Methods of Isolation and Identification of Volatile Compounds In Lipids

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Abstract

Techniques are described for the collection, separation and identification of the volatile compounds produced in lipids by oxidation or irradiation. Methods employed are high vacuum distillation followed by wide range programmed temperature gas chromatography and rapid scanning mass spectrometry. Efficacy of the methods is discussed.

Introduction

MANY TECHNIQUES are used for the qualitative and quantitative analysis of volatile compounds, but the combination of gas chromatography and mass spectrometry is the most efficacious. The choice of sampling method is vital, however, to the successful analysis of complex mixtures. Sampling methods include sampling of headspace vapors (2,13,21), flushing with an inert gas (10,18), steam distillation (3), vacuum distillation (12,14,19,23) and solvent extraction (1,6–9,20,22).

Each of the above sampling methods was devised to accomplish a purpose but each has its disadvantages. Headspace sampling will give compounds present over a sample, but many important flavor compounds are not detected. Cold trapping of the volatile compounds entrained by flushing with an inert gas fails to recover the noncondensable and some of the very volatile compounds as well as higher boiling compounds. Moreover, impurities in the flushing gas may also be condensed in the cold trap. Solvent extraction of aqueous solutions concentrates the less volatile compounds but loses the more volatile ones and contributes impurities to the sample from the solvent. Volatile compounds may be effectively isolated by steam and molecular distillation, but thermal changes may occur.

The high vacuum degassing procedures described by de Bruyn and Schogt (4) and Lea and Swoboda (11) avoid most of the problems discussed above for isolating volatile constituents from fats but are not suitable for the recovery of the more volatile compounds. The headspace and low-temperature, high-vacuum sampling procedure described in this paper was used to study volatiles produced by irradiation and autoxidation of butterfat. It was designed to prevent loss of volatiles produced by treatment, to avoid artifacts, and to make use of gas chromatography and mass spectrometry for analyses.

Experimental

The Reaction Flask

The glass container in which the treatments were carried out is illustrated in Fig. 1. The glass breakseal assembly is joined to the flask after the sample has been placed in the bottom of the flask through the wide neck. Care must be taken not to let any of the sample touch the flask near the opening since charring of the sample may occur when the breakseal assembly is attached. The breakseal obviated the need for stopcock grease during treatments and storage. The glass side arm allows evacuation of the headspace

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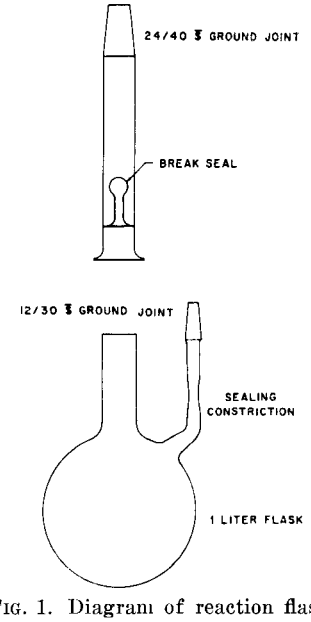

FIG. 1. Diagram of reaction flask.

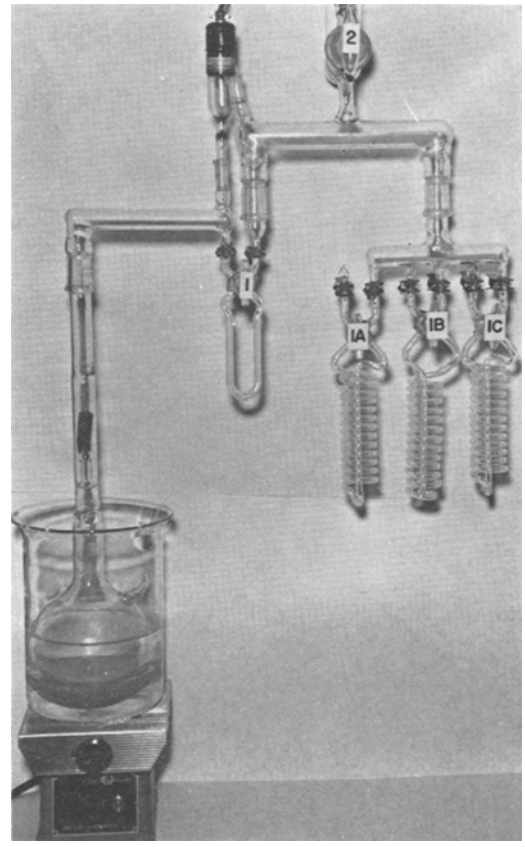

FIG. 2. Collection apparatus showing distillation of butterfat volatiles.

and degassing of the sample before treatment. Gases of known composition may also be introduced into the flask through the side arm.

The sample is sealed in the container under vacuum (10^{-4} torr) or desired gas—e.g., oxygen—at the constriction and treated.

Sampling Techniques

After treatment, the flask is attached to a high vacuum system as shown in Fig. 2. Evacuation and degassing of the complete system up to the breakseal is accomplished by opening all stopcocks. Stopcock 2 is then closed and the breakseal is broken allowing the headspace to expand into the manifold and spiral traps (50 ml volume). When the pressure in the system has reached equilibrium stopcocks 1A, 1B, and 1C are closed, trapping samples in the three spiral traps. The headspace samples are analyzed by gas chromatography and mass spectrometry.

A Dewar flask filled with liquid N_2 is placed around the U-trap. Stopcock 2 is then opened very slightly and the noncondensable gases are pumped out. The total condensate is collected in the U-trap with constant vacuum pumping (10^{-4} torr) for elimination of residual amounts of noncondensable gases. The sample is heated at 45C and magnetically stirred with a glass-encased bar added with the sample. When collection of the total condensate is complete, stopcock 1 is closed. Recovery of C_3–C_{12} methyl ketones (2 μg each), from 200 g of butterfat collecting in the manner described for 8 hr is shown in Table I. Table I shows quantitative recoveries for propanone through octan-2-one (bp 173C). Recovery falls off rapidly from nonan-2-one (bp 195C) to dodecan-2-one (bp 246C), but qualitative identifications through dodecan-2-one can be made. The temperature and time employed depend on the stability of the lipid and the volatility of the products.

The total condensate sample may be analyzed in its entirety or divided into equal aliquots by the apparatus shown in Fig. 3. The U-trap containing the sample is placed in the center position between a pair of empty matched U-traps. The system and empty traps are evacuated by opening all stopcocks except stopcock 1. When evacuation has been completed stopcock 3 is closed. Dewar flasks filled with liquid N_2 are placed around the two empty U-traps and a water bath at 50C is placed around the sample U-trap in the center. Stopcock 1 is then opened and the sample is split evenly and condensed into the two matched outside U-traps. This procedure may be repeated until a sample of the desired size is obtained. Sample splitting efficiency by this method using C_3–C_{12} methyl ketones is shown in Table II.

Analysis of Samples

The headspace and total condensable samples are separated by programmed cryogenic temperature gas chromatography (16) and a rapid scanning mass spectrometer is used in the identification of the components as they are eluted from the gas chromatograph (17).

The chromatography column chamber is a modified Barber Colman 5060 column oven fitted with a front door which is very convenient for changing columns without disturbing the connections to the ion source. These connections, which require separate heating, are also conveniently located in what was originally the detector cell housing of the gas chromatograph. The inlet system has been modified by installing a Perkin Elmer Model 4SW 4-port valve so that gaseous samples may be swept onto and condensed on the cold column with helium carrier gas. This method of sample introduction provides a uniform and very effective means of "narrow-band-on-column" injection. Provision is also made for syringe injection of the sample if required. Columns employed are 10 ft × ⅛ in. O.D. coiled stainless steel columns packed with 5% Carbowax 20M on Firebrick C-22. Liquid nitrogen is used as the coolant for the coluumn chamber and the programmer and heater are capable of providing temperature programs at rates varying from 1C to 24C per minute from −190C to over 200C.

The mass spectrometer is a Bendix Model 14 time-of-flight mass spectrometer with modified pulse circuitry to permit continuous ion generation, but operating with a spectrum scan rate of 10,000 spectra per second. These spectra may be viewed instantaneously on an oscilloscope screen. With an analog gating

TABLE I
Recovery of Methyl Ketones from Butterfat

Ketone	bp, C	Area (cm^2) of G.C. peaks of 20 μl of ketone mixture	Area (cm^2) of G.C. peaks of recovery of 20 μl of ketone mixture from 200 g butterfat	% Recovery
Propanone	56.5	1.8	1.7	95
Butanone	79.5	2.3	2.3	100
Pentan-2-one	102	4.0	4.1	103
Hexan-2-one	127.5	4.8	4.8	100
Heptan-2-one	148.5	5.8	5.8	100
Octan-2-one	173.5	6.4	6.3	98
Nonan-2-one	195	6.6	5.6	85
Decan-2-one	211	6.1	2.6	43
Undecan-2-one	223	6.6	0.8	12
Dodecan-2-one	246–7	5.7	0.1	2

Chromatograph—F&M 1609; Attenuation—1,000 × 16; hydrogen-flame detector; column 6 ft × ¼ in. I.D.; 5% Carbowax 20 M on 60–80 mesh firebrick; temperature programmed from −80C to +150C at 2 C/min; (see J. Gas. Chromatog. 2, 314 (1964)) carrier gas flow rate—40 ml/min.

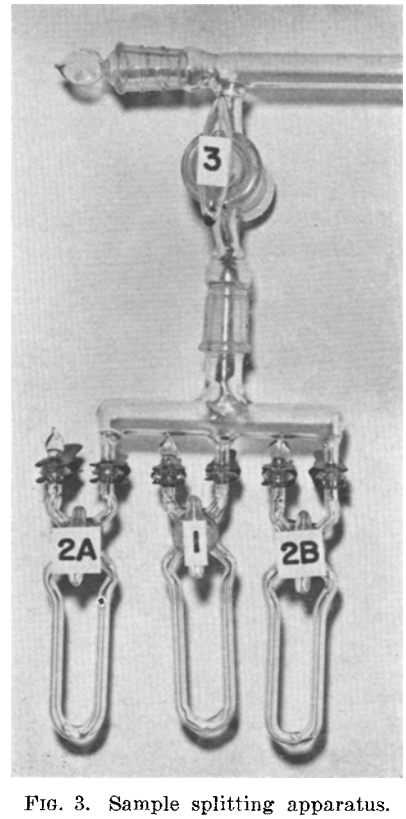

FIG. 3. Sample splitting apparatus.

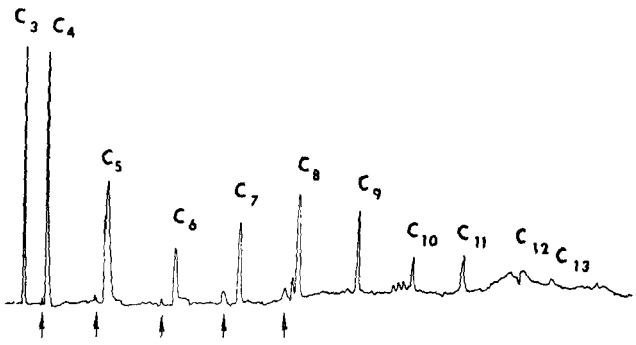

FIG. 4. Programmed cryogenic temperature "43 peak" chromatogram of irradiated butterfat volatiles. Column: 5% Carbowax 20M on 60–80 mesh, C-22 firebrick. Program: —65C to 125C at 2C/min. (Carbon numbers are indicated for homologous *n*-alkane peaks; arrows show corresponding isoalkanes).

system, a rapidly scanned chart recording of the spectrum is also displayed on a high speed direct writing oscillograph. A nominal scan speed of about 5 sec is employed to cover a mass range of about 10 to 1—i.e., from mass 20 to 200. If required, mass resolution higher than mass 200 can be achieved. In practice the oscillograph record of the mass spectrum is made, when desired, by the operator who continuously monitors the spectrum display on the oscilloscope.

A second analog scanner is used to monitor a single spectrum peak to provide a chromatographic display. Thus, the voltage corresponding to the ion current of a common mass peak, e.g., m/e-43 is displayed on one channel of the dual channel potentiometric recorder to register the chromatogram. The strip chart recording of the intensity of the m/e 43 peak produces what is called a 43 peak chromatogram. A typical chromatogram of the volatile compounds isolated from irradiated butterfat is shown in Fig. 4. By connecting a thermocouple from the chromatographic column chamber to a second channel on the stripchart recorder both a gas chromatogram and a record of the column chamber temperature may be obtained simultaneously on the same chart.

Results

The detection and identification of components of complex mixtures such as butterfat volatiles depends greatly on the effectiveness of the gas chromatographic separation achieved, and there is a particular situation where subambient programming is invaluable. For example, the mass spectra of *n*-pentane and isopentane (and other branched chain isomeric alkanes) have identical mass peaks. Only the ratios of peak intensities are different. Fig. 5 illustrates the effectiveness of subambient temperature programmed gas chromatography in separating a mixture of *n*-pentane and isopentane. A chromatogram of a mixture of *n*-pentane and isopentane, obtained isothermally at 25C and recorded as the intensity of the m/e 43 peak is shown on the left side of the figure. The mass spectrum shown above the chromatographic peak was obtained by photographing the oscilloscope screen as the mixture of components was being eluted. The chromatogram on the right shows baseline separation and a difference of one minute in retention times of the same mixture of *n*-pentane and isopentane when a subambient temperature program was used. The corresponding photographs of the mass spectra are shown over the gas chromatographic peaks. The necessity of effective gas chromatographic separation is clearly demonstrated by the mass spectra shown above the respective chromatographic peaks. From the mass spectrum of the mixture one could identify *n*-pentane and note the presence of an impurity, but could not be certain that the impurity is isopentane. From the mass spectra of the separated peaks, however, isopentane can be positively identified as the first peaks eluted and *n*-pentane as the second. The components in this case are present in nearly equal proportions. In Fig. 4, the principal components of the mixture are aliphatic hydrocarbons and the large peaks identified by carbon number correspond to an homologous series of unseparated alkanes and alk-1-enes. These compounds are easily identified by their mass spectra even in the binary mixture. The small peaks, indicated by arrows, preceding each of the larger peaks, however, correspond to an homologous series of iso-, or 2-methyl alkanes. If these were unseparated from the corresponding normal alkanes, their presence in trace amount would go unnoticed.

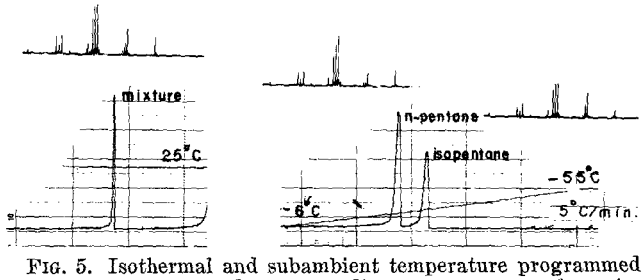

FIG. 5. Isothermal and subambient temperature programmed gas chromatograms and corresponding mass spectra of a mixture of *n*-pentane and isopentane.

Discussion

The reaction flask described in this paper provides a container which is inert, prevents loss of any volatiles produced, and allows a wide variety of treatments of the sample in any desired atmosphere.

The high vacuum distillation of volatiles works well for low and moderately high boiling compounds, but loses efficiency for recovering very high boiling compounds, e.g., methyl ketones above nonan-2-one. The low temperature and high vacuum conditions throughout the collection minimize chemical alteration of the compound and production of artifacts.

The method used for sample division is shown to be a very efficient method (Table II). Equal splitting of a sample was found to depend upon the symmetry of the apparatus. The pair of traps receiving the sample must be well matched. It is important that the stopcock bore sizes and tubing sizes be the same.

TABLE II
Splitting Efficiency

Ketone	bp, C	Area (cm^2) of G.C. peaks of 1 μl of ketone mixture	Area (cm^2) of G.C. peaks of 2 μl sample of ketone mixture, split ½	% Efficiency
Propanone	56.5	1.7	1.1	65
Butanone	79.5	2.9	3.0	103
Pentan-2-one	102	4.9	4.8	98
Hexan-2-one	127.5	6.2	6.3	102
Heptan-2-one	148.5	5.9	5.9	100
Octan-2-one	173.5	5.6	5.4	96
Nonan-2-one	195	7.3	7.4	101
Decan-2-one	211	7.9	8.0	101
Undecan-2-one	223	6.0	6.0	100
Dodecan-2-one	246–7	5.8	5.5	95

Chromatograph, F&M 1609; attenuation, 1000 × 8; hydrogen flame detector; column, 6 ft × ¼ in. I.D.; 5% Carbowax 20 M on 60–80 mesh firebrick; temperature programmed from —80C to +150C at 2C/min; carrier gas flow rate, 40 ml/min.

Alignment of openings is a necessity and any blockage by stopcock grease or other materials must be scrupulously avoided.

Some of the samples encountered may not be completely separated by wide range temperature programmed gas chromatography, due to a high complexity and wide variations in amounts of volatiles present. Nevertheless, the compounds are usually well enough separated to enable identification by rapid-scanning mass spectrometry. Moreover, two or three compounds unseparated in the one gas chromatographic peak may often be identified by the mass spectrum. These techniques have been successfully employed in recovering and analyzing the volatiles produced by irradiation and autoxidation of butterfat (5,15).

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