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Kenneth Stephen Hilderbrand Jr. for the M.S. in Food Science
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Title DIRECT INJECTION GAS CHROMATOGRAPHY OF VOLATILES FROM FISHERY PRODUCTS

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The use of gas chromatography for the separation, comparison, and subsequent identification of flavor volatiles from food products has proven highly successful in recent years. The development of various techniques for the concentration of the volatiles before gas chromatographic analysis has greatly extended the use of this important analytical tool.

The injection of vapors directly into the chromatograph without prior concentration is the simplest method and has been used successfully on many food products. However, the use of this technique on the volatiles of fishery products has met with limited success. The complexity and nature of the flavor compounds found in fishery products have required the use of highly sensitive instruments and columns with very efficient separation power. The purpose of this investigation was to develop a method for the separation and comparison of volatiles from fishery products by this direct vapor injection technique.

Preliminary investigations showed that a nine foot column of diisodecyl phthalate on 80/100 mesh, methanoic KOH treated, celite 545, operated isothermally at 35°C, would give satisfactory separation of one to three ml samples of volatiles from heated fishery products. The technique was not, however, sensitive enough to allow direct sampling of cold products unless they were highly spoiled or autoxidized.

This investigation showed that direct vapor injection, using the column and conditions described, will show differences between size and number of peaks in heated fresh, oxidized, and spoiled fishery products. Several peaks in autoxidizing menhaden oil were shown to increase with hours of oxidation and a peak with the same retention time as trimethylamine was observed in the chromatograms of spoiled fish. The direct injection technique did not show large differences between fresh dover sole, rockfish, oysters, or beef.

Tentative identification of various peaks from the chromatograms of oxidized salmon oil was attempted by comparison of retention data to known compounds and by functional group analysis by the method of Hoff and Feit (34). In this manner the possible existance of C_1 to C_7 alkanals, 2-hexen-1-al, methane, heptane, ethanol, butanol, and acetone was shown. The methods of tentative identification used were preliminary in nature and confirming tests would be necessary before positive identifications could be made.

A comparison of chromatograms from fish, oysters, beef, and fish oils showed that several similar peaks appear in every case. These peaks were found at retention times of 0.71, 0.87, 1.42, 2.21, 2.83, 3.62, 5.20, and 5.51 minutes.

DIRECT INJECTION GAS CHROMATOGRAPHY OF VOLATILES FROM FISHERY PRODUCTS

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KENNETH STEPHEN HILDERBRAND JR.

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In Charge of Major

Head of Department of Food Science and Technology

Dean of Graduate School

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INTRODUCTION

Stansby (77) has stated that "Prevention of objectionable fishy odors is a principle aim of fishery technology" and that "Application to fish of various preservative methods is primarily to stop the formation of such odors". These statements could also be true of many other food products, but it is generally considered that fishery products are more susceptible than most other food products to oxidative, bacterial, and enzymatic deteriorations which result in objectionable odors.

Efforts to characterize and identify the chemical compounds responsible for the objectionable, as well as the natural, odors of many food products have met with considerable success (9, 11). The development of gas chromatography as an analytical tool has been largely responsible for the isolation and subsequent identification of volatile compounds that contribute to these odors, many of which are present in extremely small quantities.

In spite of the characteristic odors associated with fishery products, relatively little work has been done in this area in comparison to other food products. Most procedures have been limited to classical chemical analysis. The gas chromatographic investigations have been limited to the separation of volatiles concentrated by procedures such as vacuum distillation and cold trapping (31, 38, 61).

The purpose of this investigation was to develop a method by which the components constituting fishy odors could be separated by injection of the vapors directly into the gas chromatograph without prior concentration or collection. This type of analysis, which has been used successfully on various food products (10, 11, 79), would have the advantages of simplicity and speed. However, previous investigations of this type with fishery products have met with limited success, primarily due to a lack of sensitivity in the instruments and poor resolving power of the columns employed (62).

REVIEW OF LITERATURE

Development of Gas Chromatography as an Analytical Tool

Martin and Synge (56), in a paper describing paper chromatography, suggested that the mobile phase could be a vapor as well as a liquid. This paper is credited with being the first description of what is now referred to as "gas-liquid chromatography" or more commonly "gas chromatography". Eleven years later James and Martin (42) developed a procedure for the separation and estimation of volatile fatty acids by gas chromatography. These authors began the development of what is now a highly useful analytical tool.

The present uses of gas chromatography are too numerous to mention completely, but the extent of this field can be shown by reference to one abstracting service devoted solely to this area since 1959 (45, 46, 47, 48, 49). "Gas Chromatography Abstracts" groups literature on applications of gas chromatography into five general categories of quantitative analysis, preparative, process monitoring, pure chemistry, and applied chemistry. The use of gas chromatography in the study of flavors and odors is abstracted under the category of "Applied Chemistry" under the heading of "Food".

Theory and Technique of Gas Chromatography

The various parameters influencing the operation of the gas chromatograph have been outlined and reviewed by many authors. Howard (37) reviewed the affects of stationary phase, column efficiency, sample size, support material, particle size, gas flow

rate, pressure gradient, and column temperature.

An extensive review of factors influencing the operation of the gas chromatograph was given by Hardy and Pollard (33). This paper covers 619 publications and reviews principles involved in separation by gas chromatography, as well as apparatus and techniques.

A review of the theoretical considerations and equations involved in gas chromatographic separations was reported in two papers by Pecsok (72), and Dal Nogare and Juvet (19).

Gas Chromatography of Food Volatiles

Early Investigations: The use of gas chromatography for the analysis of food volatiles is widely used today but has a history that dates back only seven or eight years. In 1956, Dimick and Corse (26) outlined a procedure for the gas chromatographic separation and collection of volatile compounds from strawberry oil trapped during flash evaporation. These authors suggested that this procedure could be extended to many other types of food.

In 1957, Jennings (43) used similar techniques to analyze an ether extract of a distillate from a fermented dairy product starter. This technique showed the presence of ethanol, acetic acid, and diacetyl.

Vacuum Distillation and Extraction Techniques: The two early investigations (26, 43) on food volatiles used a sample of food product volatiles which had been concentrated in some manner. The

concentration of volatiles by distillation, extraction, and cold trapping prior to gas chromatographic analysis is a commonly accepted technique and is still used extensively (35). Recent investigations on coffee (74, 90), fats and oils (15, 24, 50, 51), orange juice (85), wine (57, 82), potatoes (76), irradiated beef and milk (22, 23, 64, 84), spices (20), and cheese (59, 75) are a few of many examples.

The high vacuum distillation procedures, such as that developed by Libby, Bills, and Day (51), are of particular usefulness in concentrating food volatiles. This method utilized a molecular still operated under high vacuum and employed liquid nitrogen traps to collect the volatiles. Stainless steel helical traps were used to transfer the volatiles to the gas chromatograph from the nitrogen traps.

Vapor Enrichment Techniques: Several investigators have simplified sample concentration techniques in various ways to prevent chemical changes and loss of volatiles due to manipulation. Nawar and Fagerson (66) and Nawar et al. (67) recycled nitrogen gas over a food in a closed system and collected the volatiles in a "U" tube immersed in liquid nitrogen. The trapped volatiles were introduced into the carrier gas stream by a system of valves that allowed evacuation of the "U" tube prior to warming and sweeping the tube with the carrier gas. These cold trapping concepts were later modified by Hornstein and Crowe (36). The "U" tube was replaced by a two foot section of a 7.5 ft. packed chromatography column and

the volatiles from meat were trapped in a similar manner. The coil was then attached to the rest of the column, heated, and the volatiles eluted in the normal manner. Both of these procedures concentrated volatiles without the loss, contamination, or thermal destruction common to many distillation techniques.

A procedure similar to that of Hornstein and Crowe (36) was used by Burks et al. (9) to concentrate the amines involved in the flavor and odor of irradiated beef. The amines were released when concentrated NaOH was injected into the hydrochlorides placed in the carrier gas stream in an inverted "T" tube.

Direct Injection of Volatiles: Recently, workers have investigated the use of sampling techniques which allow direct injection of the vapors into the gas chromatograph without prior enrichment (44, 10). This type of sampling would eliminate the lengthy and difficult concentration steps and would appear to give a more reliable analysis of the natural compounds present. These procedures have become possible only recently and are largely the result of the development of highly sensitive ionization detectors (2).

In 1961, Buttery and Teranishi (10) reported the separation of volatiles from fruits and vegetables by direct injection of aqueous vapors. These authors tried three types of ionization detectors: The beta-ray argon triode of Lovelock (52), the single flame of Thompson (80), and the dual flame of McWilliam and Dewar (60). They found the dual flame most suitable as the argon detector was desensitized by oxygen in the vapors, and the single flame was not

as stable as the dual flame under the conditions used.

Makay, Lang, and Berdick (53) investigated the argon detector of Lovelock (52) and found it satisfactory for the direct injection of one to five ml vapor samples of peppermint oil, coffee, brandy, onion, and cigar and cigarette smoke. These authors noted the particular usefulness of the direct injection method in following the rapid changes in onion vapors with time.

Recently Teranishi, Buttery, and Lundin (79) improved their earlier efforts (10) to include temperature programming. Five to ten ml vapor samples were injected into the apparatus and the column temperature was increased as the compounds were eluted. This made possible the detection of compounds in vapors that were not eluted at lower isothermal temperatures without impairing the separation of the more volatile peaks. The dual column apparatus was especially suited to this type of work because of the baseline drift compensation of the second "blank" column.

The technique of Buttery and Teranishi (10) was again used by Jennings, Viljhalmsson, and Dunkley (44) to analyze milk vapors. These authors found that under the conditions used (1% Apiezon M on 80/100 mesh Chromosorb W at 35°C) there were no differences between fresh raw milk, milk pasteurized and homogenized (HTST), milk heated one hour at 80°C, or milk subjected to conditions which caused hydrolytic rancidity.

The most recent investigations using the techniques of direct vapor injection of food vapors were by Pippen and Nanaka (73) on the volatiles of chicken and turkey; and by Buttery and Teranishi on

vegetables (10) and auto autoxidation (11).

Direct injection sampling of food volatiles offers the advantages of simplicity and minimum changes in components. However, comparisons of enrichment sampling to direct injection have shown much reduced sensitivity in the simpler method (65). The future of direct injection sampling procedures apparently lies in the development of more sensitive gas chromatographic instruments and techniques.

Identification of Eluted Compounds: The identification of compounds eluted from the gas chromatograph is commonly carried out by chemical analysis, light spectrophotometry, or mass spectrometry when the size of sample permits collection. When using very small liquid or vapor samples, it is often extremely difficult to isolate enough compound for analysis. Tentative identifications are routinely made by comparison of actual and relative retention times or volumes (19, 72). However, tentative identification by these methods is sometimes unreliable unless confirmed by more positive analyses.

Various methods have been employed to positively identify compounds as they are eluted in small quantities (3, 6, 7, 56). The mass spectrometer is the most reliable of these and is being used by several investigators in studies of food odors (22, 23, 64). However, the use of the mass spectrometer is an exacting procedure; interpretation of the data is complicated; and the instruments are very expensive.

Several methods have been developed for identification of compounds with respect to functional groups (2, 14, 34). The use of various chemicals which react with functional groups in the sample, to remove or change peaks, was proposed by Bassette, Özeris, and Whitnah (2). Hoff and Feit (34) modified this technique and coated the sides of the injection syringe with these chemicals. In this way, the volatiles react in the syringe itself rather than in a sample flask where the reactions could be complicated by the rest of the sample. With this technique, these authors were able to distinguish between aldehydes, ethers, alcohols, saturated and unsaturated hydrocarbons, and ketones in a model system. This type of analysis is especially adaptable to direct injection sampling where the concentration of volatiles is so small as to preclude manipulations for other types of analysis.

Flavor and Odor of Fish

Occurrance of Fishy Odors: Most fresh fish have a distinctive and bland odor which is dependent on species (77). It is only after storage that most fish develop what is commonly described as a "fishy" odor, but the length of storage required may be only a matter of hours. These odors vary from those associated with bacterial spoilage (hydrogen sulfide, ammonia, trimethylamine, etc.) to the more complex odors associated with oxidative rancidity (77).

Chemical Compounds Responsible for Fishy Odors: Stansby (77) reviewed the problem of fishy odors and pointed out that there is a need for research in this area. Most efforts to associate various odors described as fishy or rancid odors with definite chemical compounds have met with limited success.

The work of Beatty and Collins (4, 5) and Collins (18) on fish spoilage in 1939-1941, and later by Dyer, Dyer, and Snow (29) and Dyer and Mounsey (28) on the amines in fish muscle, showed only that trimethylamine, methylamine, dimethylamine, ammonia, and some higher amines were formed during fish spoilage. Because of the fishy aroma which has been associated with the methylamines, it was concluded that this group of compounds was largely responsible for fishy odors. However, recent investigators (77) disagree with this assumption and have shown that pure trimethylamine has an odor similar to ammonia.

With the exception of investigations in 1950-1952 on the pyridine group compounds by Obata (71), Obata and Yamanishi (68), and Obata, Yamanishi, and Ishida (69, 70), very little progress has been made in recent years in the identification of the compounds associated with fishy odors. These investigators demonstrated that the slime on the surface of salmon produced objectionable odors when subjected to bacterial action (69, 70). It was suggested that α-amino valeric acid and α-amino valeraldehyde were partly responsible for these odors as these compounds had odors similar to the spoiled slime.

Bramstedt (8) studied the importance of free amino acids in the flavor and odor of fish. His investigations indicated that free amino acids can be precursors of special odors but generally have little odor themselves. These investigations also showed that free amino acids decrease with a corresponding increase in odoriferous substances during storage.

Many objectionable odors in fishery products are associated with autoxidation of lipids (77). In 1956-1957, Chipault (16, 17) reported the tentative identification of acrolein, ethanal, crotonaldehyde, butanal, pentanal, and heptanal in the steam distillate of oxidized menhaden oil. The oxidized oil was prepared by bubbling air through it for 16 hours at 100°C. The oil was then steam distilled for two hours at 200°C under vacuum and the distillate trapped in a dry ice bath. The tentative identifications were made on 2, 4-dinitrophenylhydrazine derivatives which were separated and compared to authentic hydrazones by paper chromatography. Chipault also suggested the presence of non-carbonyl oxygenated compounds as well as hydrocarbons.

Other workers have had more success in identifying volatile monocarbonyl compounds from autoxidizing salmon oil. Yu, Day, and Sinnhuber (89) and later Wyatt and Day (86) isolated and identified C_1 to C_{12} alkanals, C_4 to C_{12} alk-2-enals, and C_6 to C_{10} alk-2, 4-dienals by comparison of their hydrazones to authentic compounds. The carbonyls were isolated by the procedure of Day and Lillard (21).

Efforts to use hydrazones to identify carbonyls found in fish rather than fish oils met with limited success (55, 61). However,

Groninger (32) was able to identify acetoin, methanal, and ethanal in cod. Hughes (39, 40) identified ethanal, propanal, isobutanal, 2-methyl butanal, and acetone in heat processed herring.

Diemair and Schams (25) reported the identification of several carbonyl compounds in decomposed sea salmon, red perch, and herring fillets. These authors used low temperature-high vacuum fractionation and gas chromatography to isolate various fractions. The carbonyls were characterized by use of paper chromatography and infra-red and ultra-violet spectrophotometry of the dinitophenyl-hydrazine derivitives.

Gas Chromatography of Fish Volatiles: Investigations in 1958-1959 using gas chromatography on steam distillates of fresh and spoiled fish (31, 38, 41) failed to show the presence of compounds other than the methyl amines and ammonia found in the earlier work using chemical analysis (4, 5, 18, 28, 29). However, Mangan, Merritt, and Walsh (54) reported the use of a low temperature-high vacuum distillation procedure to concentrate volatiles for analysis by gas chromatography. Mass spectrometry was used to identify methanol, ethanol, ethanal, dimethyl sulfide, and trimethylamine.

A programmed cryogenic temperature gas chromatographic procedure for the separation of food volatiles was reported by Merritt and Walsh (63) in 1963. B, B'-oxydipropionitrile and squalane were used as column packings. In this procedure the column was placed in a bath of dry ice and ethanol (-65°C) and allowed to warm to room temperature as the compounds were eluted from the column.

The extension of this technique to the separation of volatiles from haddock was reported by Merritt and Mendelsohn (62). These authors reported that a high vacuum-low temperature distillation procedure for collection of volatiles proved more satisfactory than other sampling methods such as direct vapor injection. The total condensate of their distillation procedure was transferred to previously evacuated "U" traps as described by Bazinet and Walsh (3). The sample was injected into the gas chromatograph for programmed cryogenic temperature separation on the B, B'-oxydipropionitrile column. By this procedure these workers were able to show an increase in the number and size of peaks in the chromatograms of cooked haddock as compared to chromatograms of uncooked fish. Modification of this procedure allowed the use of the time of flight mass spectrometer to identify the peaks as they were eluted from the column. In this manner the volatiles from haddock stored for 18 days at 2°C were shown to contain ethanal, dimethyl sulfide, methanol, ethanol, trimethylamine, and trimethylamine oxide.

EXPERIMENTAL

Preparation of Samples for Head Space Analysis

To standardize the fish used in this study a large quantity of dover sole (Microstomus pacificus) and rockfish (Sebastodes sp.) was obtained and used throughout the investigation. The dover sole and rockfish had TBA numbers of 0.87 and 3.40 respectively. The TBA procedure was as described by Yu and Sinnhuber (88). Both species of fish were packed with a polyethylene inner wrap in five pound cartons and frozen by a commercial firm approximately three months prior to their analysis. The fish were held at -10°C until used.

The oysters (Ostrea gigas) used in this study were obtained fresh (less than three days old) in one pound jars from a local retail store. The tuna (Germo alalunga) was obtained fresh frozen from Astoria, Oregon, but was rancid and desiccated after several months storage at -10°C.

Fresh and Spoiled Fish: The fish used in this study were ground twice while still in the frozen state to insure a representative sample. Approximately two pounds were ground at one time and stored in air tight jars at -10°C until used.

Fish subjected to spoilage at various temperatures was prepared in a similar manner and stored at the desired temperature in a jar with an aluminum foil cover. Samples for analysis were taken from this main sample at the desired time intervals after a

thorough mixing. The temperatures for spoilage were 21°C (room temperature) and 1°C (refrigeration temperature).

Fish Frozen under Nitrogen and Air Atmospheres: Samples stored under nitrogen and air atmospheres to determine changes in volatiles due to oxidation were ground as previously described. Twenty grams were then placed in 250 ml erlenmyer flasks in a thin layer to expose as large a surface area as possible. Replacement of air with nitrogen in the frozen samples was accomplished by alternately evacuating and releasing the vacuum with nitrogen. The samples were stored for the desired time interval (22 days) at -10°C.

Fresh and Oxidized Fish Oils: Fresh fish oils were placed directly into the sampling flasks. Small oxidized menhaden (Brevoortia tyrannus) oil samples (approximately 20 ml) were removed at various time intervals from a larger oxidizing sample and tested for TBA numbers (88) and peroxide values (1, Cd 8-53). The remainder of the small sample was stored under nitrogen at -10°C until needed for gas chromatographic analysis.

The oxidation of the menhaden oil was accomplished by drawing air through approximately one liter of fresh clay bleached oil by means of a vacuum aspirator.

Sampling of Head Space Volatiles for Direct Injection

Fresh and Spoiled Fish and Oysters: These products were sampled for direct injection by placing a 20 g sample into a 250 ml erlenmyer flask equipped with a 24/40 ℉ joint. A 24/40 ℉ joint, modified to

accept a Barber Coleman 20 rubber septum, was then fitted to the Erlenmeyer flask making an air tight system. Three ml samples were withdrawn from the flask with a five ml syringe after the syringe was flushed two times with the vapors. The vapors were then injected directly into the chromatograph.

Ground glass syringes were used for all analyses and these were carefully cleaned with soap and water followed by a distilled water rinse. The washing was followed by drying in a 100°C oven as suggested by Buttery and Teranishi (10). All other glassware was handled in a similar manner.

Between repeated samplings of similar samples the syringes were not washed but were stored in a vacuum oven at 30 inches vacuum and at 70°C.

Cooked Fish and Oysters: These products were handled in the same manner as fresh and frozen fish but were heated for 30 min. in a boiling water bath prior to removing the volatiles for direct injection.

Fish Oils: These oils were sampled in the same manner as fresh and cooked fish with the exception that only one ml of oil was placed in a 100 ml tube with a 24/40 ℸ adapter and heated only ten min. in the boiling water bath. In most instances only one ml of vapor was injected into the chromatograph.

Operation of the Gas Chromatograph

Modification of the Gas Chromatograph: The gas chromatograph used in this study was an Aerograph, Model 600-B "Hy-Fi", with a hydrogen flame detector. This instrument was modified by adding a switch to turn off the oven fan, and a small aluminum foil hood to protect the flame head from air currents. These modifications were considered necessary due to the extreme sensitivities required. At an input impedance of 10^9, an attenuation of one, and an output sensitivity of 10x, air currents over the detector and inside the oven caused an unsteady baseline.

The recorder used was a Barber Coleman "Wheelco", model 8000-2700-J1, with one millivolt full scale sensitivity and one second full scale balancing speed.

Operating Parameters: The nitrogen flow rate used was 23 ml/min. at 20 pounds pressure with 26 ml/min. hydrogen flow at 12 pounds pressure (with a restrictor). These flow rates were measured by a soap bubble flow meter.

The air flow to the flame was approximately 300 ml/min. measured by water displacement in a 250 ml graduated cylinder.

The column temperature was 35°C in most instances but was raised to 95°C for some of the fish oil analyses.

The recorder chart speed was normally 15"/hr but was changed to 30"/hr or $7\frac{1}{2}$"/hr in instances where better separation of the early peaks was desired, or when peaks were eluted with extremely long retention times. All retention data was calculated

and reported at a basic chart speed of 15"/hr.

Preparation of Gas Chromatography Columns

Support Material: The solid support used in this investigation was Celite 545 (Johns Manville). The celite was prepared by a procedure similar to that of Farquhar et al. (30, p. 9). A 50 pound bag was size graded on a Tyler Ro-Tap shaker into 20/40, 40/60, 60/80, 80/100, 100/120, and 120/140 mesh sieve sizes. After sizing for five min. the fractions were soaked in 1 N NaOH for five to ten min., washed with distilled water to neutrality, soaked for five min. in 1 N HCl, washed to neutrality, and dried overnight in an oven at 100°C. The fractions were then resized by shaking again for five min.

After this initial acid-base treatment, part of the 80/100 mesh fraction was made slightly basic by soaking ten gram portions in 50 ml of 1 N methanoic KOH, filtering in a sintered glass filter, washing with 50 ml of methanol, filtering again, and finally drying in a vacuum oven at 70°C. This procedure is similar to that used by Farquhar et al. (30, p. 10). The celite prepared in this way was only slightly basic to neutral litmus when wetted with distilled water.

The methanoic KOH treated, 80/100 mesh, celite was used in this investigation with the exception of the preliminary investigations.

Coating the Support: The supports used in this investigation were coated using a procedure similar to that of Farquhar et al. (30, p. 10). The support was coated with the liquid phase by dissolving a weighed amount of the coating in diethyl ether and adding the proper amount of support to obtain the desired percent by weight of coating. Enough excess ether was then added to cover the celite and the mixture was stirred and partially dried in an air stream until no liquid was observed. The remainder of the solvent was removed under vacuum at 65°C with frequent mixing.

The column packing used in this investigation was 15% diisodecylphthalate on 80/100 mesh, methanoic KOH treated, Celite 545.

Packing Columns: The columns used were packed by plugging one end of a 1/8" stainless steel tube (laying flat on a table) with glass wool and attaching it to a vacuum aspirator with rubber tubing. The coated support was then added to the other end of the tube through a small funnel. The tube was vibrated until no more packing was observed entering the tube. The amount of packing added was determined by weighing the bottle of coated support before and after packing. The columns packed in this manner held approximately 0.4 g/ft.

The open end of the tube was then plugged with glass wool and then the column was coiled around a three inch diameter cardboard tube to make a smooth coil.

The columns used in this investigation were nine feet in length.

Conditioning Columns: All columns used in this investigation were conditioned in the chromatography oven for approximately three days at 120°C with a nitrogen flow rate of 25 cc/min. The conditioning was done before the column was attached to the detector.

Gas Chromatography of Known Compounds

The retention times of known compounds were obtained by injecting from one μL to one ml of vapor from the standard into the gas chromatograph. In cases where the known compounds were contaminated, plots of log of retention time against carbon number were made for a homologous series (37) to confirm the observed retention times.

Functional Group Analysis of Volatiles

Functional group analysis for carbonyls and hydrocarbons was performed by the method of Hoff and Feit (34). Confirmational checks on this procedure were made by analyzing known compounds in the same manner.

Evaluation of Data from Gas Chromatography Charts

Measurement of Retention Time: All peaks are reported by retention time measured in millimeters at a chart speed of 15"/hr and in minutes calculated from millimeters by dividing by 6.35. Measurement was accomplished by use of a ruler graduated in millimeters and a square. The measurements were taken from the injection point which was marked by attenuating momentarily to

cause a baseline deflection.

Comparison of Peaks Between Charts: The comparison of peaks between various charts and samples was accomplished by comparison of retention times and peak heights as well as visual observation of peak characteristics. Comparison of retention times alone was not considered a completely reliable comparison between peaks which eluted after several hours. Visual comparison of peak characteristics and sequence was used in these cases as small temperature changes caused large changes in retention times. Relative retention times (37) should compensate for these deviations but it was difficult to incorporate an internal standard into a vapor sample without disrupting the natural peaks. For this reason relative retention times were not used.

In comparisons of peak size, the use of peak height was found to be more convenient than peak area as unsteady baselines made calculation of areas difficult. Peak heights were calculated by multiplying the recorder response by the attenuation needed to keep the peak on scale. Full scale response is one millivolt.

Comparisons of peaks between samples was aided by giving each peak a number and using this number throughout the investigation. It should be noted, however, that even though two peaks may have the same visual characteristics and retention times, they may not be the same compound. In this investigation, peaks with the same retention time were assumed to be the same compound for the purpose of comparison.

Calculation of Peak Area: The extreme sensitivity used in this study made it difficult to obtain the steady baselines needed for accurate peak area calculation. Rough calculations were accomplished by assuming the peak to be a perfect isosceles triangle and using the formula for the area of such a triangle ($\frac{1}{2}$ base x height = area).

The calculation of areas of peaks from oxidizing menhaden oil was aided by injection of three ml vapor samples and reducing the sensitivity. This gave a much better baseline for area calculation and did not affect the resolution of the higher boiling components.

RESULTS AND DISCUSSION

Preliminary Investigations

Liquid Phases: Various liquid phases are used in the gas chromatographic analysis of food volatiles (Table 1). Several of these were examined in this study to determine their ability to resolve the components of fish volatiles at isothermal temperatures from 30 to 100°C. These temperature limits were chosen for several reasons: The lower limit because it was the lowest practical temperature obtainable on the instrument used, and the upper limit because the noise caused by column bleeding at higher temperatures made a steady baseline difficult to obtain.

The stationary phases examined are listed in Table 1. A complete systematic investigation of the effects of column temperature, sieve size of support, and type of support was not conducted with each stationary phase. However, further investigations were conducted if the stationary phase showed some separation of volatiles from heated fish at 10-15% coating on 80/100 mesh celite 545 at 50-60°C column temperature.

Four liquid phases gave good separation of volatiles (Table 1). Further investigation showed that diisodecyl phthalate gave the best separation and produced a steadier baseline at all temperatures. A coating of 15% diisodecyl phthalate was found to be most satisfactory. A coating of 6% was found to work well but only at temperatures of less than 30°C which was very difficult to obtain on the instrument used.

Table 1. Stationary phases[a] evaluated in preliminary investigations.

Stationary phases giving satisfactory[b] separation of fish volatiles	Stationary phases giving unsatisfactory separation of fish volatiles
Carbowax 400	Apiezon M
Diisodecyl phthalate	B, B'-oxydipropanitrile
Squalane	Carbowax 100
Ucon polar	Carbowax 20M
	Carbowax 1540
	Castorwax
	M-Phenyl ether
	Silicone SE-30
	Tetrahydroxyethylenediamine
	Versamide

[a] Available from Wilkens Instrument and Research, Inc., Box 313 Walnut Creek, California.

[b] Satisfactory separation in that at least 8 or 9 peaks were eluted using 10-15% coating on 80/100 mesh celite 545 at 50-60°C column temperature.

Solid Supports: It was found that 80/100 mesh celite 545 gave a better separation of volatiles than 60/80 or 100/120 mesh with the diisodecyl phthalate liquid phase. The 100/120 mesh required a high nitrogen pressure to obtain good flow rates while the 60/80 mesh did not have a small enough particle size to give good column efficiency.

Other types of 80/100 mesh supports were evaluated in an effort to reduce tailing of the peaks. Hexamethyldisilazane treated Chromosorb W (Johns Manville) did not appear to give better separation than plain celite. Celite 545 treated with methanoic KOH gave less tailing of fish volatiles and did not adsorb trimethylamine as readily as plain celite. Because of its reduced tailing

characteristics the methanoic KOH treated celite was used in this investigation.

Operating Parameters: The 15% diisodecyl phthalate column was evaluated under various conditions of temperature, nitrogen flow rate, and column length. A temperature of 35°C was found best for separation of fish volatiles but would not elute aldehydes with retention times longer than hexanal. These high boiling compounds were found in oxidized fish oils; therefore, the oil samples were also run at 95°C to allow better observation of these higher boiling components.

Eight, nine, and 12 foot columns were compared for ability to separate peaks in fish volatiles. It was found that nine foot columns gave better separation than eight foot columns but no advantage could be seen in columns longer than nine feet.

Various nitrogen flow rates were evaluated and approximately 23 ml/min. was found to give satisfactory separation. The same hydrogen flow was found to give maximum sensitivity with a minimum amount of noise due to overheating of the flame tip.

An air flow of 300 ml/min. was found to be optimum. Flows less than 250 ml/min. would not sustain the flame for extended periods and over 350 ml/min. caused an unsteady baseline.

Sampling Technique: After the nine foot 15% diisodecyl phthalate column was selected, the size of sample and method of sampling was evaluated. It was determined that the actual amount of sample in the sample flask was of little importance. In studies of fish,

20 g samples were used. Fifteen to 50 g samples would have been equally satisfactory as long as the sample size was held constant. In studies of fish oil, it was found that 0.5 to 10 ml would give approximately the same results. One ml samples were used in the studies on fish oils.

Comparisons of the size of vapor sample injected into the chromatograph indicated that up to three ml could be used satisfactorily. With three ml of vapor some peaks, which are eluted in the first few minutes, were not separated as well as with smaller samples. However, three ml samples gave more distinct peaks for the slower moving compounds.

It was found that highly oxidized oils could be sampled satisfactorily by injecting as little as one half ml of vapor into the chromatograph. In most cases, one ml vapor samples were used in studies of fish oil volatiles.

The effect of the sample temperature before injection was evaluated. With the exception of oxidized oils and highly spoiled fish, it was difficult to get a large enough concentration of volatiles into the vapor at room temperatures to give adequate detection. The chromatograms of products sampled in this manner had very few peaks even at the highest sensitivities.

It was observed that little difference could be detected in the chromatograms of cooked and uncooked fish when both were sampled at room temperature. However, cooked samples, sampled while still in the hot water bath, gave chromatograms showing many more peaks. This has been reported by several authors

(10, 44, 62, 73). This technique may produce peaks which are not originally present in the sample. However, all samples were compared to cooked controls whenever possible.

It was observed that the length of cooking time affected the number and size of peaks in both fish and oil. Because of this the heating times were standardized at 30 min. for fish and ten min. for oils. Hughes (40, p. 899) found a large increase in carbonyls of herring only after processing at 116°C for one hour.

A comparison of volatiles from fish cooked in various ways was made to determine the effect of the cooking procedure. Very little difference was noted between fish cooked in the 250 ml flask without the sample head attached and attached just before sampling; with the sample head attached during cooking; or with the sample head attached but flushed with nitrogen to remove air before cooking. Cooking with the sample head attached was the easiest method and seemed to offer less chance for contamination. The reproducibility of this technique was very good on sole cooked 30 min. This method of cooking was used throughout this investigation.

Gas Chromatography of Fresh, Cooked Products

Fish: A comparison of the volatiles from fresh, cooked dover sole and rockfish was made on the nine foot diisodecyl phthalate column at 35°C. Very little difference was observed in the chromatograms of these fish. Figure 1 shows a typical chromatogram of dover sole cooked 30 min. Table 2 lists the peaks in order of their elution with their retention times listed in millimeters (chart speed of

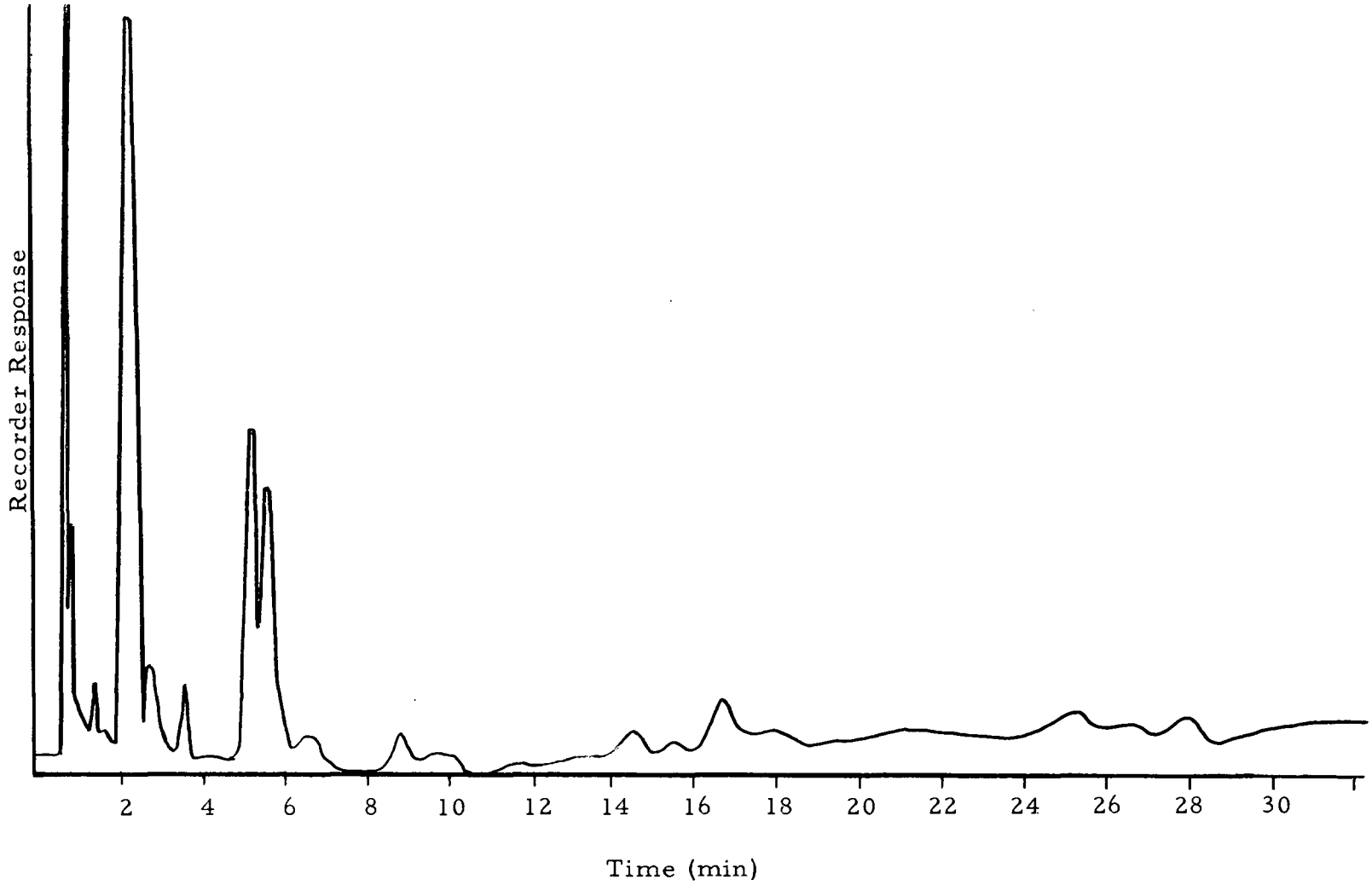

Figure 1. Typical chromatogram of fresh, cooked dover sole.

Table 2. Summary of chromatographic data from fresh dover sole and rockfish[a]

Peak No.	Sole Retention mm	Sole Retention min.	Sole Recorder Response	Rock fish Retention mm	Rock fish Retention min.	Rock fish Recorder Response
1	4.5	0.71	0.25	4.5	0.71	0.90
2	5.0	0.87	0.05	5.0	0.87	0.60
4	9.0	1.42	0.05	9.0	1.42	0.05
7	14.0	2.20	0.65	15.0	2.36	0.60
8	18.0	2.83	0.05	18.0	2.83	0.05
9	[b]---	---	---	20.5	3.23	0.05
10	23.0	3.62	0.10	23.0	3.62	0.10
12	33.0	5.20	0.30	33.0	5.20	1.70
13	35.0	5.51	0.60	shoulder ---		---
25	153.0	24.09	0.15	153.0	24.09	0.10

[a] run on 15% diisodecyl phthalate at 35°C
[b] dotted lines indicate no peak evident

Table 3. Summary of chromatographic data from fresh beef and oysters[a]

Peak No.	Oysters Retention mm	Oysters Retention min.	Oysters Recorder Response	Beef Retention mm	Beef Retention min.	Beef Recorder Response
1	4.5	0.71	1.00	4.5	0.71	0.50
2	5.5	0.87	0.15	6.0	0.94	1.10
3	[b]---	---	---	7.0	1.10	0.05
4	9.0	1.42	0.05	9.0	1.42	0.15
7	14.0	2.21	3.20	13.5	2.13	640.00
8	18.0	2.83	0.10	---	---	---
10	23.0	3.62	0.10	23.5	3.70	8.00
11	28.5	4.49	0.05	---	---	---
12	33.5	5.28	1.50	33.5	5.28	0.30
13	36.5	5.75	0.70	37.0	5.83	20.00
14	---	---	---	41.0	6.46	1.30
16	57.0	8.98	0.05	57.0	8.98	0.15
18	72.0	11.42	0.05	---	---	---
20	91.5	14.41	0.05	93.5	14.72	0.05
21	107.0	16.85	0.10	107.0	16.85	0.10

[a] run on 15% diisodecyl phthalate at 35°C
[b] dotted lines indicate no peak evident

15"/hr) and in minutes. The peak height was obtained by multiplying the observed peak height by the attenuation needed to keep the peak on scale. Full scale deflection is one millivolt.

It can be seen from these data that the only major difference between these chromatograms is in peaks 9 and 12. Peak 9 is not present in sole while peak 12 covers peak 13 in rockfish. The chromatogram of rockfish shows a shoulder after peak 12 but 12 was so large that it was not separated from peak 13.

Oysters and Beef: A chromatogram of fresh cooked Pacific oysters (Figure 2) shows a pattern of peaks very similar to those of sole and rockfish (Figure 1). Even cooked lean beef is similar in many respects to both fish and oysters (Table 3). Table 3 lists the peaks, retention times, and recorder response of the volatiles of beef and oysters run on the 15% diisodecyl phthalate column at 35°C.

The main differences between beef and oysters were in peaks 1, 3, 7, 8, 10, 13, 14, and 16. Notice particularly the large peak at 13.5 mm for lean beef (peak 7). It is so large that it covers peak 8.

From the data on fish, oysters, and beef it becomes apparent that the direct injection technique will show some differences between these products but these differences are not large.

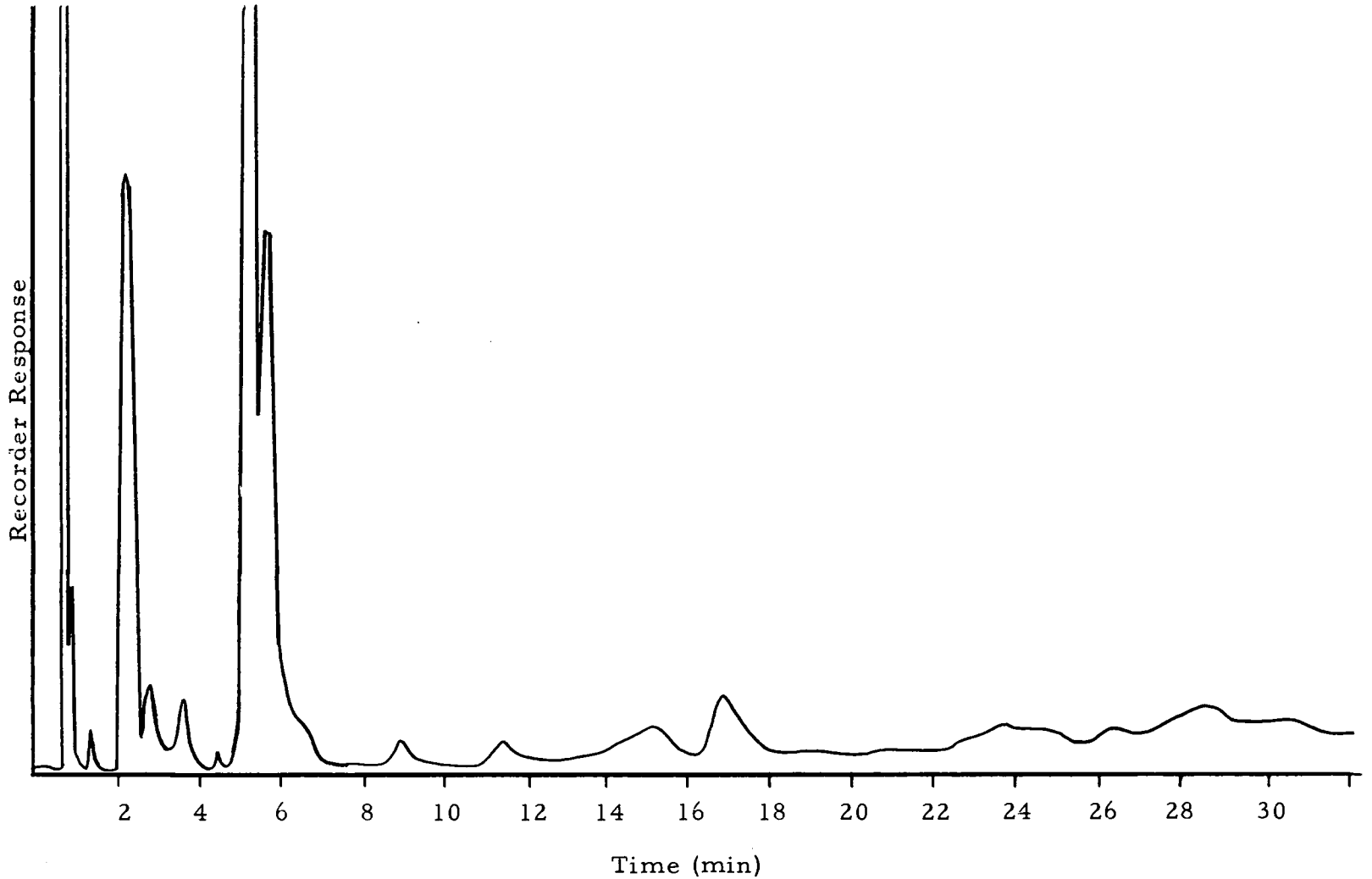

Figure 2. Typical chromatogram of fresh, cooked oysters.

Gas Chromatography of Spoiled and Oxidized Products

Spoilage of Dover Sole at Refrigeration Temperature: Table 4 shows the retention times and recorder responses of volatiles found in sole spoiled at 1°C for 295 hours. Very little change was observed in the odor or the chromatograms of the fish up to 79 hours with the exception of an increase in peak 13. However, after a longer period of time (295 hrs) some very marked changes were observed. The odor of the fish at 295 hours was not putrid but the fish would certainly have been considered inedible.

The most obvious change in the volatiles of this spoiled sole after 295 hours was the appearance of large peaks at 12, 36, and 55 mm (peaks 6, 13, 16). However, only peak 13 increased during the storage period before 79 hours. All other peaks remained essentially constant until 79 hours. The disappearance of peak 7 at 14 mm was probably due to its incomplete separation from the much larger peak 6.

Spoilage of Dover Sole at Room Temperature: The changes in volatiles of sole spoiled at room temperature (21°C) for 26 hours can be seen in Table 5. As would be expected, this fish spoiled much faster than the sole stored at refrigeration temperature and was noticeably spoiled at 21 hours. The first major change in the chromatograms appeared at 21 hours with peaks at 12, 41, and 113 mm (peaks 6, 14, and 22). Peak 14 seemed to increase with time but was covered at 21 hours by peak 6 at 12 mm. However, peak 7 at 14 mm can still be observed at 26 hours and is not covered as it

Table 4. Summary of chromatographic data from dover sole spoiled at refrigeration temperature (1°C).[a]

Peak No.	Retention Time		Recorder Response								
			0	5.5	21	26	43	55	67	79	295
	mm	min	hrs	hrs	hrs	hrs	hrs	hrs	hrs	hrs	hrs
1	4.5	0.71	0.20	0.50	0.45	0.45	0.20	0.30	0.40	0.20	0.20
2	5.5	0.87	0.10	0.10	0.10	0.05	0.10	0.10	0.05	0.05	0.05
4	9.0	1.42	0.05	0.05-	0.05	0.05 -	0.05	0.05	0.05-	0.05-	0.05-
6	12.0	1.89	[b] ---	---	---	---	---	---	---	---	1,000 +
7	14.0	2.20	3.20	3.20	3.20	2.00	2.80	4.40	3.60	2.40	---
8	18.0	2.83	0.10	0.10	---	---	0.05	0.10	0.10	---	---
10	23.0	3.62	0.20	0.20	0.50	0.40	0.50	0.50	0.60	0.40	1.60
12	33.0	5.20	0.20	0.40	0.40	0.30	0.50	0.70	0.80	0.50	0.40
13	36.0	5.67	0.80	0.90	2.60	3.20	6.40	10.40	11.20	7.20	41.60
14	40.0	6.30	0.10	0.10	0.10	0.05	0.20	0.30	0.80	---	---
16	55.0	8.66	0.05-	0.05	0.10	---	0.05	0.10	0.05	0.05	3.20
18	75.0	11.81	---	0.05	0.05	0.10	0.05	---	---	---	---
19	84.0	13.23	---	---	---	---	0.15	0.10	0.05	0.05-	0.05
20	93.5	14.72	---	---	---	---	---	0.05-	0.05-	0.05-	---
21	104.0	16.38	0.10	---	0.05	---	0.10	0.10	0.10	0.05-	---
23	127.0	20.00	---	---	---	---	---	---	---	---	---
24	137.0	21.57	---	---	---	---	---	0.05	0.05	---	---
25	153.0	24.09	---	---	---	---	---	0.05	0.05	---	---
26	179.0	28.19	---	---	---	---	---	0.05	---	---	---

[a] Run on 15% diisodecyl phthalate at 35°C

[b] Dotted lines indicate no peak evident

Table 5. Summary of chromatographic data from dover sole spoiled at room temperature (21°C)[a].

Peak No.	Retention Time		Recorder Response			
	mm	min	0 hr	5.5 hrs	21 hrs[b]	26 hrs[c]
1	4.5	0.71	0.50	0.70	0.40	0.10
2	5.5	0.87	0.10	0.15	0.05	0.05-
3	7.0	1.10	---[d]	---	0.05-	---
4	9.0	1.42	0.05	0.05	0.05-	0.05-
6	12.0	1.89	---	---	160 +	10,000 +
7	14.0	2.20	3.60	4.80	---	8.00
8	18.0	2.83	---	0.05	---	---
10	23.0	3.62	0.20	0.10	---	---
12	33.0	5.20	0.50	0.50	0.20	0.20
13	36.0	5.67	0.20	0.20	0.20	0.20
14	41.0	6.46	0.10	0.10	9.60	3.00
16	56.0	8.92	0.05	0.10	---	---
17	66.0	10.39	0.05-	---	---	---
19	84.0	13.23	0.05-	0.05	---	0.05-
20	93.0	14.65	0.05-	0.05	0.10	0.10
21	105.0	16.54	0.10	0.10	0.05-	---
22	113.0	17.80	---	---	0.10	0.05-
23	122.0	19.21	---	0.05-	---	---
24	133.0	20.94	---	0.05-	---	0.05-
25	152.0	23.94	0.05	0.05-	---	---
26	173.0	27.24	---	0.05-	---	---

[a] Run on 15% diisodecyl phthalate at 35°C

[b] Fish is spoiled and would be considered inedible

[c] Fish smells putrid

[d] Dotted lines indicate no peak evident

was at refrigeration temperature spoilage (Table 4).

It is of interest to compare the results of the sole spoiled at room and refrigeration temperatures. The main changes in fish spoiled at room temperature are in peaks 6, 8, and 14 while the main changes at refrigeration temperature are with peaks 6, 10, 13, and 16.

Spoilage of Rockfish at Room Temperature: Table 6 summarizes the retention and response data of peaks observed in the volatiles of rockfish spoiled under the same conditions as the sole at room temperature (Table 5). However, rockfish took 48 hours to spoil noticeably, while the sole took only 21 hours. The initial bacterial contamination was probably responsible for this.

Peak 6 appeared suddenly at 54 hours when the sample had a putrid odor. It is of interest to note that peak 7 at 14 mm is also present at this point as it was with the sole stored at room temperature but not at refrigeration temperature. However, these two peaks (peak 6 and 7) were so close together that they were difficult to separate.

Peak 14 at 42 mm is the only other major change in the rockfish observed during spoilage. This change was also observed in sole spoiled at room temperature but not at refrigeration temperature.

Oysters Stored at Room Temperature: The chromatograms of oysters stored six hours at room temperature (21°C) showed some differences from those of fresh oysters. Table 7 summarizes the

Table 6. Summary of chromatographic data from rockfish spoiled at room temperature (21°C)[a].

Peak No.	Retention Time		Recorder Response			
	mm	min	0 hrs	1 hr	48 hrs[b]	54 hrs[c]
1	4.5	0.71	2.00	2.40	2.40	2.00
2	5.5	0.87	0.20	0.20	0.20	0.20
4	9.0	1.42	0.05	0.05	0.05	0.05
6	12.0	1.89	---[d]	---	---	1,000 +
7	14.0	2.21	4.00	3.00	8 +	19.20
8	18.0	2.83	0.20	0.20	0.40	---
9	21.5	3.39	0.20	0.20	0.40	---
10	23.0	3.62	0.40	0.40	1.80	---
12	33.5	5.28	2.80	2.00	0.60	1.30
13	36.0	5.67	0.40	0.40	1.60	---
14	42.5	6.69	---	0.05	16.00	2.50
16	57.0	8.89	0.05	0.05	---	0.30
19	85.0	13.39	0.05	0.05	0.10	0.10
20	94.0	14.80	0.05	---	0.15	0.30
21	106.0	16.69	0.05	0.05	0.05-	---
22	115.0	18.11	---	---	0.30	0.10
24	137.0	21.57	0.05	0.05	0.05	0.10
25	152.0	23.94	---	0.05	---	---
26	173.0	27.24	---	0.05	---	---
27	186.0	29.29	---	---	0.05	0.05
30	223.0	35.12	---	---	---	0.05
33	309.0	48.66	---	0.05	---	---
35	349.0	54.96	---	0.05	---	---
36	360.0	56.69	---	---	0.10	0.10
39	655.0	103.15	---	---	---	0.10

[a] Run on 15% diisodecyl phthalate at 35°C

[b] Fish is spoiled and would be considered inedible

[c] Fish smells putrid

[d] Dotted lines indicate no peak evident

Table 7. Summary of chromatographic data from oysters stored six hours at room temperature (21°C).

Peak No.	Retention Time		Recorder Response	
	mm	min	0 hrs (Table 3)	6 hrs
1	4.5	0.71	1.00	1.00 +
2	5.5	0.87	0.15	0.30
4	9.0	1.42	0.05	0.08
7	14.0	2.21	3.20	4.80
8	18.0	2.83	0.10	0.20
10	23.0	3.62	0.10	0.20
11	28.5	4.49	0.05	0.05
12	33.5	5.28	1.50	2.40
13	36.5	5.75	0.70	1.00
14	42.0	6.61	[a] ---	0.10
15	44.5	7.01	---	0.10
16	57.0	8.98	0.05	0.10
18	72.5	11.42	0.05	---
19	84.5	13.31	---	0.80
20	93.0	14.63	0.05	0.80
21	106.0	16.69	0.10	1.50
24	137.0	21.57	---	1.20
25	154.0	24.25	---	0.60
26	181.0	28.50	---	0.80
31	239.0	37.64	---	0.40

[a] Dotted lines indicate no peak evident

data obtained from the chromatograms of oysters stored six hours and compares it to the data obtained from fresh oysters (Table 3).

The changes in the volatiles of the oysters stored six hours were not large. There was a general increase in size and number of peaks. The results show four well defined peaks at 84.5, 137, 154, 181, and 239 mm (peaks 19, 24, 25, 26, and 31) in the stored oysters that were not present in the fresh oysters.

Autoxidation of Menhaden Oil: A comparison of the chromatograms of menhaden oil at various levels of oxidation was made to determine the variations in the number and size of peaks using the direct injection sampling technique. Buttery and Teranishi (11) reported work in this area using potato granules stored in oxygen and were successful in showing some changes after several weeks storage.

Figure 3 shows a typical chromatogram of one ml of oxidized menhaden oil vapor run at 35°C on the 15% diisodecyl phthalate column. Table 8 gives a summary of the retention and response data for various levels of oxidation. A three ml vapor sample was used in this study to obtain a steadier base line for area calculations.

It can be seen from Table 8 that the size of many of the peaks is dependent on the degree of oxidation. The areas of several selected peaks were calculated to show the effect of level of oxidation on the peak area. These values are given in Table 9 and are presented graphically in Figure 4. It can be seen from Figure 4 that the areas of peaks 7, 19, 35, and 39 increase with hours of

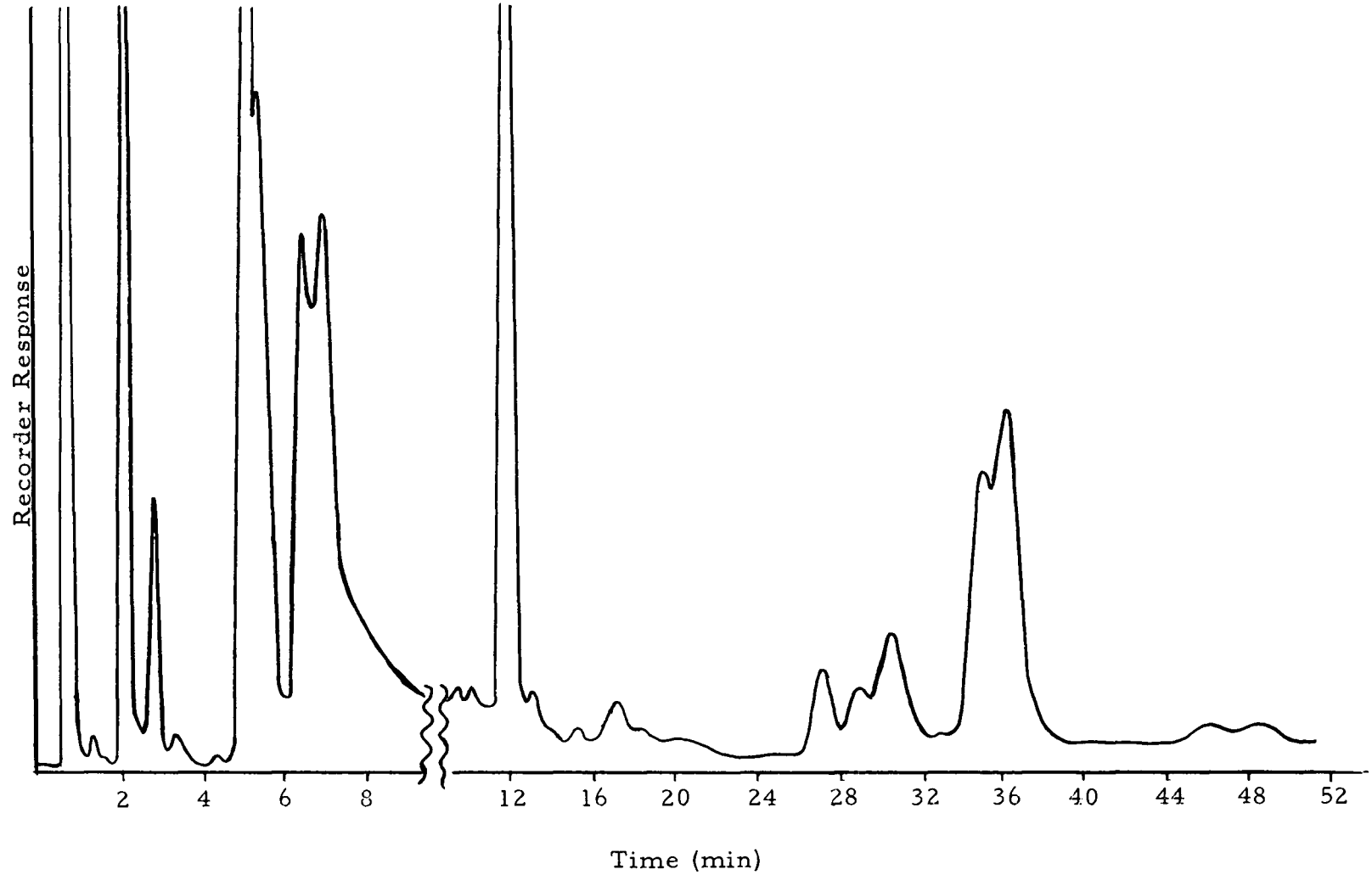

Figure 3. Typical chromatogram of oxidized menhaden oil.

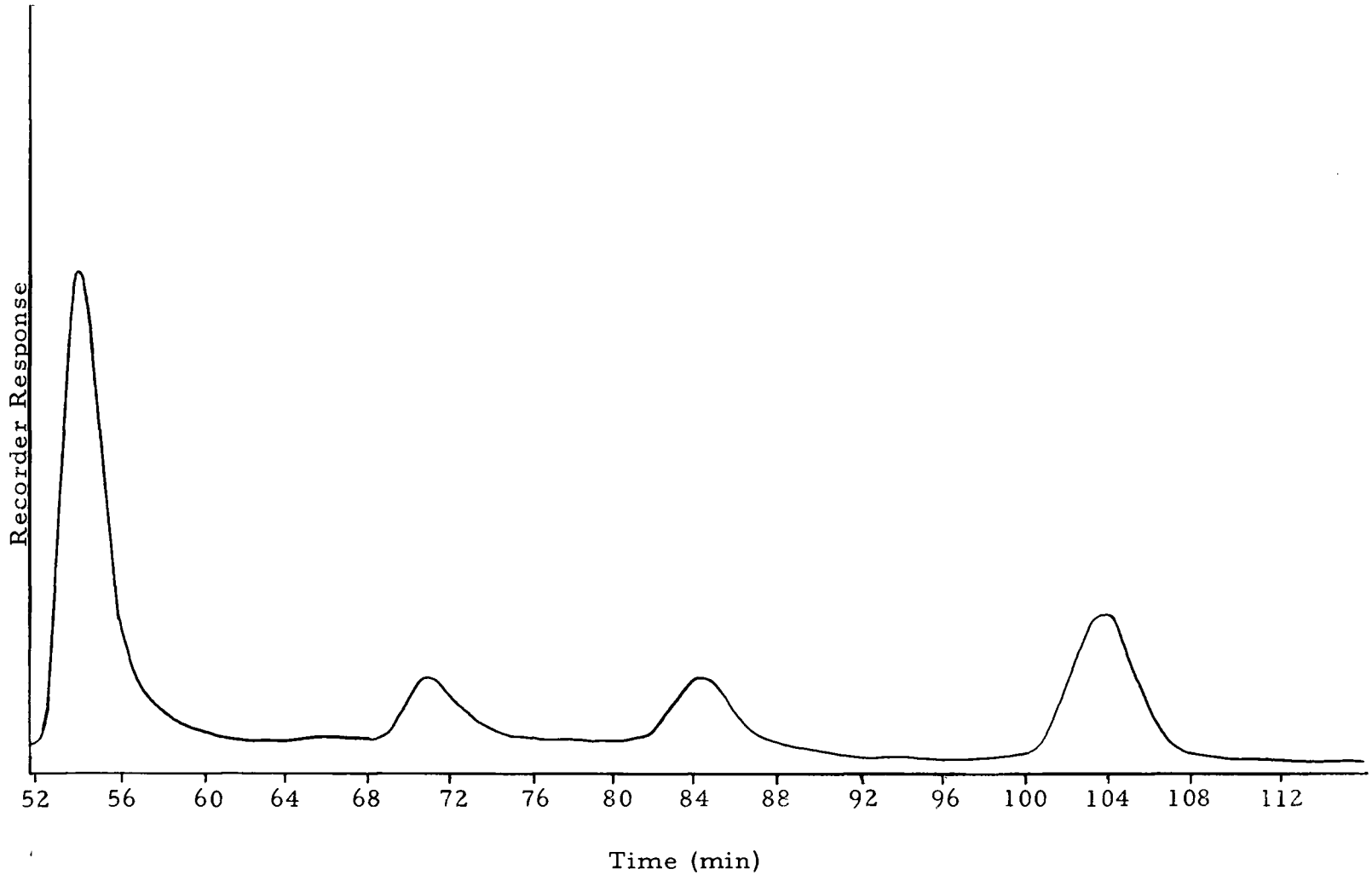

Figure 3. (Continued)

Table 8. Summary of chromatographic data from oxidizing menhaden oil. [a]

Peak No.	Retention Time		Recorder Response						
	mm	min	20 hrs	44 hrs	68 hrs	115 hrs	140 hrs	163 hrs	210 hrs
1	4.5	0.71	[b]---	0.20	0.60	5.20	8.64	13.20	20.00
2	5.5	0.87	1.56	0.90	1.60	7.20	9.12	12.16	24.00
4	9.0	1.42	---	0.04	0.08	0.16	0.32	0.32	1.20
7	14.0	2.20	1.40	2.32	2.48	6.40	9.44	12.16	16.80
8	18.0	2.83	0.24	0.40	0.78	3.76	4.80	7.04	9.80
10	23.0	3.62	0.12	0.12	0.32	0.72	0.96	1.12	2.00
11	29.0	4.57	---	0.04	0.04	0.08	0.16	0.16	0.20
12	33.0	5.20	4.16	8.32	8.80	24.96	44.80	66.56	86.40
13	35.0	5.51	8.48	19.84	17.28	32.00	60.80	81.92	94.40
14	42.0	6.66	---	---	---	---	---	---	---
15	44.0	6.93	0.08	0.28	0.24	0.40	0.88	1.20	1.90
19	82.0	12.91	0.20	0.28	0.40	1.00	1.60	2.24	3.80
21	107.0	16.85	0.04	---	---	0.08	0.12	---	0.10
22	116.0	18.27	0.04	0.08	---	0.08	0.12	0.16	0.30
24	134.0	21.10	0.08	0.08	0.20	0.12	0.12	0.24	0.50
26	178.0	28.03	0.20	0.48	0.36	0.36	0.20	0.32	0.40
27	191.0	30.08	---	---	---	---	---	---	---
28	200.0	31.50	0.12	0.36	0.20	0.36	0.60	0.72	1.10
30	228.0	35.91	0.16	0.32	0.32	1.00	1.60	1.92	3.10
31	234.0	36.85	---	---	---	0.68	1.32	1.76	3.10
32	291.0	45.83	---	---	---	---	---	---	---
33	307.0	48.35	---	---	0.12	---	---	---	---
35	344.0	54.17	0.28	0.68	0.80	2.16	3.40	4.40	6.40
36	389.0	61.26	---	---	---	0.12	---	---	---
37	454.0	71.50	---	---	---	0.20	0.20	0.32	0.60
38	538.0	84.72	---	---	---	0.16	0.28	0.40	0.50
39	658.0	103.62	---	---	0.08	---	0.24	0.32	0.50
40	1246.0	196.22	---	---	0.08	---	0.08	0.08	0.30

[a] Run on 15% diisodecyl phthalate at 35°C - 3 cc vapor sample.

[b] Dotted lines indicate no peak evident

Table 8. Continued

Peak No.	Recorder Response 234 hrs	338 hrs	362 hrs	415 hrs	554 hrs	650 hrs
1	36.00	40.00	40.00	36.00	44.00	64.00
2	38.40	28.00	33.00	28.00	---	52.00
4	1.60	1.60	1.60	1.60	0.80	1.60
7	22.40	19.20	23.20	23.00	29.60	52.00
8	14.40	10.40	10.40	8.80	8.00	13.60
10	2.40	1.60	1.60	1.60	0.80	1.60
11	---	----	0.40	0.40	0.40	0.80
12	102.40	96.00	112.00	104.00	104.00	204.80
13	96.00	80.00	104.00	89.60	88.00	137.60
14	---	----	----	----	----	3.40
15	1.80	----	2.10	1.90	2.00	3.60
19	4.30	4.20	6.00	5.80	6.20	6.00
21	0.20	0.05	0.10	0.10	0.10	0.10
22	0.40	0.30	0.30	0.30	0.30	0.30
24	0.40	0.10	0.10	0.10	0.10	0.10
26	0.60	0.30	0.40	0.40	0.50	0.50
27	---	----	----	----	0.30	0.40
28	1.00	0.80	1.20	0.10	0.90	0.80
30	3.20	2.70	3.40	2.90	2.90	1.80
31	3.60	3.00	3.60	3.20	3.10	2.20
32	---	----	0.10	0.10	0.10	0.10
33	---	----	0.10	0.10	0.10	0.10
35	6.00	4.50	5.90	4.90	4.30	3.00
36	---	----	0.10	0.10	0.10	0.40
37	0.60	0.60	0.80	0.70	0.50	0.40
38	0.80	0.60	0.70	0.70	0.50	0.40
39	0.60	0.70	0.90	0.90	1.10	1.00
40	0.40	0.40	0.70	0.50	0.30	0.20

Table 9. Areas of selected peaks from chromatograms of oxidizing menhaden oil.

Hours oxidation	Peroxide value	Peak No. 7 14 mm	Peak No. 19 82 mm	Peak No. 35 344 mm	Peak No. 39 650 mm
20	29.4	[b]241	120	640	---
44	38.6	420	184	1320	---
68	55.3	450	248	2380	---
115	251.0	1146	700	3750	360
140	415	1700	825	5900	900
163	505	2210	1120	7900	880
210	640	3600	1350	10800	1540
234	697	3960	2100	10800	1600
338	978	6960	1785	11100	1610
362	1033	[a]----	2140	[a]-----	2400
415	1050	----	2100	-----	2400
554	982	----	2250	-----	3300
650	1020	----	2250	10900	5000

[a] Areas marked ---- were not measured due to poor base lines.

[b] Areas are given in square millimeters at 15"/hr chart speed.

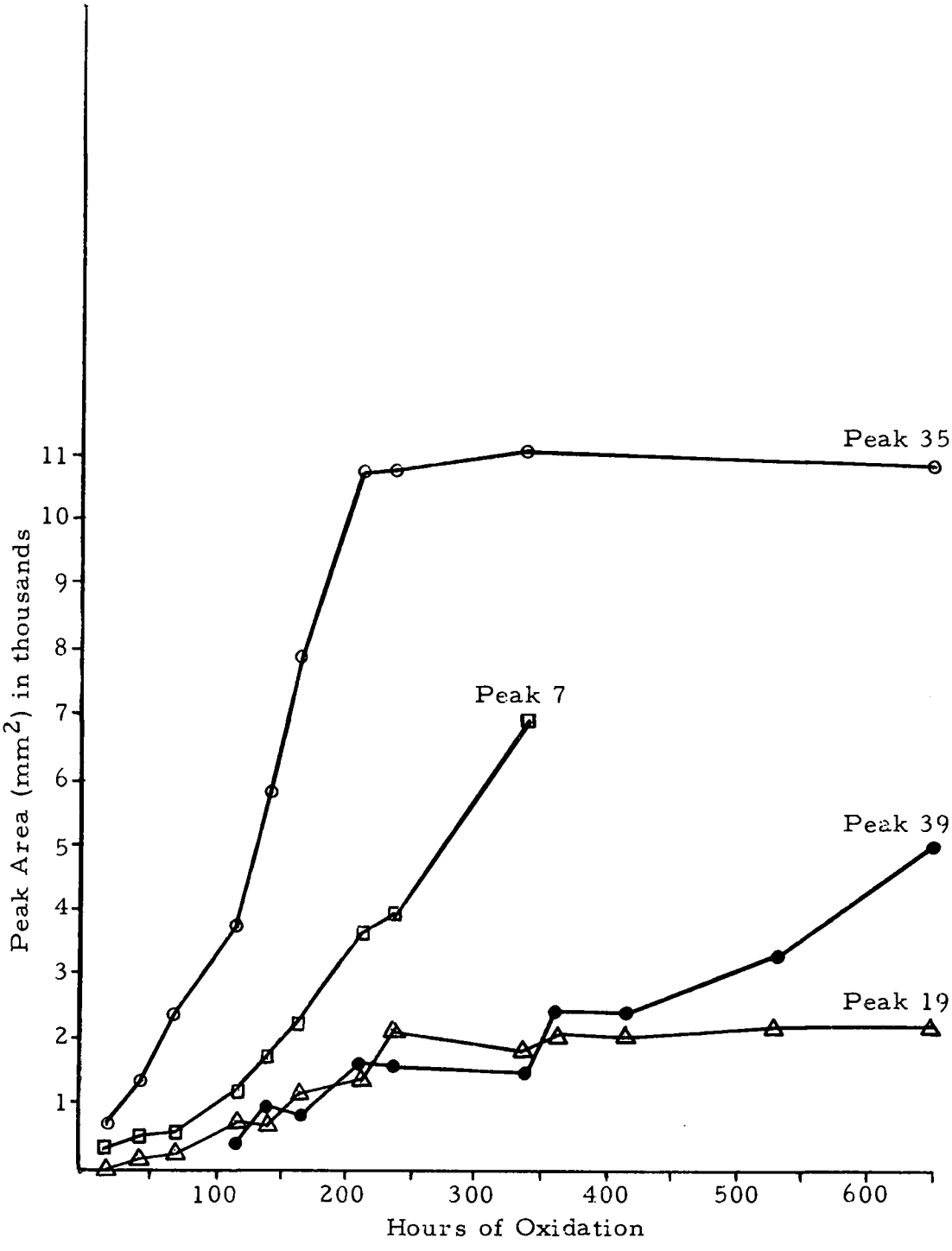

Figure 4. Change in peak areas of volatiles from oxidizing menhaden oil with hours of oxidation.

oxidation. Other investigators have noted a similar increase in the carbonyls of autoxidizing salmon oil (86, 89).

Oxidized Salmon Oil: Direct vapor analysis of salmon (Oncorhynchus sp.) oil, with a peroxide value of over 1,000, gave a chromatogram very similar to that of menhaden oil (Figure 3). Table 10 gives a comparison of the chromatograms of salmon oil to menhaden oil (POV = 1,000 +). The similarities between the volatiles of these two oils is obvious. The size of sample injected from the menhaden oil was three ml while only one ml of vapor from the salmon oil was used. The recorder response from the chromatogram of salmon oil was multiplyed by three in Table 10 to compensate for this difference in sample size.

Tuna Fish Stored at -10°C: While the chromatograms of fresh sole, rockfish, oysters, and even beef were very similar (Tables 2 and 3) the chromatograms of tuna appeared to be more like those of the oxidized oils. This would seem to confirm the earlier observation that considerable lipid oxidation had occurred in the tuna during storage. Table 11 shows a comparison of retention and response data from fresh sole (Table 2), menhaden oil (Table 10), and the tuna.

Oxidation of Dover Sole under Air and Nitrogen Atmospheres: Table 12 gives a summary of chromatographic data from sole stored 22 days at -10°C in both air and nitrogen atmospheres in an effort to produce chromatograms similar to those of tuna and oxidized oils.

Table 10. Summary of chromatographic data comparing oxidized menhaden and salmon oils[a]

Peak No.	Menhaden[b] Retention mm	min	Recorder Response x1	Salmon Retention mm	min	Recorder Response x1	x3
1	4.5	0.71	64.00	4.5	0.71	4 +	12 +
2	5.5	0.87	52.00	5.5	0.87	2.40	7.20
4	9.0	1.42	2.40	9.0	1.42	1.60	4.80
5	[c]---	---	----	10.5	1.65	0.08	0.24
7	14.0	2.21	52.00	13.5	2.13	19.20	57.60
8	18.0	2.83	14.40	18.00	2.83	1.44	4.32
10	23.0	3.62	1.60	21.5	3.39	0.48	1.44
11	29.0	4.57	0.80	28.00	4.41	0.32	0.96
12	33.0	5.20	204.80	32.5	5.12	225.00	675.00
13	35.0	5.51	137.60	34.0	5.35	37.4	112.20
14	42.0	6.61	0.50	41.0	6.56	0.16	0.48
15	45.0	7.09	1.50	44.0	6.93	0.16	0.48
17	68.0	10.71	0.10	---	---	---	----
18	72.0	11.34	0.20	72.0	11.34	0.08	0.24
19	82.0	12.91	1.20	83.5	13.15	1.68	5.04
20	91.0	14.33	0.60	92.0	14.49	0.63	1.89
21	107.0	16.85	0.20	---	---	---	----
22	116.0	18.27	0.60	116.0	18.27	0.48	1.44
24	134.0	21.10	0.20	135.0	21.26	0.08	0.24
26	178.0	28.03	1.20	179.0	28.19	1.60	4.80
27	192.0	30.24	0.80	---	---	---	----
28	200.0	32.00	2.00	204.0	32.13	0.40	1.20
30	228.0	35.91	3.30	230.0	36.22	0.64	1.92
31	234.0	36.85	4.20	236.0	37.17	0.64	1.92
33	302.0	47.56	0.20	---	---	---	----
34	318.0	50.08	0.20	---	---	---	----
35	344.0	54.17	6.00	339.0	53.39	2.80	8.40
36	----	----	---	371.0	58.43	0.40	1.20
37	454.0	71.50	0.80	458.0	70.13	0.08	0.24
38	538.0	84.72	0.80	537.0	84.57	0.08	0.24
39	658.0	103.62	2.00	653.0	102.83	0.40	1.20
40	1246.0	196.22	0.40	---	---	---	----

[a] Run on 15% diisodecyl phthalate at 35°C

[b] Sample size = 3 ml as compared to 1 ml for salmon oil

[c] Dotted lines indicate no peak evident

Table 11. Summary of chromatographic data comparing fresh dover sole, oxidized tuna, and oxidized menhaden oil[a]

Peak No.	Sole[b] Retention mm	Sole[b] Retention min	Sole[b] Recorder Response	Tuna Retention mm	Tuna Retention min	Tuna Recorder Response	Menhaden oil[c] Retention mm	Menhaden oil[c] Retention min	Menhaden oil[c] Recorder Response
1	4.5	0.71	0.25	4.0	0.71	32.00	4.5	0.71	64.00
2	5.0	0.79	0.05	5.5	0.87	9.28	5.5	0.87	52.00
4	9.0	1.42	0.05	9.0	1.42	25.60	9.0	1.42	2.40
7	14.0	2.21	0.65	14.0	2.21	9.76	14.0	2.21	52.00
8	18.0	2.83	0.05	18.5	2.91	1.40	18.0	2.83	14.40
10	23.0	3.62	0.10	23.0	3.62	3.00	23.0	3.62	1.60
11	[d] ---	---	---	28.5	4.49	0.08	29.0	4.57	0.80
12	33.0	5.20	0.30	33.5	5.28	6.00	33.0	5.20	204.80
13	36.5	5.75	0.60	---	---	----	35.0	5.51	137.60
14	---	---	---	43.0	6.77	1.80	42.0	6.61	0.50
15	---	---	---	---	---	----	45.0	7.09	1.50
16	---	---	---	57.0	8.98	0.12	---	---	----
17	---	---	---	---	---	----	68.0	10.71	0.10
18	---	---	---	72.5	11.42	0.06	72.0	11.34	0.20
19	---	---	---	84.0	13.23	0.20	82.0	12.91	1.20
20	---	---	---	92.0	14.48	0.10	91.0	14.33	0.60
21	---	---	---	106.0	16.69	0.02	107.0	16.85	0.20
22	---	---	---	117.0	18.43	0.20	116.0	18.27	0.60
23	---	---	---	126.0	19.84	0.01	---	---	----
24	---	---	---	137.0	21.57	0.05	134.0	21.10	0.20
25	153.0	24.09	0.15	153.0	24.09	0.20	---	---	----
26	---	---	---	181.0	28.50	0.60	178.0	28.03	1.20
27	---	---	---	---	---	----	192.0	30.24	0.80
28	---	---	---	---	---	----	200.0	32.00	2.00
30	---	---	---	---	---	----	228.0	35.91	3.30
31	---	---	---	239.5	37.64	0.07	234.0	36.85	4.20
32	---	---	---	290.0	45.67	0.15	302.0	47.56	0.20
34	---	---	---	---	---	----	318.0	50.08	0.20
35	---	---	---	356.0	56.06	0.15	344.0	54.17	6.00
37	---	---	---	---	---	----	454.0	71.50	0.80
38	---	---	---	---	---	----	538.0	84.72	0.80
39	---	---	---	---	---	----	658.0	103.67	2.00
40	---	---	---	---	---	----	1246.0	196.22	0.40

[a] Run on 15% diisodecyl phthalate at 35°C

[b] From Table 2

[c] From Table 10

[d] Dotted lines indicate no peak evident

Table 12. Summary of chromatographic data comparing dover sole stored 22 days in air and nitrogen to oxidized menhaden oil[a]

Peak No.	Sole stored in nitrogen			Sole stored in air			Oxidized in menhaden oil[b]		
	Retention		Recorder	Retention		Recorder	Retention		Recorder
	mm	min	Response	mm	min	Response	mm	min	Response
1	4.5	0.71	0.55	4.5	0.71	1.00	4.5	0.71	64.00
2	5.5	0.87	0.10	5.5	0.87	0.28	5.5	0.87	52.00
4	9.0	1.42	0.06	9.0	1.42	0.05	9.0	1.42	2.40
7	14.0	2.21	2.00	14.0	2.21	9.60	14.0	2.21	52.00
8	[c] ---	---	---	---	---	---	18.0	2.83	14.40
10	23.0	3.62	4.00	23.0	3.62	0.20	23.0	3.62	1.60
11	---	---	---	30.0	4.72	0.06	29.0	4.57	0.80
12	33.0	5.20	0.20	34.0	5.35	0.90	33.0	5.20	204.80
13	36.0	5.67	1.68	35.0	5.51	32.00	35.0	5.51	137.60
14	---	---	---	39.0	6.14	3.20	42.0	6.61	0.50
15	---	---	---	---	---	---	45.0	7.09	1.50
16	56.0	8.82	0.03	---	---	---	---	---	----
17	---	---	---	---	---	---	68.0	10.71	0.10
18	---	---	---	---	---	---	72.0	11.34	0.20
19	83.0	13.07	0.01	85.0	13.39	0.05	82.0	12.91	1.20
20	---	---	---	94.0	14.80	0.03	91.0	14.33	0.60
21	105.0	16.54	0.04	106.0	16.69	0.08	107.0	16.85	0.20
22	---	---	---	---	---	---	116.0	18.27	0.60
23	---	---	---	---	---	---	---	---	----
24	135.0	21.26	0.06	137.0	21.57	0.05	134.0	21.10	0.20
25	---	---	---	155.0	24.41	0.04	---	---	----
26	---	---	---	181.0	28.50	0.02	178.0	28.03	1.20
27	---	---	---	---	---	---	192.0	30.24	0.80
29	---	---	---	212.0	33.39	1.00	200.0	32.00	2.00
30	---	---	---	---	---	---	228.0	35.91	3.30
31	---	---	---	---	---	---	234.0	36.85	4.20
33	---	---	---	---	---	---	302.0	47.56	0.20
34	---	---	---	---	---	---	318.0	50.08	0.20
35	---	---	---	---	---	---	344.0	54.17	6.00
37	---	---	---	---	---	---	454.0	71.50	0.80
38	---	---	---	---	---	---	538.0	84.72	0.80
39	---	---	---	---	---	---	658.0	103.67	2.00
40	---	---	---	---	---	---	1246.0	196.22	0.40

[a] Run on 15% diisodecyl phthalate at 35°C

[b] From Table 10

[c] Dotted lines indicate no peak evident

Table 12 shows that not all the peaks of oxidized menhaden oil were produced in the sole stored in air. However, almost all the peaks from sole stored in air are larger than those of sole stored under nitrogen. Table 12 also shows that peaks at 39, 94, 155, 181, and 212 mm were found in the sole stored in air but were not found in sole stored in nitrogen.

Tentative Identification of Peaks Eluted from Oxidized Salmon Oil

Monocarbonyls: The volatile monocarbonyls from autoxidizing salmon oil have been investigated by Wyatt and Day (86). The C_1 to C_{12} alkanals, C_4 to C_{12} alk-2-enals, and C_6 to C_{10} alk-2, 4-dienals were identified by comparison of their hydrazones to the derivatives of known compounds. A chromatogram of oxidized salmon oil was obtained and compared to the retention data from a group of selected standards in an effort to tentatively identify some of the peaks. Table 13 summarizes the data from the chromatograms of oxidized salmon oil run at 35°C and compares it to the data obtained from the standards which is summarized in Table 15. Table 14 shows the data obtained from the chromatograms of oxidized salmon oil run at a column temperature of 95°C and compares it to the data obtained from the standards run under the same conditions. A functional group analysis for carbonyls and hydrocarbons was run by the method of Hoff and Feit (34). The results of this analysis are included in Tables 13 and 14.

Table 13. Summary of chromatographic data comparing oxidized salmon oil to known compounds (35[a]).

Peak No.	Oxidized salmon oil Retention mm	min	Functional group analysis (34)	Known compounds from Table 15
1	4.5	0.71	hydrocarbon	[a]methane (19), ethane (20)
2	5.5	0.87		
4	9.0	1.42		
7	14.5	2.28	carbonyl	ethanal (2)
8	19.0	2.99		
10	23.0	2.62		
11	30.0	4.72		methanal (1)
12	34.0	5.35	carbonyl	propanal (3), acetone (35)
13	37.0	5.83		
14	41.0	6.46		ethanol (15)
15	46.0	7.24		
16	51.0	8.03		
17	64.5	10.16		
18	71.0	11.18		
	76.0	11.97		
19	88.0	13.86	carbonyl	butanal (4)
20	96.0	15.20		
22	122.0	19.21	hydrocarbon	heptane (120)
24	142.0	22.36	hydrocarbon	
25	171.0	26.93		
26	188.0	29.61		
27	215.0	33.86	carbonyl	
	229.0	36.06		
30	shoulder		carbonyl	pentanal (34), 3-pentanone (27)
31	248.0	39.06		
	288.0	45.36		
	313.0	49.29		butanol (17)
34	331.0	52.13	hydrocarbon	
	342.0	53.86		
35	358.0	56.38		
	393.0	61.89		
37	440.0	69.29		
	458.0	72.13		
	484.0	76.13		
	542.0	85.35		
38	570.0	89.76		
39	696.0	109.61	carbonyl	hexanal (6)
	755.0	118.90		
	895.0	140.94		
	1314.0	206.92	carbonyl	4-heptanone (29)
	1462.0	230.23		
	1906.0	300.15		
	2696.0	424.40		
	3736.0	588.34		

[a] Numbers in parentheses refer to compound number in Table 15.

Table 14. Summary of chromatographic data comparing oxidized salmon oil to known compounds (95°C).

Peak No.	Oxidized salmon oil Retention mm	min	Functional group analysis (34)	Known compounds from Table 15
	20.0[a]	3.15		
	21.0	3.31	carbonyl	[b]butanal (4), glyoxal (35) methylvinyl ketone (31) diacetyl (33)
	25.0	3.94	hydrocarbon	heptane (23)
	31.0	4.88		
	34.0	5.35		
	37.0	5.83	carbonyl	3-pentanone (27), ethylvinyl ketone (32) pentanal (5)
	40.0	6.30		butanol (17)
	45.0	7.09		
	55.0	8.66		
	59.0	9.29		
	63.0	9.92	carbonyl	
	72.0	11.34	carbonyl	2-hexanone (24), acetoin (72)
	87.0	13.70	carbonyl	hexanal (6)
	101.0	15.91		
	127.0	20.00	carbonyl	4-heptanone (29)
	138.0	21.73	carbonyl	2-hexen-1-al (38)
	143.0	22.52	carbonyl	
	168.0	26.46	carbonyl	heptanal (7)
	195.0	30.71		
	208.0	32.76		
	215.0	33.86		
	250.0	39.37		
	287.0	45.20	carbonyl	
	306.0	48.19		
	351.0	55.28	carbonyl	

[a] Peaks appearing before 20.0 mm were not resolved at 95°C.

[b] Numbers in parentheses refer to the number of the compound in Table 15.

Table 15. Summary of retention data from chromatograms of known compounds (35 and 95°C).[a]

Standard	Retention Time 35°C		95°C	
	mm	min	mm	min
Alkanals				
1 methanal	29.0	4.57	13.0	2.05
2 ethanal	14.0	2.20	7.3	1.15
3 propanal	34.0	5.35	11.8	1.85
4 butanal	83.5	13.15	20.0	3.15
5 pentanal	239.0	37.64	40.0	6.30
6 hexanal	661.0	104.09	80.0	12.60
7 heptanal	1860.0	292.91	161.0	25.35
8 octanal	----	----	344.0	54.17
9 nonanal	----	----	709.0	111.65
10 decanal	----	----	1428.0	224.88
11 isopentanal	151.5	23.86	29.0	4.57
12 isobutanal	55.0	8.66	15.5	2.44
13 2-methyl pentanal	411.0	64.72	58.0	9.13
Alkanols				
14 methanol	32.0	5.04	10.0	1.57
15 ethanol	41.5	6.54	12.0	1.89
16 propanol	113.0	17.80	20.5	3.23
17 butanol	320.0	50.39	41.0	6.46
18 heptanol	----	----	356.0	56.06
Hydrocarbons				
19 methane	4.0	0.63	4.5	0.71
20 propane	5.0	0.79	5.5	0.83
21 butane	8.5	1.34	6.5	1.02
22 hexane	43.5	6.85	13.5	2.13
23 heptane	120.0	18.90	24.0	3.78
Ketones				
24 2-hexanone	600.0	94.49	73.0	11.50
25 2,4-dimethyl-3-pentanone	494.0	77.80	67.0	10.55
26 acetoin	----	----	72.0[b]	11.34
27 3-pentanone	233.0	36.69	38.0	5.98
28 3-methyl-2-butanone	158.0	24.88	29.0	4.57
29 4-heptanone	1246.0	196.22	123.0	19.37
30 acetone	35.0	5.51	11.5	1.81
31 methylvinylketone	96.0	15.12	21.0	3.31
32 ethylvinyl ketone	228.0	35.91	38.0	5.98

[a] Run on 15% diisodecyl phthalate

[b] Badly contaminated - questionable retention time.

Table 15. Continued

Standard	Retention time 35°C mm	35°C min	95°C mm	95°C min
Diones				
33 diacetyl	104.0	16.38	22.0	3.46
34 2,4-pentan-dione	----	----	96.5[a]	15.20
35 Glyoxal	95.0[b]	14.96	22.0[b]	3.46
36 2,5-hexane-dione	----	----	307.0	48.35
37 2,3-heptane-dione	----	----	147.0[b]	23.15
Enals				
38 2-hexen-1-al	----	----	137.0	21.57
Amines				
39 trimethyl-amine	12.0	1.89	8.0	1.26
40 dimethyl-amine	----[c]	----	----	----
41 methylamine	----[c]	----	----	----
42 ethylamine	----[c]	----	----	----
43 ammonia	----[d]	----	----	----

[a] Tails badly - doubtful retention time

[b] Badly contaminated - doubtful retention time

[c] Adsorbed and tails badly - retention time unobtainable

[d] Not detectable by flame detector

It can be seen from Table 13 and 14 that the alkanals from C_1 to C_7 can be tentatively identified by retention data and functional group analysis. Another monocarbonyl which might be present is 2-hexen-1-al as the chromatogram of the salmon oil shows a carbonyl peak at 95°C which has a retention time similar to that of this alkenal.

It should be noted that the tentative identifications of the monocarbonyls are supported by the work of Wyatt and Day (86). However, it should also be noted that the peak corresponding to methanal did not appear to give a positive carbonyl functional group test; the peak for propanal could also have been acetone; and the peak tentatively identified as pentanal is not well separated and could very well be 3-pentanone. Further investigation is needed before identification of these compounds can be made with confidence.

Miscellaneous Compounds: The hydrocarbons which can be tentatively identified by retention data and function group analysis are methane and heptane. However, it is difficult to tell if the peak at 4.5 mm was methane or ethane as they have approximately the same retention time at 35°C column temperature. It is possible that both methane and ethane are present as the peak at 4.5 mm does appear to be two peaks at a column temperature of 8°C.

Other compounds which have retention times similar to peaks found in the volatiles of oxidized salmon oil are acetone, ethanol, 3-pentanone, butanol, and 4-heptanone. Although the

alcohols were not specifically tested for by functional group analysis, the peaks tentatively identified as ethanol and butanol did not appear to be carbonyls or hydrocarbons.

Peaks Reoccurring in All Chromatograms of Fish and Oils

In looking at the various chromatograms and tables it becomes apparent that several peaks are common in various intensities to fresh, spoiled, and oxidized fish as well as oxidized and fresh oils. A list of these peaks and their retention times is shown in Table 16.

The peaks which seem to be common to all products sampled are peaks 1, 2, 4, 7, 8, 10, 12, and 13. In the few cases where these peaks were not apparent the preceding or following peak was so large that it obscured the missing peak.

It is also interesting to note that one peak was observed only in spoiled fish (Tables 4, 5, and 6). This was peak 6 at 12 mm and could very well have been trimethylamine which has a retention time of 12 mm (Table 15, No. 39) at a column temperature of 35°C.

Table 16. Summary of chromatographic data from peaks re-occurring in chromatograms of all products.[a]

Peak No.	Retention mm	Retention min
1	4.5	0.71
2	5.5	0.87
4	9.0	1.42
7	14.0	2.21
8	18.0	2.83
10	23.0	3.62
12	33.0	5.20
13	35.0	5.51

[a] Run in 15% diisodecyl phthalate at 35°C

SUMMARY AND CONCLUSIONS

The volatiles of fresh, spoiled, and oxidized fish and fish oils were successfully separated and compared by direct injection gas chromatography. A nine foot column of 15% diisodecyl phthalate on 80/100 mesh, methanoic KOH treated, celite 545 operated isothermally at 35°C was used.

It was found that injection of one to three ml of vapor from the heated product directly into the gas chromatograph gave satisfactory separation. Only in the case of highly spoiled or oxidized products was direct sampling of vapors from cold products possible.

Several peaks found in autoxidizing menhaden oil were shown to increase with the degree of oxidation of the oil.

It was observed that spoiled fish developed several peaks not present in fresh fish, but only after the product was considered inedible. One peak had a retention time very similar to that of trimethylamine.

Tentative identification of some monocarbonyls found by Wyatt and Day (86) in oxidized salmon oil was made by comparison of retention data at 35 and 95°C column temperature and functional group analysis by the method of Hoff and Feit (34). Peaks with retention times similar to the alkanals C_1 to C_6 and C_4 to C_7 were observed at 35 and 95°C respectively. The dienal, 2-hexen-1-al, was tentatively identified at 95°C column temperature.

The hydrocarbons methane and heptane were also tentatively identified. Other compounds which may have been present were acetone, ethanol, and butanol.

A comparison of chromatograms from all products show that several peaks appear in varying intensities in almost every product investigated. These peaks were 1, 2, 4, 7, 8, 10, 12, and 13 at 4.5, 5.5, 9.0, 14.0, 18.0, 23.0, 33.0, and 35.0 mm respectively (15"/hr chart speed).

The following conclusions were drawn from the results of this investigation:

1. Satisfactory separation of volatiles from heated fishery products is possible by direct injection of one to three ml of vapor using a nine foot column of 15% diisodecyl phthalate on 80/100 mesh, methanoic KOH treated, Celite 545 operated isothermally at 35°C. However, higher boiling compounds with retention times longer than hexanal can be satisfactorily eluted only at higher column temperatures such as 95°C.
2. The methods and conditions described in this investigation will show differences between the volatiles of fresh and badly spoiled fish and fresh and oxidized oils. However, pronounced differences between species of fish or types of meat with pronounced differences in odor are not as easily detected.

3. The direct injection technique described in this thesis will detect increases in peak area, with increases in degree of oxidation, for compounds found in the volatiles of oxidizing menhaden oil.

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