A Simple Technique for Determining the Gas Liquid Chromatography Profile of Volatile Compounds in Vegetable Oils

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ABSTRACT

A simple procedure has been developed for the isolation, concentration and gas liquid chromatographic detection of the volatile compounds in vegetable oils. The volatile compounds are isolated by bubbling purified helium through a measured quantity of vegetable oil heated in an oil bath having a temperature of 350 F. These compounds are collected on activated charcoal and then extracted from the charcoal with carbon disulfide containing an internal standard. The distribution of the volatile compounds is determined with a flame ionization detector. A 400-fold concentration of the volatile compounds is achieved with this procedure. The technique provides good reproducibility (94.3% to 105.5%) and has been successfully used for measuring the increase of volatile compounds in vegetable oils during storage and food production.

INTRODUCTION

Gas chromatography has contributed significantly to the analysis of volatile compounds contained in a wide variety of foods and food ingredients. Many approaches have been suggested for preparation and introduction of the volatile compounds into the gas chromatograph. The simplest approach involves the direct injection of an aliquot of the head space vapors (1-4). The success of such methods is limited by the fact that they require the injection of massive quantities of head space vapors in order to detect dilute vapor components. These trace components may be so diluted with carrier gas that they escape detection.

Vacuum distillation techniques have been employed to concentrate volatiles prior to gas liquid chromatographic (GLC) analyses (5-8). While these techniques have often been successful, they require elaborate and expensive equipment.

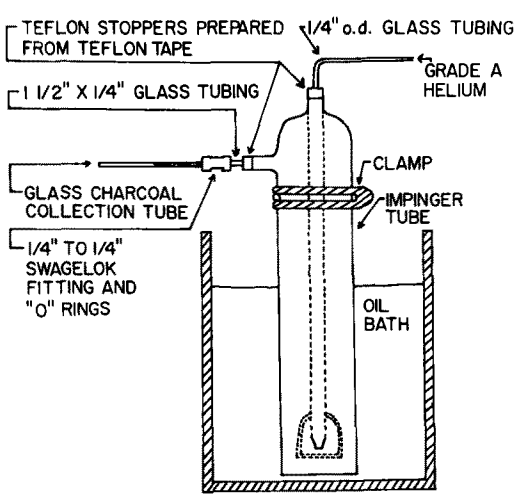

FIG. 1. Diagram of apparatus for volatiles analysis.

Low temperature GLC precolumns have been used to concentrate the volatiles from head space vapors (2,4,9,10). Since appreciable diffusion occurs in these precolumn systems, the quantity of head space vapors that can be used is severely limited.

Scott et al. (11) proposed the use of a gas-solid chromatographic precolumn to concentrate volatiles from head space vapors prior to elution to a GLC system. Walls (12), Dhont et al. (13) and Heinz et al. (10) used activated charcoal for absorbing volatiles for subsequent analysis. Jennings et al. (14) substituted charcoal in the gas-solid chromatographic step of the method proposed by Scott et al. (11) and devised a technique for concentration of volatiles from large vapor samples containing high quantities of water vapors.

A modification of Jennings' et al. (14) technique was used to concentrate the volatile compounds swept from heated vegetable oil (350 F, oil bath temperature) with purified helium. The purpose of this work was to devise a simple and rapid technique for measuring changes occurring in the volatiles profile of vegetable oil during processing or storage tests.

EXPERIMENTAL PROCEDURE

Apparatus

Volatile Collection Apparatus. An Impinger Tube (Corning #6800-6820) was modified as shown in Figure 1. The inlet port of the Impinger Tube was fitted with a 1/4 in. o.d. glass tube centered in a teflon stopper prepared from teflon tape. During volatiles analysis, the 1/4 in. o.d. glass tube is attached with Swagelok fitting and Hewlett Packard F&M O rings (#5080-4982) to a helium tank equipped with a flow regulator.

The exit of the Impinger Tube was fitted with a 1 1/2 in. x 1/4 in. o.d. glass tube centered in a teflon stopper prepared from teflon tape. A 1/4 in. to 1/4 in. stainless steel Swagelok fitting having a 1/4 in. i.d. bore was attached to the exit end of the 1 1/2 in. x 1/4 in. o.d. glass tube using a 1/4 in. Swagelok nut, farrow and O rings. A 6 in. x 1/4 in. o.d. glass tube packed with activated charcoal and silanized glass wool (Applied Science) was attached to the 1/4 in. to 1/4 in. Swagelok fitting in a similar manner. An asbestos covered clamp was used to secure the ground glass fitting of the Impinger Tube. The Impinger Tube and contents were heated with a Blue M Model MW 1145A oil bath.

Gas Chromatographic System. A Hewlett Packard F&M Model 5750 gas chromatograph was used, it was equipped with dual flame ionization detectors and a 6 ft. x 4 mm i.d. glass column packed with 10% DC 200 (12,500 cst) on 100 to 200 mesh Gas Chrom Q.

Gas Chromatograph Conditions. Temperatures were the following: column, 110 C; detector, 200 C; and injector port block, 200 C. Gas flows were the following: helium (carrier gas), 50 ml/min; hydrogen, 40 ml/min; and compressed air, 450 ml/min. Sensitivity was as follows: range, 100, and attenuator, 2 to 128. The chart speed was 1/2 in./min.

TABLE I

Method Reproducibility Data for a Commercially Prepared Oil

Peak No.	Test 1, intensity ratio[a]	Test 2, intensity ratio[a]	Test 3, intensity ratio[a]	Average (3), intensity ratio[a]	Per cent reproducibility[b,c] Test 1	Test 2	Test 3
1	1379.3	1323.0	1293.0	1331.8	103.5	99.3	97.2
2	137.9	137.4	149.2	141.5	97.4	97.2	105.5
3	6.3	5.9	4.8	d			
4	5.4	5.6	5.7	d			
5	19.1	21.0	20.8	20.3	94.3	103.4	102.5
6	35.5	37.4	36.8	36.6	97.1	105.4	103.7
7	4.7	5.2	4.9	d			
8	11.5	14.1	12.4	d			
9	3.9	4.2	5.0	d			
10	9.7	11.2	11.4	d			
11	1.8	2.4	2.6	d			
12	7.5	10.9	10.4	d			
13	1.7	2.2	2.4	d			
14	2.3	2.8	2.8	d			
15	0.6	0.7	0.5	d			
16	0.9	1.2	1.2	d			
17	0.9	1.0	0.8	d			
18	0.7	0.9	0.7	d			
19	1.2	1.7	1.2	d			
Total intensity	1631	1589	1611	1610	105.5	98.7	101.3

[a]See Experimental Procedure for calculation of this value.

[b] $\frac{\text{Intensity ratio X 100}}{\text{Average intensity ratio}}$

[c]Reproducibility range for individual volatiles representing 1% or more of the total peak height = 94.3 to 105.5%.

[d]Volatiles representing less than 1% of the total peak height.

[e]Reproducibility range for total intensity values = 98.7 to 105.5%.

Preparation of Activated Charcoal. A modification of the procedure of Jennings et al. (14) was used to prepare the activated charcoal.

Twenty grams of 20 to 40 mesh cocoanut charcoal (Analabs) was mixed with 100 ml of carbon disulfide, stirred for 15 min and the solvent decanted. A second 50 ml portion of carbon disulfide was added to the charcoal, the mixture was allowed to stand for 30 min with occassional stirring and the solvent decanted.

The charcoal was partially dried using a Buchner funnel, Shark Skin filter paper (Schleicher & Scheull Co.) and reduced pressure. The partially dried charcoal was transferred to a 250 ml beaker and heated for 16 hr at 50 C, for 8 hr at 120 C and for 16 hr at 180 C. (Charcoal should be heated under a hood).

Determination of Volatiles Profile for Vegetable Oil. A 1/4 in. loosely packed wad of silanized glass wool was positioned approximately 2 in. from one end of a clean 6 in. length of 1/4 in. o.d. glass tubing. One tenth of a gram of activated charcoal was added to the 2 in. section of the glass tube and secured with a second 1/4 in. loosely packed wad of silanized glass wool. This wad of glass wool was followed by a second 0.1 g portion of activated charcoal and capped with a third 1/4 in. loosely packed wad of silanized glass wool.

With the exception of the glass-charcoal collection tube, the apparatus was assembled as shown in Figure 1. The system was purged for 30 min using 500 ml/min helium and an oil bath temperature of 350 F.

The Impinger Tube was then removed from the oil bath and cooled to room temperature. Four hundred milliliters of oil were added to the Impinger Tube. (Addition of a few

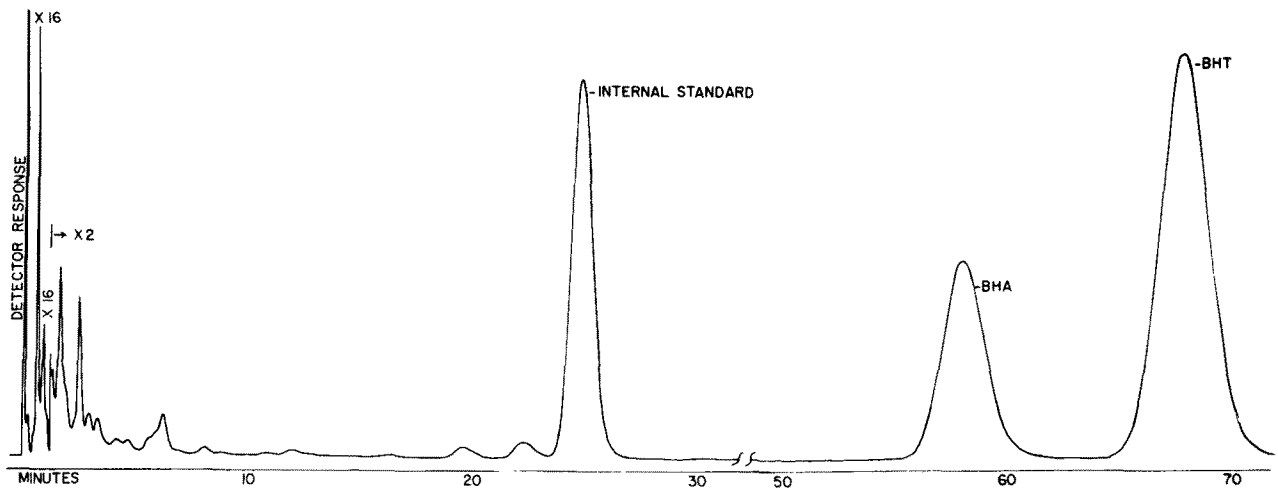

FIG. 2. A 6 ft X 4 mm i.d. glass column packed with 10% DC 200 on 100-120 mesh Gas Chrom Q; column, 110 C; detector, 200 C; injector port, 200 C; flow-rate, 50 ml/min helium; sensitivity, 6.4×10^{-11} to 4.0×10^{-12} amp full scale deflection; sample size injected, 5.0 μl.

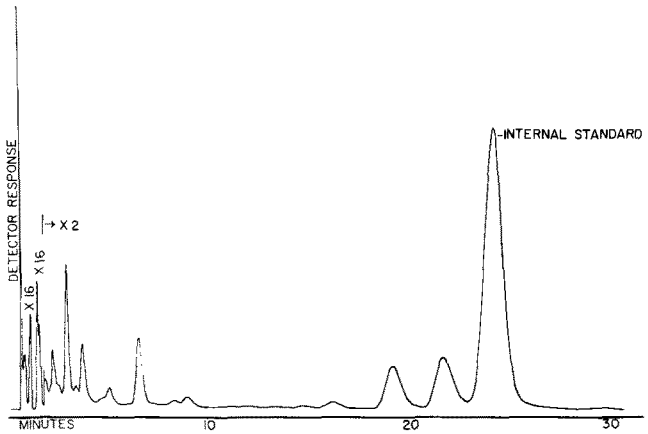

FIG. 3. GLC conditions were the same as listed for Figure 2; sample, volatiles isolated from high oleic safflower oil; sample size injected, 4.6 μl.

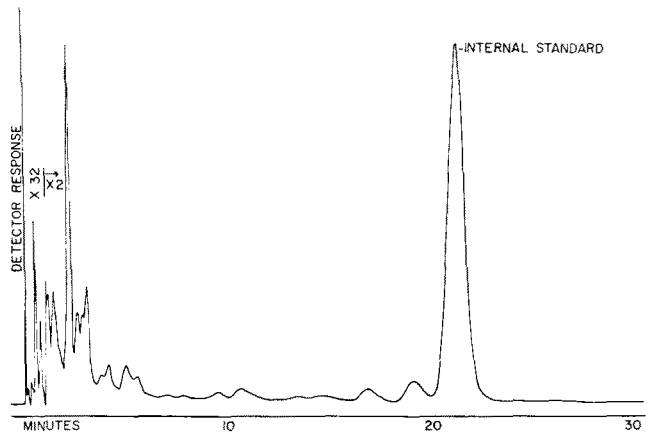

FIG. 4. GLC conditions were the same as listed for Figure 2 with the exception of sensitivity which was 1.28×10^{-10} to 4.0×10^{-12} amp full scale deflection; sample, volatiles isolated from soybean oil; sample size injected, 4.6 μl.

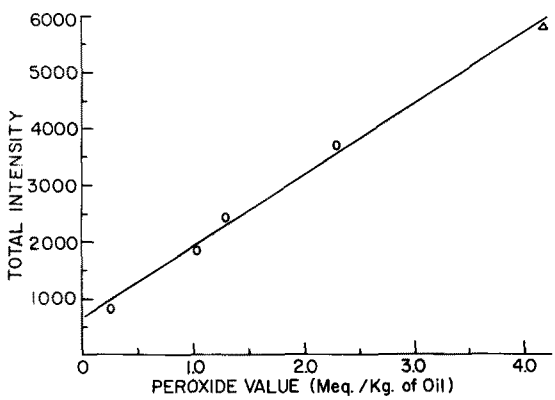

FIG. 5. Plot of total intensity values versus peroxide value; sample, volatiles isolated from commercially prepared oil; o, represents vegetable oil stored at 100 F; Δ, represents vegetable oil stored at 145 F.

drops of oil to the ground glass connections of the Impinger Tube assures a gas tight fitting).

The apparatus was reassembled, the glass-charcoal collection tube attached, and the Impinger Tube immersed in the oil bath to a level 1 in. above the 250 ml calibration mark. The helium flow rate was set at 250 to 300 ml/min, the oil bath temperature was checked to assure a temperature of 350 F and the system was checked for gas leaks.

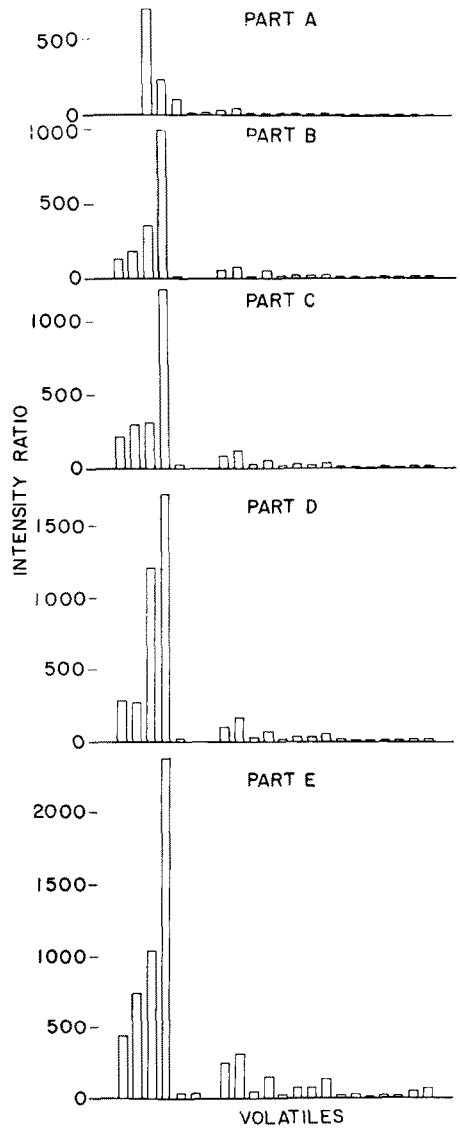

FIG. 6. Intensity ratios for volatiles from oil storage tests; sample, commercially prepared oil (same as in Figure 5); Part A, initial oil - peroxide value 0.26 mEg/kg of oil; Part B, 100 F storage - peroxide value 1.09 mEq/kg of oil; Part C, 100 F storage-peroxide value 1.30 mEq/kg of oil; Part D, 100 F storage - peroxide value 2.29 mEq/kg of oil; Part E, 145 F storage - peroxide value 4.24 mEq/kg of oil; elution order from GLC column: left to right.

After 2 hr, the glass-charcoal collection tube was removed from the system. (A blank determination was completed in the same manner as was the sample determination except that the vegetable oil was deleted from the system).

The charcoal was pushed from the tube using a clean 1/16 in. diameter stainless steel rod, the glass wool discarded and the charcoal collected in a 4 ml glass vial.

One milliliter of carbon disulfide containing 300 μg/ml of methyl decenoate was added to the charcoal, the cap (with clean aluminum foil liner) secured on the vial, the mixture shaken vigorously for 3 min and the charcoal allowed to settle for 1 to 2 min. The solvent was removed from the charcoal using a clean dry 1 ml syringe equipped with a 25 gauge needle and transferred to a clean and dry 4 ml vial.

Three to 7 μl of the extract was injected into the gas chromatograph. The chromatograms were calculated as follows;

$$\text{Per cent of peak height} = \frac{\text{Peak height of volatile compound X 100}}{\text{Total peak height (without internal standard) of volatile compounds}}$$

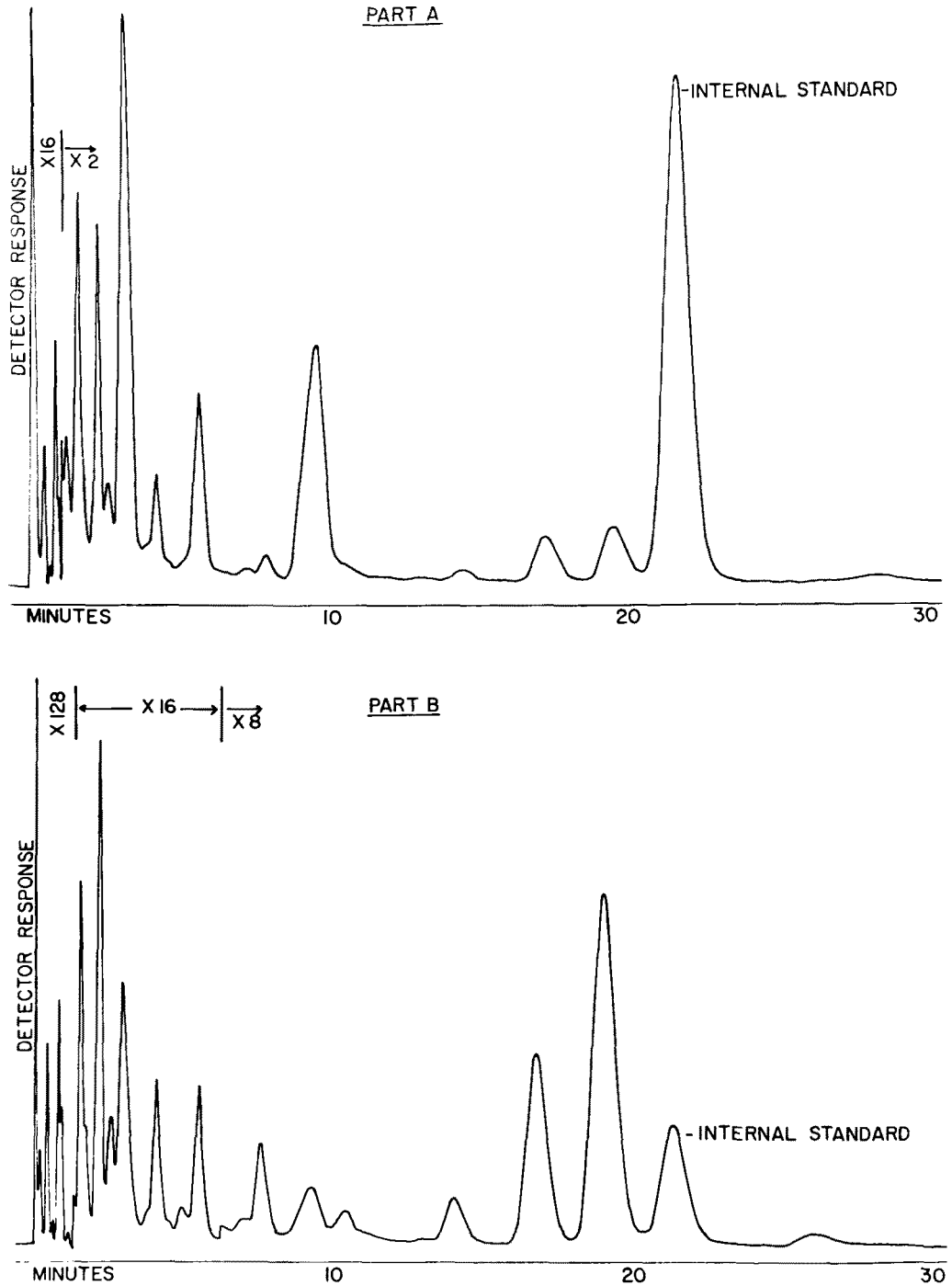

FIG. 7. GLC conditions were the same as listed for Figure 2 with the exception of sensitivity which was 2.56×10^{-12} to 4.0×10^{-12} amp full scale deflection; sample, Part A, commercially prepared oil (Lot 1) having a desirable flavor; Part B, commercially prepared oil (Lot 2) having an undesirable flavor; sample size injected, Part A, 4.6 µl, Part B, 3.4 µl.

$$\text{Peak intensity ratio} = \frac{\text{Peak height of volatile compound X 100}}{\text{Peak height of internal standard}}$$

Total intensity = Sum of peak intensity ratios

Analysis of Vegetable Oils. Peanut, high oleic safflower and soybean oils were analyzed using the described technique.

A commercially prepared vegetable oil was subjected to controlled storage conditions, periodically sampled and the volatile profiles determined. The volatile profiles were determined for two samples of a second commercially prepared oil. These samples represented a different refining process from that of the previously mentioned commercially prepared oil.

RESULTS AND DISCUSSION

Temperatures of 170, 200, 260, 350 and 400 F were tested for the collection of volatile compounds from vegetable oil. During these tests the helium sweep gas was set at a flowrate of 250 to 300 ml/min. These tests showed that with the exception of antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), all other volatile components were stripped from the vegetable oil in 1 hr with a 350 F oil bath temperature. Lower oil bath temperatures required considerably greater periods of time to remove the volatiles from the oil. Some destruction of oil was indicated with a 400 F oil bath temperature. Volatile profile obtained at this temperature

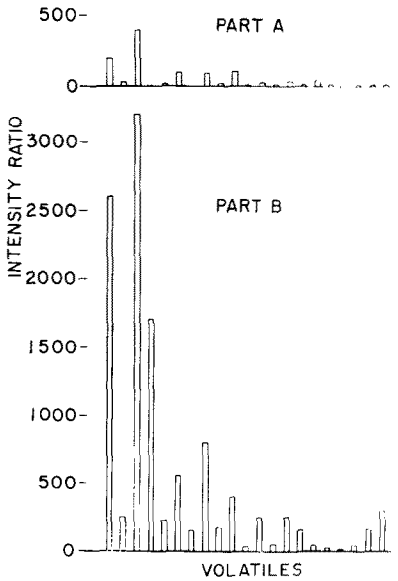

FIG. 8. Intensity ratios for volatiles from desirably and undesirably flavored commercially prepared oil; Part A and Part B, see Figure 7; elution order from the GLC column, left to right.

contained components not found at lower oil bath temperatures.

Peroxide values (15) determined for vegetable oils before and after volatiles analysis showed a higher value for the oil prior to analysis. Stability values obtained by the Active Oxygen Method (16) showed no significant differences before and after volatiles analysis. These tests indicate that no detectable thermal oxidation occurred during volatiles analysis.

Triplicate analysis for a commercially prepared vegetable oil showed a reproducibility range of 94.3% to 105.5% for the major volatile components, i.e., components representing 1% or more of the total volatiles peak height (Table I).

Typical volatile profiles for peanut, high oleic safflower and soybean oils are seen in Figures 2, 3, and 4.

A commercially prepared oil was stored in the dark at 100 F, sampled periodically and the volatiles profile and peroxide values determined. A plot of the total intensity of the volatile compounds versus peroxide value was found to be a linear function. A second portion of the commercially prepared oil was stored in the dark at 145 F for 20 days. The total intensity value and peroxide value were determined for the oil. The values for this oil were found to fall on the linear plot obtained for the 100 F storage tests (Fig. 5). These tests indicated that while the intensity ratios of some of the volatiles changed considerably during storage; the overall trend was toward a build up of volatile compounds with increased oxidation of the oil (Fig. 6).

The technique was also tested using two samples of a second commercially prepared oil. The first sample was a fresh oil having a desirable flavor while the second oil had developed a very undesirable flavor during storage. The peroxide value of the desirable oil was found to be 0.40 mEq/kg of oil while the undesirable oil had a value of 13.5. The chromatograms for these oils are shown in Figure 7. The volatile profiles for these oils reveal many differences in the intensity ratios of the components (Fig. 8). The total intensity value of the desirable oil was found to be 1026 units while the undesirable oil had a value of 11,297.

In addition to oil storage and processing tests, the technique has been used to study the changes in volatile compounds occurring during the processing of foods.

Preliminary attempts at characterization of the volatile compounds have involved the use of a column stream splitter and a "sniff" tube. These tests have provided a general indication of the odor of the volatile compounds and the threshhold level of the volatiles as detected by the human nose.

Studies are presently being conducted to determine if certain volatile compounds are associated with particular flavor characteristics of vegetable oils. Identification of such compounds could be achieved by IR and mass spectra analysis following GLC separation.

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[Received July 27, 1970]