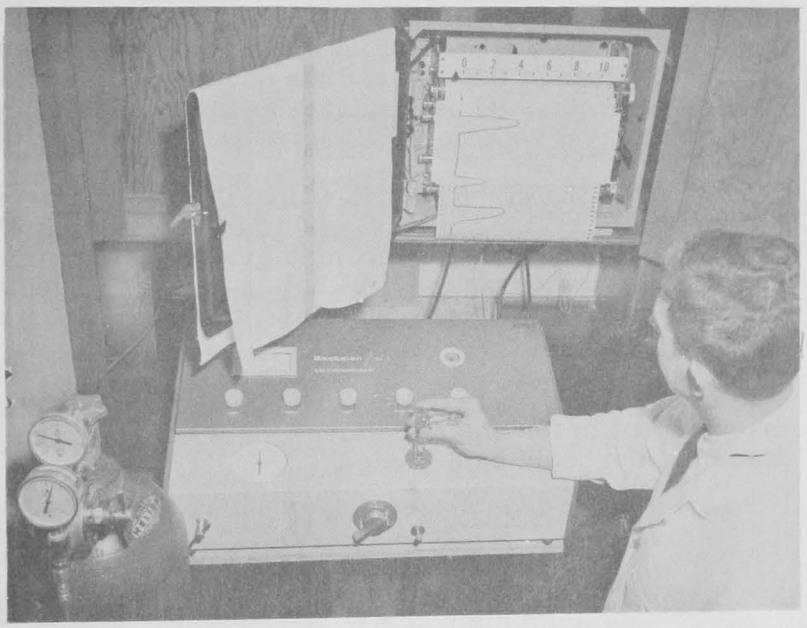

Fig. 1 - The injection of a liquid sample onto the chromatographic column.

The development of new analytical methods for measurement of bacterial metabolites would be an important aid to these studies. Since gas chromatography--a relatively new analytical tool--promises to be useful in a problem of this nature, the objectives of the present research were (1) to investigate the use of gas chromatographic techniques in the study of spoilage patterns and (2) to fractionate, by gas chromatography, the volatile nitrogen constituents (a major group of metabolites) of fish.

USE OF GAS CHROMATOGRAPHY IN THE STUDY OF SPOILAGE PATTERNS

METHODS: Gas chromatographic techniques offer a method in which qualitative and quantitative information can be obtained on a mixture of volatile compounds. These techniques thus can be used to fractionate and estimate the individual volatile bacterial metabolites from fish. The sequence of formation of the metabolites, with reference to spoilage time, would describe the pattern of spoilage.

There are acidic, basic, and probably neutral volatile bacterial metabolites formed in spoiling fish. Since a low-temperature-distillation method was chosen

to remove the volatiles from fish and since the acidic volatiles have high boiling points, attempts were made to separate only the neutral and basic volatiles.

Removal of Volatiles: Samples of ground cod and halibut were spoiled under controlled conditions for various periods of time. To remove the volatiles, the samples were adjusted to a pH of 9.3 and distilled at a temperature of 30° C. (86° F.), 30 millimeters pressure, for 2 hours. To aid in the removal of the volatiles, nitrogen gas was bubbled through the fish slurry and then passed through a series of traps that were maintained at temperatures of 0°, -77°, and -195° C. (32°, -106.6°, and -319° F.). The condensate from the -195° C. (-319° F.) trap was chromatographed immediately after collection. (Tests had shown that only water was collected in the traps held at the two higher temperatures.)

Gas Chromatography: After testing a number of column packings, Carbowax 1,000 was found to give the best separation. Volumes of 0.005 to 0.03 milliliter of condensate were chromatographed at 100° C. (212° F.) and a flow rate of helium of 60 milliliters per minute.

RESULTS AND DISCUSSION: A chromatogram of the volatiles removed from fish (in alkaline media) showed 3 to 4 incompletely separated components. Owing to this incomplete separation, it was not possible to determine the identity of the components or to determine if qualitative differences occurred in the chromatogram during the period of spoilage. Too few components were separated to describe the state of spoilage adequately. A correlation was observed, however, between the degree of spoilage of the fish and the quantity of individual volatiles. Fairly consistent results were obtained on duplicate samples, but the distillation method gave low recoveries on test volatile solutions of known composition.

CONCLUSIONS: Attempts to use the chromatograms of volatiles removed from fish (in various stages of spoilage) to describe the state of spoilage were only partially successful because the chromatograms gave insufficient information. It appears that gas chromatography might better be used in spoilage studies for the fractionation and estimation of specific classes of bacterial metabolites, rather than of heterogenous groups of metabolites.

Limiting the study to specific classes would permit use of a column material that is specific for a particular class of compounds. As a result, more efficient fractionation could be expected.

FRACTIONATION OF THE VOLATILE NITROGEN CONSTITUENTS BY GAS CHROMATOGRAPHY

METHODS: The principal constituents of the volatile nitrogen of fish are trimethylamine (Me_3N), ammonia (NH_3), dimethylamine (Me_2NH), and methylamine ($MeNH_2$). James, Martin, and Smith (1952) successfully separated ammonia and the low-molecular-weight aliphatic amines by gas chromatography. They used a column that was packed with celite No. 545 coated with a mixture of 85 percent hendecanol (5-ethyl-2-nonanol) and 15 percent paraffin oil. Hughes (1958) separated trimethylamine, ammonia, dimethylamine, and methylamine from herring meat, using the procedure of James, Martin, and Smith. Burks (1957) found that triethanolamine supported on C-22 firebrick gave more complete separation of ammonia and of some of the low-molecular-weight aliphatic amines than did the column packing used by James, Martin, and Smith. These various investigators introduced the amines and ammonia onto the column as the hydrochloride salts and liberated the bases by reaction with alkali. This procedure eliminated a large amount of the water that would have been introduced into the column had the bases been analyzed in free form.

Since it was desired to analyze the ammonia-amine mixture from fish as the free base, different column materials were tested for their efficiency of separation of these compounds in the presence of large amounts of water. After examining a number of materials, it was found that a mixture of 75 percent triethanolamine and 25 percent Amine 220 (1-hydroxyethyl-2-heptadecenyl glyoxalidine) gave fair separation of trimethylamine, ammonia, dimethylamine, and methylamine.

Column Preparation: Six grams of triethanolamine and 2 grams of Amine 220 were dissolved in 30 milliliters of acetone, and the solution was mixed with 14 grams of GC-22 Super-support. The solvent was removed from the packing material by heating on a water bath under reduced pressure. The material was poured into a 6-foot-long, 0.25-inch-outside-diameter aluminum tubing and packed with the aid of a vibrator.

Chromatography of Fish Volatile Nitrogen: The constituents that make up the volatile nitrogen were removed from fish meat by extracting 60 grams of ground meat with 100 milliliters of 60-percent ethanol for 3.5 minutes in a blender. The extract was made alkaline with 4 grams of sodium borate, and the free bases were steam distilled into dilute hydrochloric acid. The solution of amine and ammonium hydrochlorides was concentrated to a volume of several milliliters by distillation, made alkaline with 10-percent potassium hydroxide solution, and quickly steam distilled into a receiving flask cooked with dry ice. The solidified distillate was melted by placing the flask in cool water, and the solution was chromatographed immediately.

Peaks on the chromatograms were tentatively identified by comparing their elution times with those of known compounds. The elution time was determined by measurement of elapsed time between the appearance of the air peak and the maximum of the component peak. The concentration of material represented by a chromatographic peak was determined by measurement of the area of the peak.

RESULTS AND DISCUSSION: The elution times of trimethylamine, ammonia, dimethylamine, methylamine, propylamine, butylamine, and water were, respectively, 2.1, 3.3 to 3.8, 5.5 to 5.6, 6.6 to 7.1, 13, 24, and 41 minutes. The column was operated at 130° C. (266° F.) with helium flowing at the rate of 24.5 milliliters per minute. The high elution time obtained for water permits the use of this column for the detection of the higher aliphatic amines (C_2 to C_4). The relationship of peak area to concentration of component was determined for trimethylamine, ammonia, dimethylamine, and methylamine; and a satisfactory linear relationship was obtained for trimethylamine, ammonia, and dimethylamine.

When the volatile nitrogen from refrigerated-brine-chilled cod was fractionated, three peaks were found with elution times of 2.1, 4.1, and 5.6 minutes. These peaks apparently corresponded to those of trimethylamine, ammonia, and dimethylamine, respectively. The elution time for the peak that was thought to be that of ammonia is greater than that found for the known compound. This discrepancy might be due to the variability of elution time and peak shape when low concentrations of material are chromatographed.

The total volatile nitrogen fractionated by gas chromatography was compared to the total volatile nitrogen obtained by the method of Stansby, Harrison, Dassow, and Sater (1944). Taking the latter method as a standard, 90 percent of the volatile nitrogen could be accounted for in samples containing a large amount of nitrogen, and 76 percent could be accounted for in samples containing small amounts of volatile nitrogen.

CONCLUSIONS: 1. Using known compounds for preliminary test purposes, trimethylamine, ammonia, dimethylamine, methylamine, propylamine, and butyla-

mine were fractionated by gas chromatography by means of a column made of a mixture of 75 percent triethanolamine and 25 percent Amine 220 applied to GC-22 Super-support.

2. The volatile nitrogen from cod meat was fractionated into three components that appear to be trimethylamine, ammonia, and dimethylamine. The recovery of the total volatile nitrogen chromatographed from fish meat ranged from 90 percent for samples high in volatile nitrogen to 76 percent for samples low in volatile nitrogen.

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TECHNICAL NOTE NO. 47 - STEELHEAD TROUT - DESCRIPTION AND PROXIMATE COMPOSITION

ABSTRACT

The average proximate chemical composition of steelhead trout fillets analyzed in this study was 21.3 percent protein, 9.7 percent oil, 68.2 percent moisture, and 1.3 percent ash. The yield of fillets, which varied from 49 to 73 percent, averaged 62 percent.

BACKGROUND

The steelhead trout (Salmo gairdnerii) is similar to salmon in that it ascends rivers of the Pacific Coast to spawn. Its spawning habits and pattern of migration vary greatly from one area to another. The steelhead near Ketchikan ascends the streams from late winter to early summer. It is unlike the salmon, however, in that it feeds while in fresh water and it may return to the sea after spawning. The fingerlings migrate to the ocean after spending 1 or 2 years in fresh water.

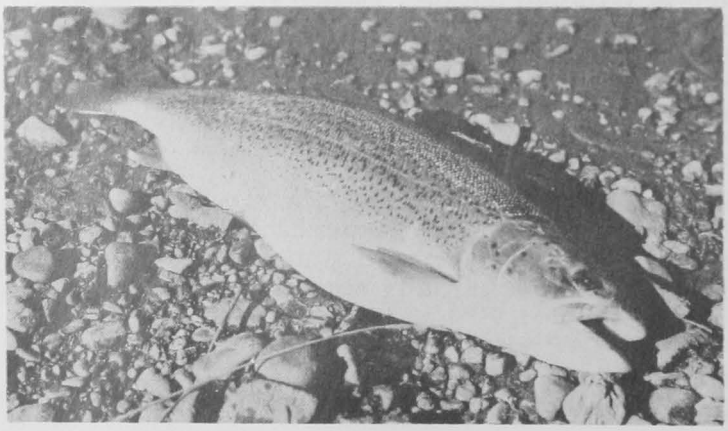

Fig. 1 - A steelhead trout caught in Alaska.

The steelhead varies greatly in size from one habitat to another. While in salt water, it often associates with migrating salmon and may be difficult to distinguish from them. The mature steelhead, when in marine waters, is a metallic-blue on the dorsal surface turning

to a silver on the sides and silver-white on the ventral surface. The steelhead may be distinguished from similar fish by the trout-like head and the black spots on its dorsal surface and tail. It inhabits coastal streams from southern California to southeastern Alaska.

There is little information available on proximate composition of fish indigenous to Alaska. This paper therefore presents proximate analyses made on 15 steelhead trout caught in salt water, 4 of which were made available to the Laboratory in 1954 and 11 of which were made available in 1957.

PROXIMATE COMPOSITION

A representative portion of one fillet (skin on) was taken from each fish and was analyzed for proximate composition, using standard techniques of the Association of Official Agricultural Chemists (1950 and 1955). The average yield of fillets, which varied from 49 to 73 percent, was 62 percent (table 1). The average proximate chemical composition of the fillets was 21.3 percent protein, 9.7 percent oil, 68.2 percent moisture, and 1.3 percent ash. The larger fish tended to give a higher yield of fillets and to contain a higher content of oil than did the smaller fish, although individual samples varied considerably in oil content. This variation is probably related, at least in part, to their particular time of spawning. Steelhead trout caught at another time and at another location might differ somewhat in proximate composition.

Table 1 - Proximate Composition of Fillets from Steelhead Trout (Salmo gairdnerii) Caught in Salt Water Near Ketchikan, Alaska

Date Caught	Sample[1/]	Length	Weight	Fillet Yield	Proximate Composition			
					Protein	Oil	Moisture	Ash
	Number	Inches	Pounds	(Percent)				
August 1953	1	29	8.6	70	22.8	8.6	68.2	1.34
	2	25	6.6	73	20.8	16.4	62.8	1.23
	3	27	5.9	71	22.0	11.8	66.4	1.28
	4	22	3.7	71	22.2	10.1	67.8	1.24
August 8, 1957	1	26.5	8.6	70	21.5	12.2	65.0	1.28
	2	24.0	7.0	70	22.4	11.2	66.2	1.35
	3	22.5	5.4	68	19.2	9.7	69.7	1.30
	4	24.0	6.4	65	22.3	10.6	65.5	1.29
	5	24.0	6.0	69	21.5	8.7	68.6	1.40
August 23, 1957	1	18.0	4.4	50	20.0	6.6	72.0	1.30
	2	17.5	4.0	49	20.2	6.4	71.2	1.32
	3	17.5	4.2	52	19.9	8.1	71.5	1.30
	4	17.5	4.3	52	22.5	7.2	69.8	1.22
	5	14.0	2.5	50	19.0	9.8	69.4	1.34
	6	17.5	4.4	52	22.7	8.2	68.3	1.40
Average		21.7	5.5	62	21.3	9.7	68.2	1.31

1/Each sample is a representative portion of one fillet (skin on) from one fish.

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POSSIBLE NEW USE FOR OYSTER LIQUORS

It was brought to the attention of the Bureau's Pascagoula Fishery Technological Laboratory that large quantities of highly-nutritive natural juices, expressed from oysters when they are steamed prior to shucking, are lost each year in the shucking plants. In an effort to utilize this juice, an acceptable canned oyster stew using the juice, milk, and oysters has been developed. A major technological problem was surmounted in the prevention of curdling when the canned stew was sterilized in the retort. This was accomplished by careful control of the quantities of salt and protein used in relation to the processing times and temperatures. This development may have significance because, at the present time, soups containing quantities of milk are not normally marketed in a canned unfrozen state.

CLUE TO CAUSES OF ODOR IN FISH OIL

Workers at the Hormel Institute, Austin, Minn., under a contract with the Bureau, have developed a new approach to the problem of chemistry of fishy odors. Two fish oils, one having a strong fishy odor and one a bland odor, are under investigation. The one having the fishy odor was found to contain about 200 times as much nitrogen as the other. The form in which the nitrogen occurs is now under investigation. If, through this research effort, a solution to the development of undesirable odors in fish oil can be found, a much broader marketing base may be available for this important industrial fishery product.

THE FISH PARASITE *ARGULUS LATICAUDA* AS A FORTUITOUS HUMAN EPIZOON

In June 1955 I was called upon to remove a foreign object from the eye of a 10-year-old boy who was suffering intense discomfort. The irritant, a parasitic copepod, was moving vigorously about on the surface of the eyeball. The copepod persistently resisted dislodgment, but was finally detached and preserved for identification. According to his account, the patient had been struck while swimming open-eyed in the clear inshore waters of the Tred Avon River (average summer salinity, 12 o/oo) off Oxford, Maryland. Several days later while crabbing across the river from Oxford, I captured a toadfish, *Opsanus tau*, which bore several argulids externally. These copepods were also preserved.

The argulids from human and fish were tentatively identified as *Argulus laticauda*, an ubiquitous ectoparasite of North Atlantic fishes. This identification was confirmed by Dr. David G. Causey of the University of Arkansas. Numbers of toadfishes are present in these inshore estuarine areas and it seems likely that the copepod had been attracted to the first moving object after accidental dislodgment from its normal fish host.

Although a single occurrence of this nature is of little general parasitological or medical consequence, it is believed noteworthy as a rare record of a parasitic copepod occurring on a human, albeit temporarily. (Contribution No. 77 from the Virginia Fisheries Laboratory.)—WILLIAM J. HARGIS, JR., *Virginia Fisheries Laboratory, Gloucester Point, Virginia.*

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