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Techniques for Collecting Volatile Components from Haddock Flesh for Gas Chromatographic Analysis

SUMMARY

The volatile compounds isolated from haddock (*Melanogrammus aeglefinus*) flesh were analyzed by cryogenic gas chromatography. Four methods of collecting the volatile compounds were studied and evaluated. Chromatograms showed that the best samples were obtained from the equilibrium vapor above the total condensate removed from the haddock flesh by vacuum distillation. Chromatograms illustrate the changes in the relative amounts of volatile compounds collected from samples of both raw and cooked fish after storage at 2°C for 1, 5, and 10 days. The amount and number of volatile compounds increased during storage, and cooking released additional compounds, some in large amount.

INTRODUCTION

Little is known of the volatile compounds which contribute to the flavor and odor of fishery products. Isolation and identification of these compounds are essential to discover their chemical nature and to understand their contribution to the flavor and odor of the product. Basic studies of these compounds are also needed to determine how they are affected by various storage and processing conditions. Although gas chromatography is widely used for separating the volatile components associated with the flavor and odor of foods, analysis is meaningful most generally only when the sample is collected with little or no change from its natural state. Several techniques have been used by various workers for removing the volatile compounds from foods for analysis by gas chromatography. The most widely used methods are solvent extraction (Hornstein and Crowe, 1960; Hunter *et al.*, 1961; Jennings, 1961) and distillation (Slater, 1961; Yueh and Strong, 1960) or a combination of both (Brandenberger and Müller, 1962; Hiu and Scheuer, 1961; Smith and Coffman, 1960; Vorbeck *et al.*, 1961; Weurman, 1963; Wick

et al., 1961). The disadvantages of solvent extraction are the inability of solvents to remove all of the odor components quantitatively and the possibility of contaminating the samples with impurities from the solvent. Distillation at elevated temperatures may lead to chemical changes. Other methods have been reported, such as high-vacuum distillation (Merritt *et al.*, 1959), flushing with an inert gas (Kramlich and Pearson, 1960; Rhoades, 1958), and direct sampling of equilibrium vapors (Buttery and Teranishi, 1961; Carrol and O'Brien, 1959; Mackay *et al.*, 1961; Nawar and Fagerson, 1962; Teranishi *et al.*, 1962). In a previous study (Mangan *et al.*, 1959) of the volatile components isolated from fish, high-vacuum distillation was used, but results obtained from further investigations have revealed that samples differ markedly in composition with the method of isolation. Therefore, a systematic study was undertaken of certain techniques for removing volatiles from haddock flesh. In assessing the significance of the analysis, of paramount importance in addition to the method of sampling is the efficiency of the gas chromatographic procedures in both degree of separability and level of sensitivity. Although, ultimately, it is the objective of the current research to determine the effects of storage and cooking on the composition of the volatile components in fish and their relation to flavor, this study was initiated primarily to evaluate certain methods of collecting an odor concentrate. In the course of the investigation considerable insight has been gained, however, concerning the change in the composition of the volatiles under the various conditions of storage for both raw and cooked fish.

This paper deals with 4 techniques for collecting volatile compounds from cold-stored haddock and the gas chromatographic analy-

ses of these mixtures. It also shows the changes in the volatile compounds from both raw and cooked haddock flesh which are due to storage.

EXPERIMENTAL TECHNIQUES

Storage conditions. The samples of haddock fillets and storage conditions have been described (Mendelsohn and Steinberg, 1962). Sensory odor evaluation of the fillet was made by the experimenters prior to storage and after each storage period. The first sample of volatiles was removed from the flesh the day the fish arrived at the laboratory. Since these fish were stored on ice almost 24 hr prior to arrival, they were considered as 1 day old. Subsequent samples, taken after the fourth and ninth days of storage in the laboratory, are referred to as the 5- and 10-day storage samples. Since haddock stored for 10 days at refrigerated temperatures, though edible, is of poor organoleptic quality, no samples were stored beyond this period. The composition of the volatiles isolated from haddock probably vary slightly with the time of the year, landing area, and handling aboard the boat. Therefore, all samples were obtained from the same source, and storage conditions in the laboratory were held constant. At each storage interval, duplicate samples were removed from storage. Various weights of samples were taken depending on the method of collection to be used. One sample was used for analysis of the volatile compounds from the raw fish, while the other fish sample was placed in a sealed glass vessel and was steamed in a pressure cooker at 110°C. This sample was placed in the cooker as soon as the water had started to boil and cooked for 20 min.

Methods of collecting volatile concentrates.

Direct sampling of equilibrium vapor. Three hundred g of the raw haddock fillets were placed in a 1-L flask fitted with a sidearm over which was placed a rubber septum. Ten ml of the vapor in equilibrium with the fish in the flask ("head gas") were removed directly by means of a gas syringe. The raw fish was equilibrated to room temperature, whereas the cooked fish volatiles were removed through the septum while the sample was still hot. Vapors were injected directly onto the chromatographic column.

Flushing with an inert gas. One hundred g of minced raw haddock flesh were placed in the collection trap shown in Fig. 1. and equilibrated for at least 1 hr at room temperature. The volatiles were flushed for 1 min with argon carrier gas onto the head of a gas chromatographic column held at -65°C (see chromatographic procedure below). For analysis of the cooked fish volatiles, the trap was sealed and was cooked in the pressure cooker

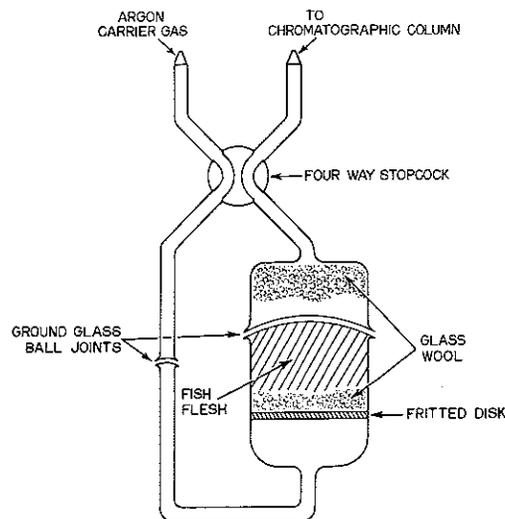


Fig. 1. Sample container used for removal of fish volatiles by flushing.

for 20 min. The hot volatiles were then removed in the same way by flushing for 1 min.

Vacuum collection of equilibrium vapor. At room temperature the equilibrium vapors above 600 g of raw haddock fillets and cooked haddock fillets were collected in gas bottles evacuated to a pressure of about 1 μ Hg. The collection bottles were then immersed in liquid nitrogen and connected to the vacuum manifold, and any uncondensable gases were removed from the system. The collection bottles were brought to room temperature again, and about 1.4×10^{-4} moles of the volatiles (measured by the vapor pressure and calculated by $PV = P'V'$) were transferred to a gas trap, according to the procedure of Bazinet and Walsh (1960). The trap was placed in the stream of carrier gas leading to the chromatographic column.

High-vacuum distillation. The total condensate from 600 g of minced, raw, or cooked haddock flesh was collected with the high-vacuum distillation technique of Merritt *et al.* (1959). The collection was allowed to proceed for 24 hr. The sample was obtained by allowing the equilibrium vapor above the total condensate to expand freely into an evacuated glass collection bottle. By measurement of the vapor pressure of the gases which were allowed to expand into the collection bottle, approximately 1.4×10^{-4} moles of the total condensate vapor were then transferred to a gas trap, and the contents of the trap were flushed by the argon carrier gas onto the gas chromatography column for separation. A comparison was made of the gas chromatograms obtained of the equilibrium vapor above the total condensate with the volatile

material collected in the usual way as "center cut" (Merritt *et al.*, 1959).

Gas chromatography procedure. Preliminary studies showed that isothermal gas chromatography at room temperature or above gave poor resolution. Therefore, all the mixtures of volatile compounds were separated by programmed cryogenic temperature gas chromatography (Merritt and Walsh, 1963) to achieve a high degree of separability. An argon ionization detector (Lovelock, 1958) was used to attain high sensitivity. The gas chromatography column was a 6-ft \times 0.25-in.-OD U-shaped glass column with 5% (wt/wt) β,β' -oxydipropionitrile (OPN) on 80-100-mesh untreated Chromosorb-W. The column, before introduction of the sample, was immersed in a dry-ice-ethanol coolant, and when the temperature had come to -65°C the sample was swept onto the column with carrier gas. Elution of the components from the column was allowed to proceed as the temperature of the column was allowed to rise to room temperature. The column was surrounded by a glass jacket to contain the coolant and was attached at the column exit end to the argon ionization detector of a Barber Colman gas chromatograph, model 10. The argon carrier gas flow was 40 ml per min measured before the start of the chromatogram at the detector outlet by a Fisher Porter Tri-Flat precision-bore flow meter. The ionization detector (20 mc Sr^{90}) was operated at 190°C .

RESULTS AND DISCUSSION

Weurman (1963) investigated various methods of removing the volatile compounds from foods and showed that the compounds detected and identified were directly dependent upon the removal method. The effectiveness of 4 methods of collecting the volatile components was evaluated with the gas chromatograms obtained used as criteria. Chromatograms were obtained of the volatile components from haddock filets collected by each of the 4 methods.

Direct sampling of equilibrium vapors above the filets with a syringe provides a gaseous sample of the odor components actually perceived and is simple and rapid. Fig. 2 shows the chromatograms obtained of a 10-ml sample of gas removed by syringe from both raw and cooked haddock filets that had been stored 10 days at 2°C . Only relatively few peaks are observed. The bulk of the gaseous components of this sample are air and carbon dioxide and the argon ionization

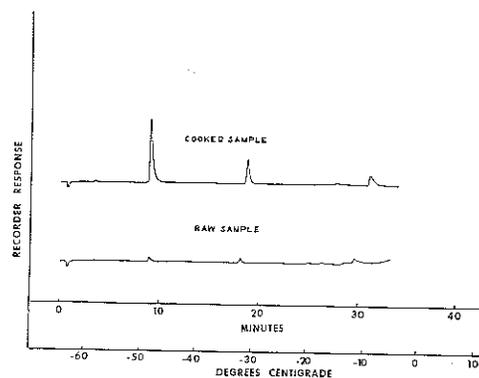


Fig. 2. Programmed cryogenic-temperature gas chromatograms of volatile components collected by syringe from haddock "headspace."

detector does not respond to these components. The peaks observed on the chromatograms are those due to the volatile flavor and odor components in the sample and are shown to be present in extremely low concentration. Since a 10-ml gas sample is considered to be fairly large, and since it was composed almost entirely of air and carbon dioxide, it was concluded that removal of the volatile compounds with a syringe produced a sample which is too dilute to be suitable. Chromatograms for filets stored 1 and 5 days showed fewer peaks than chromatograms for filets stored 10 days.

Flushing the equilibrium vapors directly onto the chromatographic column concentrates the volatile odor components on the head of the column. In addition, it may be expected that neither the fish flesh nor the composition of the volatiles is altered by this method of sampling. Another advantage is the fact that components which are normally lost by condensation when liquid nitrogen cold-trapping procedures is employed (see below) are detected on the chromatograms obtained by this method of sampling. Chromatograms obtained by this technique from both raw and cooked fish samples are shown in Fig. 3. Only the sample which was stored for 10 days and then cooked showed large peaks. As with the syringe, the sample flushed onto the column is mainly air and carbon dioxide and contains but a very small proportion of the flavor and odor components. Although the cooked 10-day-old fish shows an abundance of volatiles which is much

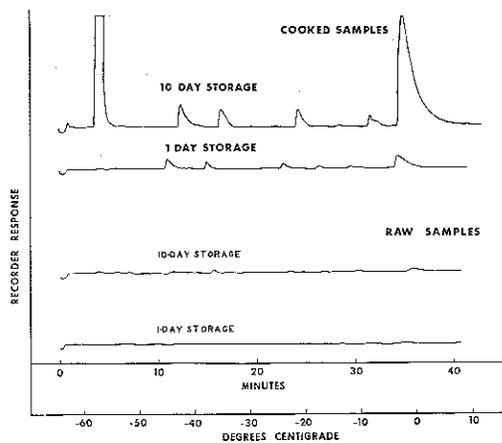


Fig. 3. Programmed cryogenic-temperature gas chromatograms of volatile components in haddock "headspace" flushed onto the column.

greater than that of the other samples, the amounts indicated by the chromatograms are too small for purposes of comparison.

The method of collecting the equilibrium vapor over a sample by free expansion into an evacuated receiver was devised in an attempt to concentrate the volatile components. The chromatograms subsequently obtained from the series of raw fish samples are shown in Fig. 4. As expected, both a larger number as well as a greater quantity of the volatile components are indicated by the chromatograms. This procedure provides the greatest concentration of volatile odor compounds attainable without alteration of the equilibrium composition of the vapor. For comparing the chromatograms from different

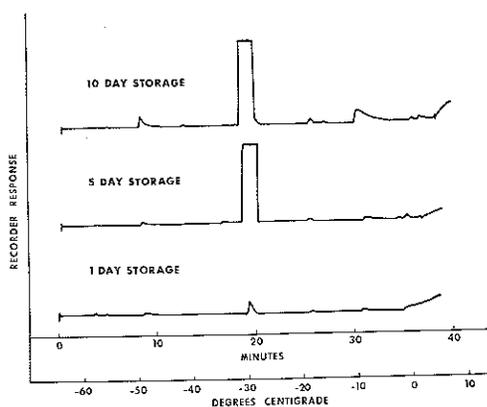


Fig. 4. Programmed cryogenic-temperature gas chromatograms of volatile components collected by vacuum from haddock "headspace."

samples, however, the quantity of volatiles collected is too small to provide much information about composition except for major components of the mixture.

The largest yield of volatile material is provided by the vacuum distillation method (Merritt *et al.*, 1959). In most prior studies using this technique, the total condensate from the distillation was taken for subsequent separation and analysis. In this study the procedure has been simplified by allowing the vapor over the total condensate to expand into an evacuated sample trap. The relative amounts of the compounds in the vapor sample tend to differ, however, from the corresponding amounts in the distillate. Nevertheless, the validity of the qualitative correspondence of the sample composition is maintained since the same peaks are observed on total condensate vapor chromatograms as on chromatograms of liquid total condensate. As with the other methods, the bulk of the material collected consisted mainly of carbon dioxide and water. Since these components are separated from the volatile odor compounds by the gas chromatography procedure subsequently employed, they do not interfere with the evaluation of the sampling procedures. Many more components are found in total condensate vapor samples than in samples which retain the unaltered "headspace" composition. For comparing the change in composition of the volatile components in the samples of fish flesh itself, the greater number of components and larger yield provided by total condensate vapor are superior to the other methods of collection. Chromatograms of samples collected by this procedure (Fig. 5) clearly demonstrate the larger number and higher concentration of compounds collected.

This investigation shows that a vacuum distillation method for collection of volatile compounds can provide a sample of sufficient concentration for further study. This technique has been used in studying the effect of temperature, storage time, cooking, and any other variables on the composition of volatile odor and flavor components in fish. The data provided by this study show that both the number and amount of components increase with storage time and with cooking. Apparent absence of volatile compounds in the

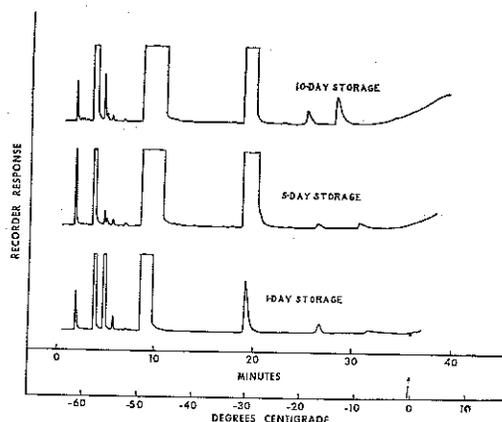


Fig. 5. Programmed cryogenic-temperature gas chromatograms of volatile components in cooked haddock total condensate vapor.

chromatogram of a 1-day raw sample confirms analytically the sensory observation that fresh raw fish is practically odorless. The significance of these results is difficult to evaluate, however, without a knowledge of the identity of the volatile components. The method used to accomplish such identifications by rapid-scanning mass spectrometry of the gas chromatographic eluate has been described (Merritt *et al.*, 1964). A subsequent paper will describe further studies of the composition of the flavor and odor components of fishery products.

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