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Chemical Components of the Odor  
of Fish

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Dr. Strong, ladies and gentlemen:

Knowledge of the flavor and odor components of fishery 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.

This paper is concerned with some initial studies conducted by the Bur. of Comm. Fisheries in collaboration with the PRD of Q. M. R. & E. Center to define the odor of fishery products in terms of its chemical composition.

An outline of the general approach used in the investigation of an unknown odor is shown in slide 1. This approach was developed by the Analytical Laboratory of the Pioneering Research Division and has proven highly successful in defining the chemical components in the odors emanating from onions, irradiated foods, insect secretions and a variety of vegetable products. The general method is divided into seven steps. Steps 1 and 2, of course, refer to the method of preparing the sample for subsequent manipulation. Preparation of the sample will be governed by the type of material and the odor involved. It may be simple comminution of the sample, solvent extraction, freezing and thawing in the case of biological materials or air sampling techniques in the case of atmospheric contaminants or pollutants. At this step of the procedure it may also be useful, if possible, to have an odor panel evaluation to assist in defining the odor spectrum and to aid in the confirmation of later findings.

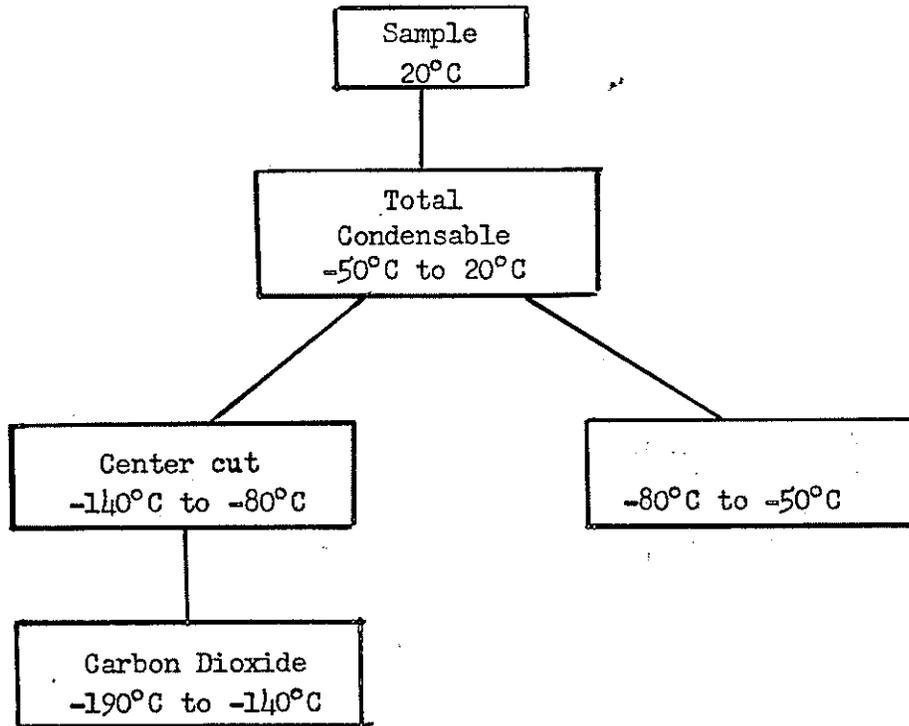
The next step in the procedure involves the use of high-vacuum low temperature fractionation techniques. These techniques were described in detail by Dr. Merritt before a meeting of this Division, last September in Chicago. Reviewing briefly; the sample is first placed in a vacuum flask and frozen to liquid nitrogen temperature ( $-190^{\circ}\text{C}$ ). Air is pumped out of the system, and with our apparatus, using a Hg diffusion pump, a vacuum of about 1 micron is attained. The sample is then allowed to come to room temperature and distillation at this temperature is begun. The distillate is collected in a receiver cooled to liquid nitrogen temperature. This first fraction is designated the total condensible fraction and in the case of most food samples, is composed mainly of water, carbon dioxide and minute quantities of odorous substances. Using low temperature-high vacuum bulb to bulb distillation techniques in the usual way, the total condensate is further separated into a carbon dioxide fraction, a center cut and a water fraction. The separation scheme is shown on the next slide.

#### Slide 2

It can be seen that we obtain 5 fractions; the solid residue remaining from the sample, an aqueous residue remaining from the total condensibles, a center cut fraction composed of material volatile between  $-140^{\circ}$  and  $-80^{\circ}$ , and a carbon dioxide fraction composed of material volatile between  $-190^{\circ}$  and  $-140^{\circ}$ . A fifth fraction containing material volatile between  $-80^{\circ}$  and  $-50^{\circ}$  may be obtained by distilling the total condensable residue at a temperature of  $-50^{\circ}\text{C}$ . If desired, further and finer fractionations may be attained by the proper choice of appropriate coolants. The mass spectrometer may be used advantageously at this point to determine if further fractionation is necessary. Each of the fractions are examined and if the mixtures are not too complex, identification of the components present in the fractions may be completed. This is, however, usually not the case and further separations may be necessary.

May I have the next slide.

Slide 2



### Slide 3

This is the first slide again. Referring now to step 4, gas chromatography has proven to be a very useful tool and has been employed in our laboratories primarily as a means for the separation of compounds from complex mixtures. In dealing with mixtures isolated from natural products, it is difficult to lay down a set of rules for the proper selection of best operating temperatures, sample size, column substrates or flow rates. These must be determined experimentally and unfortunately, are usually different for each type of sample. A general rule of thumb, however, is that if one uses a non-polar high boiling substrate, materials will separate according to boiling point. If the problem is to separate materials having the same boiling point but different chemical structure, or to separate azeotrope-forming compounds, one makes use of polar type substrates.

When operating conditions have been determined and satisfactory separations achieved, the next problem is that of collecting the column eluate. In some cases, a simple cold finger immersed in a suitable coolant is satisfactory. We have used a "U-tube" type of cold trap immersed in liquid  $N_2$  and have found this to be quite satisfactory. Stuffing the U-tube with glass wool aids in trapping any aerosol that may be formed.

The sample is then taken to the mass spectrometer (step 6) and the non-condensable gas (helium) is pumped off, while the sample is held at  $N_2$  temperature. The sample is then warmed to room temperature and the mass spectral pattern is obtained. Identification of the unknown is obtained, of course, by comparison of the spectra of the unknown with the spectra of known compounds on file. If the compound is not on file, some idea of the molecular structure of the unknown may be deduced from its spectra and one then obtains

or synthesizes suspected compounds until identification is completed. Confirmatory identification may be made by any of a number of other methods such as infrared, ultraviolet, molecular weight, etc; and finally, of course, the ultimate step is the reconstitution of the odor with known compounds and comparison with the original starting odor. This is the general scheme and serves as a starting point. It will be seen later, however, that we do not restrict ourselves to these steps, but modify the scheme according to our findings.

The general scheme of analysis outlined above, was followed in our examination of the odor of haddock samples. A 1500-g. sample of flesh was obtained from fish still in rigor. These fish were probably less than 8 hours out of the water, were well iced and of prime quality. An odor panel evaluation, by an untrained panel composed of laboratory personnel, indicated that this sample had no "fishy odor", but was, in fact, practically odorless. This is no surprise to anyone who has ever cooked a fish immediately after it had been taken from cold Atlantic waters. There just isn't any trace of what most people refer to as a "fishy odor".

The sample was finely divided in a Hobart food chopper, placed in a vacuum flask, and the volatile odor components were collected and separated by the procedures previously described. Total condensables were collected for 16 hours and yielded approximately 800 ml.

Mass spectrometric analysis of the fractions obtained, indicated that compounds were present in all fractions, but positive identification of other than carbon dioxide and water could not be made because of the extremely minute quantities of these compounds present. It appeared then that if we were to identify any of the components present in the neutral volatile distillate, we would have to go to a much larger sample size. However, before we did this, we

decided to test our methods using an older sample of fish and one which we knew had some odor associated with it. A sample of haddock was obtained which had been held on ice (0°C) for 8 days. This sample was of excellent quality as judged by odor, firmness of flesh and clarity of eye fluids. The "odor panel" decided that the sample possessed an agreeable type of "fishy odor". The sample was prepared in the same manner as the sample of fresh fish. It was fractionated by the usual high-vacuum techniques and mass spectral patterns were obtained on each of the fractions. In the center cut, four compounds could be identified. These were dimethyl sulfide, acetaldehyde, methanol and ethanol. These compounds appear to be common components of the volatile fraction of many foodstuffs and have been reported in beef, coffee, and vegetables. It is apparent they are not uniquely characteristic of fish odor.

Traces of material were found in the other fractions but no identifications could be made by mass spectrometry because of the minute amounts of material present. It was, however, obvious that the aqueous residue still retained most of the fish odor but the gross amounts of water in the vapor obscured the mass spectra of the trace components.

It was at this point we had to deviate from our general scheme of analysis. Our problem now was to concentrate the odor material which remained in our aqueous fraction and which was not removed by our preliminary separations. Distillation of the aqueous residue under reduced pressure allowed the separation of a fraction which had an ammoniacal odor, was basic to litmus and gave a positive Kjeldahl-Nessler test for nitrogen. The residue remaining in the flask after distillation still retained a fish-type odor.

The distillate was then further fractionated by gas chromatography on a Dow-Corning 703 silicone column. The temperature of the column was gradually increased from room temperature to a temperature of 95°, and the helium flow rate was maintained at 50 ml. per minute.

The next slide shows a typical chromatogram obtained.

Slide 4

Peaks (1) and (2) are due to air and CO<sub>2</sub> respectively. Peak (3) was subsequently shown by mass spectrometry to be mainly trimethylamine. The mass spectral analysis also showed that traces of trimethylamine oxide were present in this fraction. Upon collecting, in a cold trap, the material corresponding to base line between the CO<sub>2</sub> and (CH<sub>3</sub>)<sub>3</sub>N peaks, methanol and ethanol were identified by mass spectrometry. Methanol, ethanol, trimethylamine and trimethylamine oxide are the only components which we were able to find in this fraction.

The residue remaining from the distillation which still possessed a "fishy odor" was then treated with 2,4-dinitrophenyl-hydrazine in perchloric acid and allowed to stand for 24 hours. At the end of this time, a flocculant precipitate had formed with 30 per cent perchloric acid, then distilled water, and was dried in air at 50°C. The melting point of the precipitate was indeterminate and decomposition occurred indicating that a mixture of phenylhydrazine derivatives was obtained. The derivatives were only partially soluble in methanol, benzene, and in dioxane but completely soluble in ethanolic sodium hydroxide. In ethanolic sodium hydroxide, the phenylhydrazine derivatives gave rise to a deep blue color which is indicative of dicarbonyl or  $\alpha$ -hydroxy carbonyl type compounds. An infrared spectra indicated that the compounds were aliphatic, and that the major portion of the compounds probably contained less than four carbon atoms. We have not as yet been able to identify the specific carbonyl compounds present in the precipitate.

Slide 5

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.	Fish Stored for 8 Days at 0°C. and 3 Months at -10°C.
<u>Compounds</u>	<u>Compounds</u>	<u>Compounds</u>
none	acetaldehyde	* -
none	dimethyl sulfide	* -
none	methanol	methanol
none	ethanol	ethanol
none	trimethylamine	trimethylamine
none	trimethylamine oxide	trimethylamine oxide
2,4 DNP Derivatives	2,4 DNP Derivatives	2,4 DNP Derivatives

\*center cut of this sample not analyzed

Concurrently with the studies of 8 day old haddock, analysis were also made of the neutral volatiles from a sample of haddock fillets which had been stored on ice at 0°C for 8 days, then stored for 3 months at -10°C and finally thawed at room temperature for 8 hours. This fish was judged to be only fair in quality. It possessed a definite "fishy" by not repulsive odor. Distillation of the aqueous residue of this sample gave a fraction with a definite ammoniacal odor which again was shown to be trimethylamine.

The residue remaining after distillation, which still retained a "fishy odor" was treated with 2,4-dinitrophenylhydrazine and a heavy precipitate was obtained. This fraction is still under investigation. We then returned to our original sample of fresh haddock, treated our aqueous residue with 2,4-dinitrophenylhydrazine and obtained a few milligrams of derivative. The amount of 2,4 DNP derivative obtained from the various samples permitted us to make semi-quantitative estimate of the relative and quantities present. We found that the amount of the 2,4 DNP derivatives of the 8 day old fish was about 10 times the amount of the derivatives of the fresh fish.

Finally, I would like to summarize our results to date. (next slide). In the first column are our results obtained on the sample of fresh fish. Here it is seen that the only positive identification we have is the presence of 2,4 DNP reactive material. In the second and third columns are listed the compounds we have identified in the volatile of fish. It can be seen that fish "odor" is a complex mixture of organic compounds and that we have not yet identified all of the compounds present. Certainly trimethylamine has long been implicated in fish odor, but we feel, however, that the material that reacts with 2,4 DNP may, to a large extent, be responsible for some of the unique characteristics of fish odor.

THANK YOU