Recovery and Measurement of Volatiles from Lipids

Hydrocarbons in Irradiated Fats

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Techniques are described for the isolation and quantitative measurement of a wide range of volatile compounds in lipids. The higher-boiling compounds are collected by cold-finger vacuum distillation and their quantities in the fat are determined by relating their gas chromatographic peak areas to that of an appropriate internal standard. The lower-boiling compounds are recovered on a short precolumn packed with stainless steel helices, which can be fitted inside the gas chromatographic oven before the inlet of an alumina column. Recovery data are given for a series of hydrocarbons from C_1 to C_{22}. The methods described in this report were used mainly for the measurement of hydrocarbons in a number of irradiated fats, but can also be applied for the analysis of other classes of compounds.

Several investigators have been interested in the analysis of trace volatile components, present or formed in lipid systems, because of their significance from the standpoint of flavor or public health and their value in explaining certain reaction mechanisms. The various techniques which have been employed for the isolation of volatiles from fat include steam distillation (Chang, 1961; Day and Lillard, 1960; Lea and Swoboda, 1962; Patton and Tharp, 1959), sweeping with inert gas (Nawar and Fagerson, 1962), headspace analysis (Buttery and Teranishi, 1963; Nawar, 1966; Nawar *et al.*, 1962), vacuum distillation (de Bruyn and Schogt, 1961; Merritt *et al.*, 1959) and chemical derivatization (Schwartz *et al.*, 1963).

Although high-vacuum distillation methods appear to offer the most efficient approach to the separation of these compounds from fat (Forss and Holloway, 1967), the quantitative measurement of such compounds is frustrated by a lack of reproducibility and a significant difference in recovery of the individual components present. While the short chain compounds (C_1 to C_8) suffer from partial escape prior to analysis or from incomplete condensation in conventional cold traps, the higher molecular weight members of the volatile spectrum give increasingly lower distillation recoveries as their solubility in fat increases and their vapor pressure decreases.

Angelini *et al.* (1967) obtained 100% yields of the C_{3-8} methyl ketones from butterfat after 8 hours of vacuum distillation at 45° C., but recovery fell rapidly from nonanone (85%) to dodecanone (only 2%). Forss and Holloway (1967) recovered less than 50% of the methyl ketones above C_8 and of the alcohols above C_5 by high vacuum degassing of butter oil at 50° C. for several hours, but better recoveries were obtained when vacuum degassing was followed by cold-finger molecular distillation.

Merritt and coworkers (1966, 1967) employed a glass break-seal assembly and a sample splitting apparatus for the collection of volatiles from meats and irradiated fats. They resorted to subambient temperature-programmed gas chromatography for the separation of these compounds and used the intensity of mass spectral peaks at m/e 43 as their quantitative criterion. These workers identified a series of hydrocarbons, but recognized the limitations of their method, since compounds with boiling points above 250° C. could not be detected.

This article describes the methods used in the course of an over-all study on the radiolysis of lipids.

EXPERIMENTAL

The term volatiles is used here to describe those compounds which are separated from the sample by the specified vacuum distillation technique. Because the compounds investigated had a wide range of boiling points—from about −100° to above 300° C.—it was practical to divide the volatiles into two groups—i.e., lower-boilers with boiling points up to about 150° C., and higher-boilers, which have higher boiling points. The apparatus and procedures are dealt with separately for each group.

Since hydrocarbons appear to be the major radiolytic products of fats (Champagne and Nawar, 1968; Dubravcic and Nawar, 1968; Khatri *et al.*, 1966; Merritt *et al.*, 1966), a group of normal alkanes and alkenes ranging from C_1 to C_{22} was selected to represent a wide spectrum of volatile compounds.

HIGHER-BOILING COMPOUNDS

Apparatus and Isolation Procedure. The system used for collection of the higher-boiling compounds is a modification of the cold-finger apparatus described by

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de Bruyn and Schogt (1961). As shown in Figure 1, the sample flask was fitted with a cold-finger (F) which extends upwards into a funnel-shaped reservoir (R) to accommodate a relatively large volume of liquid nitrogen. Contamination from the vacuum pump was prevented by inserting two liquid nitrogen traps between the distillation flask and the pump. A known amount of an appropriate internal standard was added to the sample of oil, which varied from 1 to 20 grams. The distillation was carried out at 10^{-3} torr for 2 hours, during which the sample was maintained at 80° ± 1° C. and stirred continuously by means of a magnetic stirrer. At the end of the distillation period, vacuum was released by disengaging the spherical joint (S) and the cold-finger part was removed for quick rinsing with 10 ml. of diethyl ether. The solution was concentrated at room temperature under a gentle stream of nitrogen to approximately 0.5 ml. for gas chromatographic and mass spectral analysis.

Blank distillations with no sample in the apparatus, as well as control runs with unirradiated oil samples, were carried out regularly to ensure the absence of interfering impurities.

Separation and Identification. The solution obtained by cold-finger distillation was analyzed by flame ionization gas chromatography (GC) on a variety of columns. The following columns were efficient in the separation of a wide range of hydrocarbons and other radiolytic products: a 12-foot × ⅛-inch Carbowax 20M, 15% on 60- to 80-mesh Gas-Chrom P; a 6-foot × ⅛-inch silicone rubber SE 30, 10% on Chromosorb W; a 200-foot × 0.02-inch Carbowax 20M capillary; and a 200-foot × 0.02-inch DEGS (LAC 728) capillary. The oven temperature was programmed from 60° to 200° C. at 2° per minute.

For quantitative analysis, the total column effluent was passed through the flame ionization detector. For identification, however, the effluent was split and a portion was admitted through a heated line to the ion source of a Hitachi Perkin-Elmer RMU-6A mass spectrometer. Identification of unknowns was based on the agreement of mass spectra and GC retention times with those of authentic compounds.

Recovery. The efficiency of distillation for the saturated and unsaturated normal alkanes and alkenes from C_9 to C_{22} was evaluated by calculating the recovery of these compounds from a lipid phase. One milligram of each authentic compound was added to 10 grams of previously stripped corn oil, and the mixture distilled as described. On injection of an aliquot of the concentrated distillate into a Carbowax column, the areas of GLC peaks were compared with the corresponding peak areas obtained by direct injection of the known mixture into the gas chromatograph. The results shown in Table I are averages from five separate distillations. The coefficient of variation for the recovery of individual compounds ranged from 0.04 to 0.09.

Quantitative Measurement. A known quantity of an internal standard was added to the sample prior to distillation to achieve reproducibility of quantitative analysis. GLC peak areas of the compounds being determined could then be related to that of the internal standard. This method compensates for errors which may arise from variations in distillation recovery, GC response, size of sample injected, etc.

Table I. Recovery of the Higher-Boiling Hydrocarbons

Compound[a]	%	Compound	%	Compound	%
9 *A*	90	12 *Y*	98	16 *E*	98
9 *E*	85	13 *A*	98	17 *A*	91
10 *A*	90	13 *E*	98	17 *E*	94
10 *E*	85	14 *A*	99	18 *A*	81
11 *A*	96	14 *E*	99	18 *E*	69
11 *E*	95	15 *A*	98	19 *A*	60
12 *A*	98	15 *E*	99	20 *A*	45
12 *E*	98	16 *A*	99	22 *A*	12

[a] Numericals indicate the number of carbon atoms. *A*= alkane, *E*= alkene, *Y*= alkyne.

Table II. Conversion Factors for the Higher-Boiling Hydrocarbons

Compound[a]	*f*	Compound	*f*	Compound	*f*
9 *A*	1.43	13 *A*	0.99	17 *A*	0.99
9 *E*	1.50	13 *E*	1.06	17 *E*	1.11
10 *A*	1.26	14 *A*	0.99	18 *A*	1.22
10 *E*	1.29	14 *E*	1.06	18 *E*	1.55
11 *A*	1.12	15 *A*	0.99	19 *A*	1.77
11 *E*	1.06	15 *E*	1.05	20 *A*	2.93
12 *A*	0.99	16 *A*	0.99		
12 *E*	1.06	16 *E*	1.05		

[a] Numericals indicate the number of carbon atoms. *f*= conversion factor, *A*= alkane, *E*= alkene.

After screening a number of compounds, 6-dodecyne was found to be suitable as an internal standard for the analysis of radiolysis products in fats. A conversion factor for each of the individual compounds was established by the distillation of the authentic compound mixture and the internal standard from fresh stripped fat. The data are presented in Table II. The weight of each hydrocarbon in the irradiated sample can be calculated according to the following formula:

$$\text{Mg. hydrocarbon} = \frac{\left(\begin{array}{c}\text{hydrocarbon}\\ \text{peak area}\end{array}\right) \times \left(\begin{array}{c}\text{conversion}\\ \text{factor}\end{array}\right) \times \left(\begin{array}{c}\text{mg. internal}\\ \text{standard}\end{array}\right)}{\text{peak area of internal standard}}$$

In cases where the sample contains relatively high-boiling compounds (as in irradiated fish oil), it is preferable to include a second internal standard of a higher molecular weight.

LOWER-BOILING COMPOUNDS

Apparatus and Isolation Procedure. The samples to be analyzed for low-boiling compounds were irradiated in the special ampoules shown in Figure 2. The irradiated samples were first warmed to a temperature slightly above their melting point to liquefy the fat and then frozen again in an inverted position with the narrow end down (B). On opening of the ampoule (at C) the frozen fat acted as its own seal, preventing the escape of low-boiling compounds. Before melting the fat, the inverted ampoule was inserted into the distillation flask, (D), which was then flushed with nitrogen and attached to a special precolumn, (P), by means of swagelock fittings. (S). The precolumn consisted of an 8- × ¼-inch stainless steel tube packed with 1/16-inch stainless

steel helices and fitted on both sides with high-vacuum, temperature-resistant valves, (V). The flask with the ampoule was then gently warmed to melt the fat. Gradual application of vacuum resulted in the discharge of the sample from the ampoule into the distillation flask. After a vacuum of 10^{-3} torr was reached, collection of the volatiles on the precolumn was continued for 1 hour, during which the sample was maintained at 70° C. and the precolumn immersed in liquid nitrogen.

Separation and Identification. After completion of the collection period, the valves on both sides of the precolumn were tightly closed to prevent the lower molecular weight components, particularly the gaseous compounds, from escaping. The precolumn assembly was then disconnected from the distillation apparatus and immediately fitted, by means of swagelock fittings, between the injection port of the gas chromatograph and the inlet of a 12-foot × ⅛-inch column packed with 60- to 80-mesh activated alumina, type F-1 (Figure 3). The carrier gas flow was resumed after opening the precolumn valves and closing the oven. The oven temperature was programmed from 60° to 350° C. at a rate of 15° per minute. Separation of hydrocarbons on alumina was originally suggested by List *et al.* (1965). For identification, a portion of the GC effluent was introduced into the mass spectrometer as described above for the higher-boilers.

Recovery. Three-tenths of a microliter of each of the normal alkanes C_5 to C_{10} and 7 μl. of the gaseous hydrocarbons C_1 to C_4 were added to 10 grams of previously stripped corn oil in an ampoule similar to that shown in Figure 2. The volatiles were recovered on the precolumn and analyzed as described. The areas of GC peaks were compared with the corresponding peak areas obtained by direct injection of the known mixture into the gas chromatograph. Average recovery values from five separate tests were as follows (in per cent): C_2 94, C_3 103.8, C_4 113.0, C_5 100.5, C_6 100.2, C_7 100.4, C_8 101.9, C_9 99.0, and C_{10} 102.3. The coefficient of variation for recovery was less than 0.05 for the C_4 to C_{10} compounds and less than 0.08 for the C_2 and C_3 compounds. Recovery of the lower-boiling compounds was almost complete. This method is not suitable, however, for the recovery of methane. The vapor pressure of the latter is too high for adequate trapping on the cooled helices. Complete recovery of methane can be achieved if the precolumn is packed with alumina rather than the stainless steel helices.

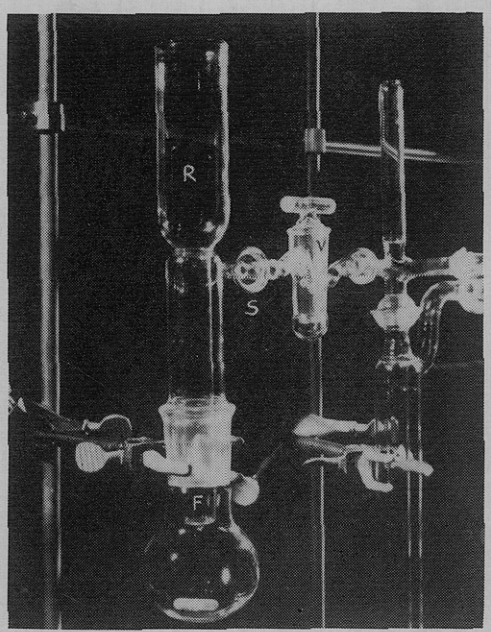

Figure 1. Apparatus for distillation of the higher-boilers

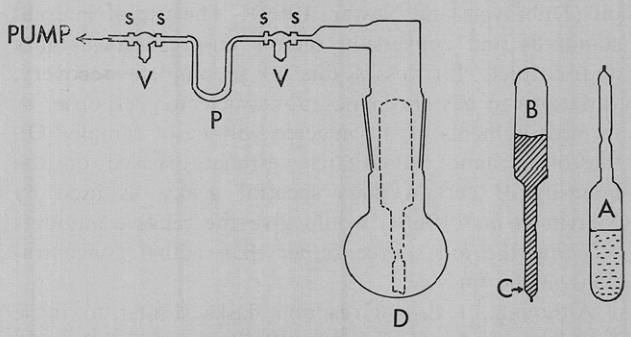

Figure 2. Apparatus for distillation of the lower-boilers

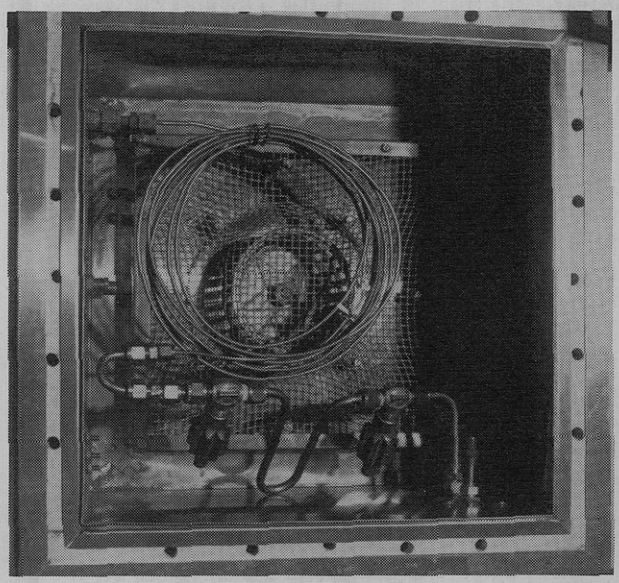

Figure 3. Positioning of precolumn assembly in gas chromatographic oven

Quantitative Measurement. Peak areas of the low-boiling compounds from experimental samples were measured, and the quantities of the individual hydrocarbons calculated from standard curves—i.e., peak area *vs.* quantity—established separately for each compound.

DISCUSSION

Quantitative analysis of the volatile compounds present in food lipids is important in assessing their flavor significance (Champagne and Nawar, 1968) and in interpreting mechanisms of their formation (Dubravcic and Nawar, 1968). The techniques outlined here provide simple and practical means for the measurement of a wide range of hydrocarbons. The proximity of the cold-finger to the surface of the sample allows better recovery of the higher molecular weight compounds (Table I), such as the major radiolytic products in fish

oil (Dubravcic and Nawar, 1969). The use of internal standards and conversion factors ensures repeatability and corrects for losses due to incomplete recovery, variations in gas chromatographic detector response, or in measurements of the injected volume of sample. On the other hand, quantitative estimates based on the intensity of certain mass spectral peaks, as used by Merritt *et al.* (1966), would give the relative amounts reaching the ion source rather than actual concentrations in the fat.

Although break-seal reaction flasks similar to those described by Angelini *et al.* (1967) were used in conjunction with the precolumn for the analysis of the low-boilers, the use of ampoules (Figure 2) was much simpler. The packing of the precolumn with stainless steel helices provides a large surface area for trapping without seriously impeding the vacuum efficiency, and thus complete recoveries of the lower-boiling compounds can be achieved. The placement of the precolumn inside the gas chromatographic oven obviates the need for exterior heating and pressure-injection systems. Gas-solid chromatography on alumina columns allows the fractionation of hydrocarbons without resorting to cryogenic programming equipment.

While the methods described in this report were used mainly for the measurement of hydrocarbons, they are applicable to the analyses of other groups of compounds as well. Departure from the parameters of distillation, however, would require the establishment of new conversion factors.

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