FLAVOR AND ODOR OF FISH - PROGRESS REPORT

INTRODUCTION

Knowledge of the flavor and odor components of fish products has a direct bearing on numerous problems of fishery technology. Since the odor of fishery products is often used as a subjective method of quality assessment, a better knowledge of the odor components could lead to the development of objective methods for quality assessment. This knowledge could be further utilized to study such questions as species identification through qualitative or quantitative differences in odor components, changes which occur in flavor and odor components on freezing and storage, the composition of off-odors, irradiation odors, flavor loss, and numerous other problems with which fishery technology is concerned.

With the objective of isolating and identifying the chemical components of the flavor and odor of fresh raw fish, an agreement was made March 10, 1958, for a collaborative project between the U. S. Bureau of Commercial Fisheries Fishery Technological Laboratory, East Boston, Mass., and the Analytical Section, Pioneering Research Division, Quartermaster Research and Engineering Command, Natick, Mass.

The experimental work on the project is being performed at the Quartermaster Research and Engineering Laboratories, Natick, Mass., under the direction of the U. S. Bureau of Commercial Fisheries, and the Chief, Analytical Section, Pioneering Research Division, Quartermaster Research and Engineering Command.

METHODS OF INVESTIGATION

The general methods of investigation of an unknown odor, shown in figure 1, were developed by the Analytical Section of the Pioneering Division and have proven highly successful in defining the chemical components in the odors emanating from onions, irradiated beef, insect secretions, and a variety of vegetable products (1, 2, 3). The general method is divided into seven separate steps. Steps 1 and 2 are obvious and need no further explanation. Step 3 consists of low temperature, high vacuum bulb-to-bulb distillation of the composite odor, allowing a separation of widely boiling components, so that when put into the chromatographic column finer separations can be achieved.

Steps 4 and 5 involve the use of gas chromatography for further fractionation of the odor components. The principles involved in the use of gas chromatography have been discussed in numerous publications (4, 5). However, in this application gas chromatography is not used as an analytical technique but simply as an elegant means of separation. This is made possible by attaching a trap to the exit part of the chromatographic column and condensing each fraction as it is eluted. Since we are primarily interested in presenting the contents of the trap for mass spectral analysis, a high vacuum system is used to pump out the carrier gas and leave only the condensables. These are then run on the mass spectrometer.

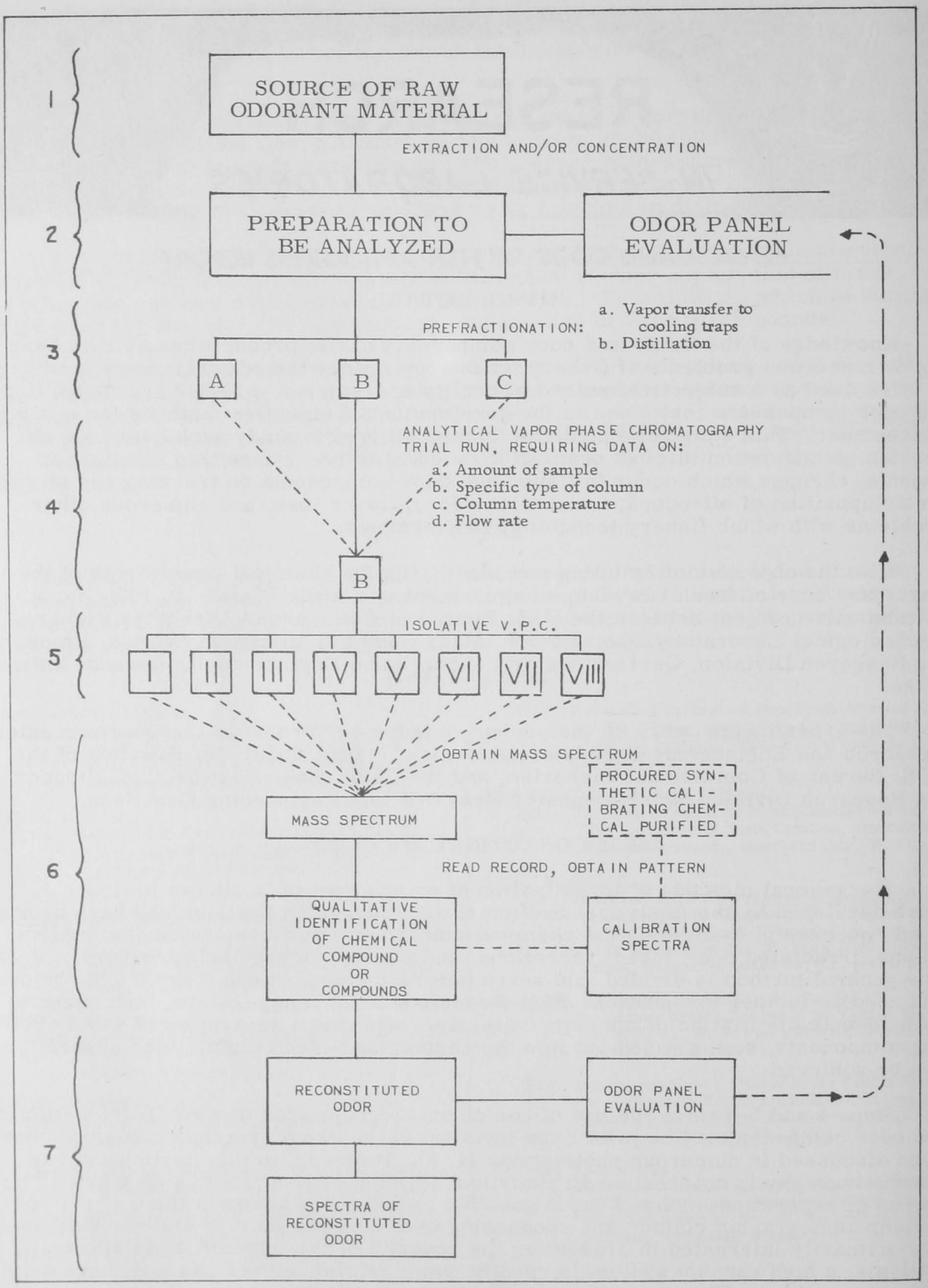

Fig. 1 - Scheme for investigation of an unknown odor.

Figure 2 is a diagram showing the principal parts of a mass spectrometer. The sample, usually in the vapor state, is admitted to the spectrometer through the inlet

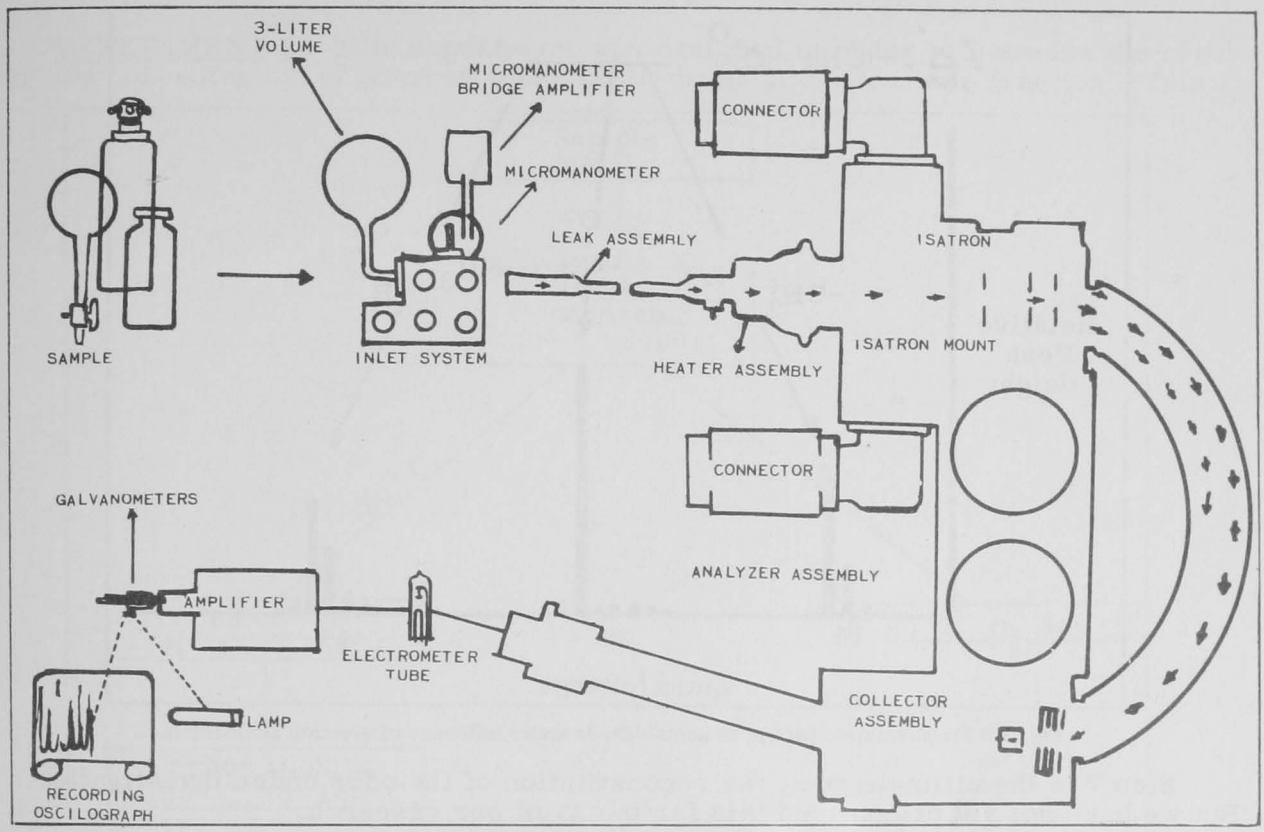

Fig. 2 - Diagram showing principal parts of a mass spectrometer.

system. In the isotron the molecules which are electrically neutral are bombarded by electrons, some become positively charged ions, while others are fragmented and become positively charged fragments. These ions are then accelerated to a high velocity and are sorted according to weight by magnetic means. As each beam of separated ions sweeps across the collector, its intensity is recorded. Figure 3 shows the fragmentation pattern of a simple molecule, acetaldehyde. It is noted that this molecule is broken up into 3 major positively-charged fragments $-CH_3^+$, $-CHO^+$, and a parent mass peak of CH_3CHO^+. The abscissa is fragment mass while the ordinate reflects the comparative quantity of each fragment; thus this relationship is specific for acetaldehyde and no other molecule. Each chemical compound has a pattern unique to it.

There are, however, limitations in the use of the mass spectrometer insofar as identifying food odors is concerned. Under normal operating conditions, that is, without a special heated inlet system, data can only be obtained on compounds up to molecular weight 300. A C_{12} hydrocarbon is typical of the upper limits of usefulness of this spectrometer. A second and more serious limitation is the following: if one has an unknown mixture of 12 components, contributions will be obtained at all mass peaks, and one will be unable to make a start without assuming the presence of a certain compound and thereafter, by dint of laborious calculations, try and fit them into a pattern. Thus in the case of unknown compounds, certain identification is improved by the lesser number of compounds present for mass spectrometric analysis. Again, if only a single pure compound is presented, and although one does not know what it is, a structural analysis of the spectrogram will allow one to predict what it is; this is followed up by comparison to a known standard spectrum, and the identification can be verified. It is for these reasons that preliminary

separation of a complex odor into single compounds or simple mixtures by vapor phase chromatography or low temperature fractional distillation is desirable.

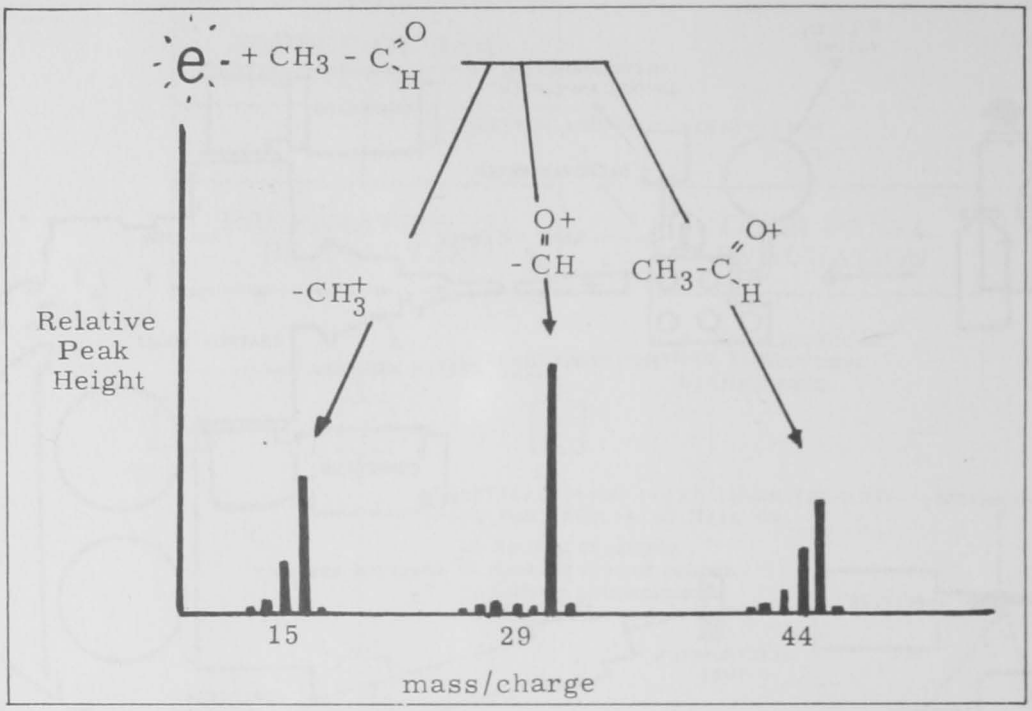

Fig. 3 - Fragmentation pattern of acetaldehyde under influence of electron bombardment.

Step 7 is the ultimate goal, the reconstitution of the odor under investigation. But we have not yet progressed this far in any of our research.

RESULTS

Operating within the framework of the procedures outlined above, the determination of the chemical components of the odor of fish was carried out in three separate experiments.

EXPERIMENT 1: The haddock used in this experiment were in rigor when obtained from a local distributor. The samples were prepared for analysis as soon as they were received. Two-hundred grams of finely chopped fillet were placed in a gas bottle and frozen with liquid nitrogen. Air was then pumped from the sample bottle to a pressure of less than 1 micron. The sample was allowed to come to room temperature and the volatile components from the sample were vacuum distilled into a receiving flask cooled with liquid nitrogen. The distillation was allowed to continue for six hours. The fraction obtained, designated as total condensables, was further fractionated by freezing to -80° C. (-112° F.). The vapors which did not condense at this temperature were collected in a flask cooled by liquid nitrogen. The fraction obtained was designated as the center cut. The center cut was then cooled to -145° C. (-229° F.) and the vapors not condensing at this temperature were collected in another liquid nitrogen trap. This fraction is the carbon dioxide fraction. Figure 4 is a schematic of the fractionating procedure. A photograph of the apparatus used in high-vacuum low temperature fractionation is shown in figure 5.

Mass spectra were then obtained of the fractions separated by the above procedures. Only water vapor and carbon dioxide could be positively identified. However, the mass spectra did indicate the presence of other components in the center cut. Positive identification of these substances could not be made because of their low concentration. Attempts were made to increase the concentration of these substances by doubling the size of the sample and increasing the distillation time.

Considerable difficulty was encountered with these samples due to the large amounts of water in the distillate. Fractionation and analysis of the larger samples gave the same results that were obtained with the smaller samples (6, 7).

EXPERIMENT 2: This experiment was designed in order to increase the number and concentration of odorous material in the total condensable fraction. This

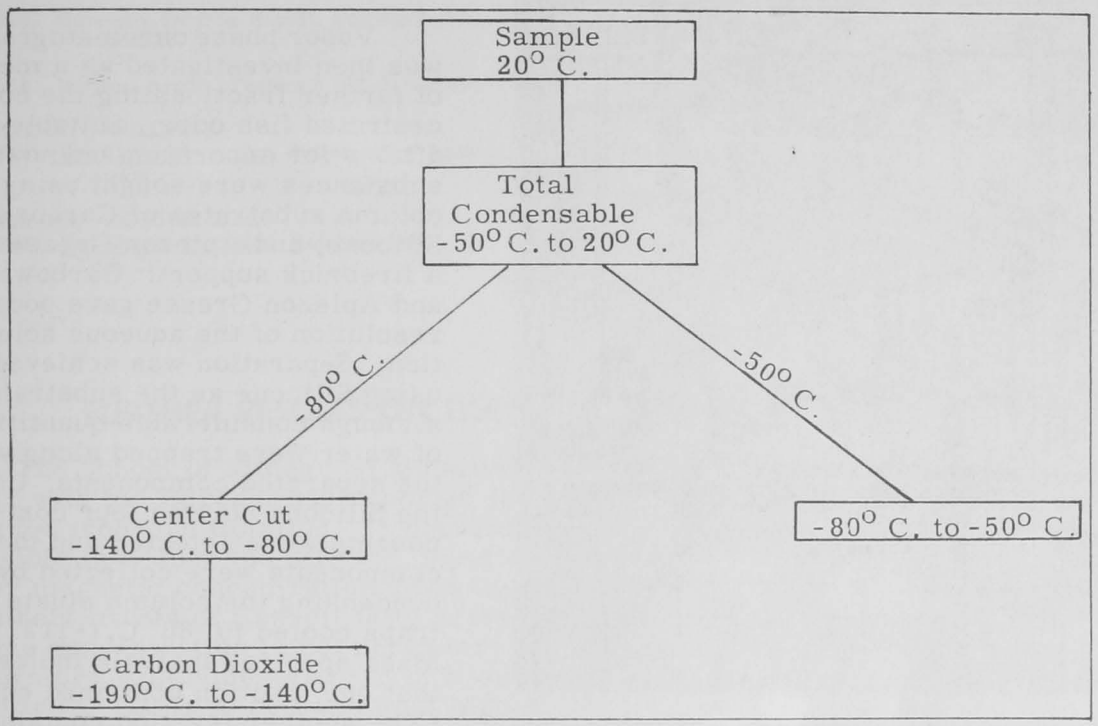

Fig. 4 - Low temperature vacuum fractionation of neutral volatile substances from fish fillets.

was done for two reasons: (1) to test the separation methods, and (2) to obtain some indication of the type of compounds one could expect to find in the neutral volatile distillate.

In this experiment the sample used was 1,500 grams of haddock fillets which had been held at ice temperature (0° C. or 32° F.) for 8 days. The sample was prepared in the same manner as the sample used in Experiment 1 and carried through the same low-temperature, high-vacuum prefractionation procedures. Three compounds could be identified in the center cut from this sample. Positive identification was made of dimethyl sulfide, acetaldehyde, and ethanol. However, these compounds are not unique constituents of fish odor but appear to be present in the volatile fraction of many foodstuffs. Further examination of the total condensable fraction of this sample indicated that most of the fish odor remained in the aqueous residue and was not removed by low temperature fractionation. Solvent extractions of this residue with diethyl ether and isopentane were carried out. It was possible to transfer some of the odorous material to the organic phase. However, attempts to increase the concentration of the odorous material by reducing the volume of solvent were unsuccessful. Most of the odorous material distilled off with the solvent.

Table 1 - Compounds Identified in Neutral Volatiles of Haddock Samples Held at Different Temperature Levels.

Fish in Rigor	Fish Stored for 8 Days at 0° C. (32° F.)	Fish Stored for 8 Days at 0° C. (32° F.) and 3 Months at -10° C. (14° F.)
	(Compounds)	
None	Acetaldehyde	-
"	Dimethyl sulfide	-
"	Methanol	Methanol
"	Ethanol	Ethanol
"	Trimethylamine	Trimethylamine
"	Trimethylamine oxide	Trimethylamine oxide

A more concentrated odor solution was obtained by straight distillation of the total condensable fraction. The cut coming over at a temperature of 90° C. (194° F.) appeared to have the highest concentration of odorous material. The residue remaining after distillation was odorless (8, 9).

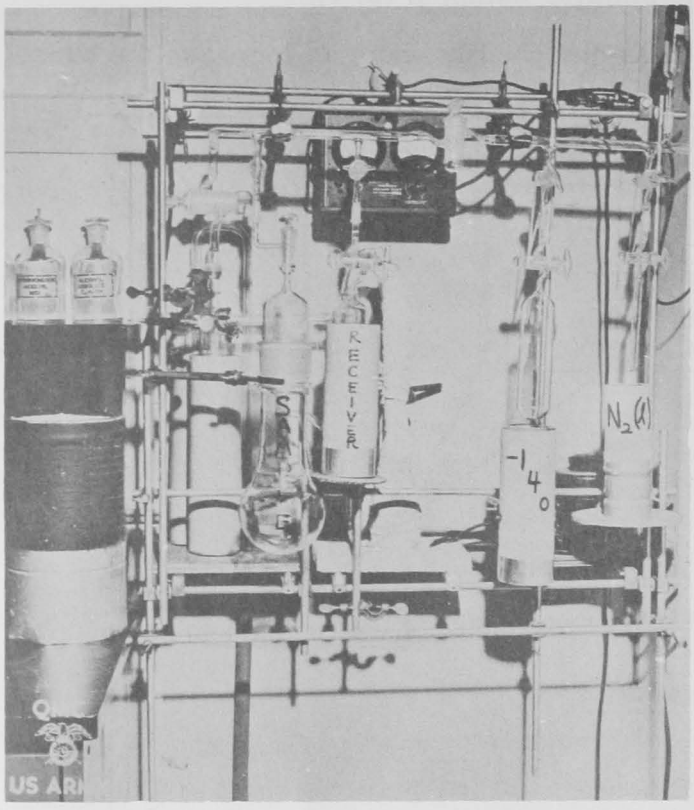

Fig. 5 - Apparatus for high-vacuum low-temperature fractionation of odor components.

Vapor phase chromatography was then investigated as a means of further fractionating the concentrated fish odor. Suitable conditions for separating unknown substances were sought using column substrates of Carbowax, Silicone, and Apiezon Grease on a firebrick support. Carbowax and Apiezon Grease gave poor resolution of the aqueous solution. Separation was achieved using Silicone as the substrate although considerable quantities of water were trapped along with the separated components. Using the Silicone column four components were detected and these components were collected by condensing the column eluate in traps cooled to -80° C. (-112° F.). Mass spectral analysis indicated that one fraction contained only CO_2, another fraction contained ethanol and methanol, a third fraction contained trimethylamine and water, and the fourth fraction contained trimethylamine, trimethylamine oxide, and water.

EXPERIMENT 3: The sample used in this experiment was 1,500 grams of haddock fillets which had been at ice temperature (0° C. or 32° F.) for 8 days, stored at -10° C. (14° F.) for three months and thawed at room temperature for 8 hours. Total condensables were collected; the fish odor was concentrated by distillation under reduced pressure; the concentrate was chromatographed on a Silicone column and fractions separating were collected and analyzed by mass spectrometry. The same compounds which were identified in Experiment 2 were also found in this sample. There were indications that this sample contained larger quantities of trimethylamine than the previous sample and that the residue remaining after distillation contained other compounds which contribute to the odor of fish. Methods for removing these remaining compounds from this residue are presently being investigated. The results obtained in this investigation to date are summarized in table 1.

DISCUSSION

The approach used in this study has so far been of a qualitative nature only. Considerable effort has been devoted to developing and testing methods in order that future work may be of a quantitative nature. The results to date indicate that "fish odor" is a complex mixture of organic compounds occurring in minute concentration in gross amounts of water. The results stated in this report are subject to change on the basis of additional evidence.

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FURTHER RESULTS ON USE OF FISH OIL FOR ORE FLOTATION

The School of Mines and Metallurgy at the University of Minnesota, under a contract with the Bureau, has reported further very promising results in the use of fish oils for iron-ore flotation. In previous work using fish-oil fatty acids, it has been possible to reduce the silicate impurities in the ores from the 8 percent level left after magnetic concentration down to about 5 percent. Recent work employing chemical derivatives of fish-oil fatty acids has reduced this level to one percent. Such extreme concentration is not necessary for efficient commercial operation. When these laboratory experiments are tried in commercial-scale experiments, the level of impurities is likely to be somewhat higher.

The contractor also reports that high temperature iron-ore flotation, first proposed in our research program more than a year ago as a means of improving flotation efficiency, has been adopted by a commercial iron-ore flotation plant in Michigan.

SHARK REPELLENT

The Office of Naval Research recently awarded a research contract to the East Boston Fishery Technological Laboratory. This work, to be done in Boston, will include chemical studies on decomposing shark meat with the eventual aim of finding an effective shark repellent.

Shark meat will be decomposed under various conditions. The resulting product will be extracted, concentrated, and an attempt made to isolate and identify the active material. The biological testing of the effectiveness of the resulting products will be done by the Navy who will maintain close contact with Bureau technologists.

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