Automated Gas Chromatographic System for Volatile Profile Analysis of Fats and Oils

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ABSTRACT

A system is described for automating the direct gas chromatographic method for examining volatile profiles of vegetable oils. The system couples an external inlet device to a microprocessor-based gas chromatograph for providing automatic control of the analysis cycle. Automation of the analysis cycle permits consistent control over sampling and analysis to obtain rapid and reproducible volatile profiles. Manual control is completely eliminated and the analyst is freed for other tasks. Volatile Profile Analysis (VPA) is employed to monitor "protected oil" quality of oils being processed, as well as in the finished product, in an objective manner, thus eliminating the subjectivity found in many flavor panels. Automated VPA is also a useful tool in modifying operating parameters when handling various oil types.

INTRODUCTION

A simple direct gas chromatographic method was first reported by Dupuy et al. (1) for analyzing volatile flavor compounds in salad oils and shortenings which has been used for a wide variety of applications including: residual hexane in oils (2); correlation of raw peanut volatiles with flavor scores (3); flavor quality and residual solvents in soy products (4); analysis of neutral lipids in mayonnaise (5); and assessment of egg flavor (6).

An objective method (e.g., gas chromatographic [GC]) for measuring the quality of fats and oils that would be less costly, less subjective and more specific than the currently used flavor panel scores has generated continuing interest. In addition, flavor panelling is not an acceptable means of monitoring in-process oil quality requiring on-the-spot adjustments for consistent product quality.

Implementation of Dupuy's direct GC technique (7) required a GC with an appropriate inlet design. To overcome this, other workers developed modifications for implementing direct GC volatile analyses on any GC. Jackson and Giacherio (7) reported high correlations of aged soybean-oil volatiles to flavor scores using an aluminum U-tube for isolating volatiles. An aluminum U-tube containing the sample was heated in an oven and volatiles were collected on an analytical column connected to the outlet of the U-tube outside the oven. The analytical column was disconnected from the U-tube, then connected to the GC for analyzing the isolated volatiles.

Min (8) refined the U-tube technique by using potassium carbonate and sodium sulfate to remove acidic compounds and water from the isolated volatiles. Good correlations between sensory data and instrumental analysis were reported for soybean oil, hydrogenated soybean oil and corn oil. This modification was further applied to the analysis of Dowtherm A (9) and phenolic antioxidants in oil (10). Legendre et al. (11) reported an external inlet device consisting of a heated inlet block assembly and a 6-port sample valve for direct GC/MS (mass spectroscopy) analysis of volatiles in aqueous and nonaqueous systems.

Each of these modified approaches had the shortcoming of requiring manual involvement during volatile isolation, e.g., changing columns, switching valves or controlling the volatile isolation times. In a plant environment, where rapid turnaround time of results is essential, a manual approach would be too labor intensive and dependent on consistent technique by individual analysts on different shifts. For this reason, we undertook an investigation for developing an automated method that would overcome the disadvantages of the manual approaches.

This paper reports on: (a) an automated GC system for volatile profile analysis (VPA) using an external inlet block that eliminates manual involvement after the sample tube is placed in the inlet block; and (b) the use of the automated system for the analysis of actual in-process oils through hard copy "fingerprints" of volatile content.

EXPERIMENTAL PROCEDURES

Materials

Glass sample tubes—84 mm × 9 mm borosilicate, square cut and fire polished, were obtained from Teklab, Baton Rouge, LA. Silicone septa—12.5 mm with 1/8-inch hole bored through the center; glass wool (Corning 3950); and Pasteur pipettes, 5¼ inches, were used. The above materials were preconditioned at 200 C overnight before use. Samples were collected in 125 mL amber plastic bottles (Nalge 2004 linear polyethylene).

The reference oil was 200 g freshly deodorized salad oil spiked with 5 μL each of butanal, hexanal, nonanal, heptane, decane and pentadecane (Polyscience Corporation). Reference oil was prepared with minimum agitation, transferred to individual 3 mL sample vials with teflon-lined caps and stored in a freezer at −10 C.

Samples of in-process oils were collected in 125 mL amber polyethylene bottles. Each bottle was completely filled to minimize headspace.

Equipment

Gas chromatograph — Hewlett-Packard Model 5880A equipped with a flame ionization detector (FID), external event board, heated valve compartment, 6-port high temperature Valco valve with an automatic actuator, in-line rotameter to monitor carrier gas flow visually and auxiliary heater and thermocouple. A Hewlett-Packard Model 3353 data system was used for integration.

GC column—10 ft × 1/8 in. nickel-200 column packed with 60/80 mesh Tenax GC coated with 8% polymeta-

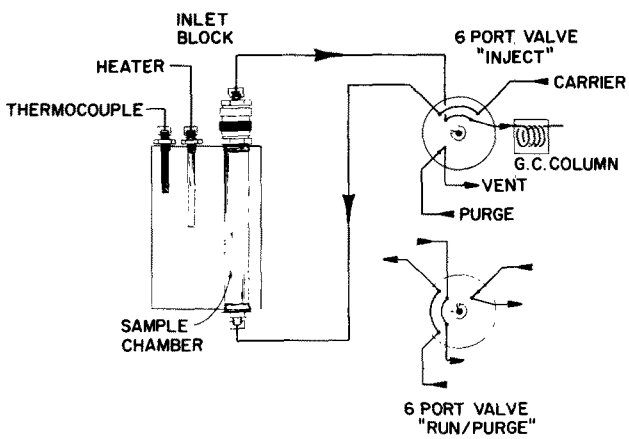

FIG. 1. Inlet block schematic.

[1] Presented at the AOCS annual meeting, Toronto, 1982.

phenoxylene (Teklab, Baton Rouge, LA) conditioned at 275 C for 16 hr.

Inlet block—commercially available from Scientific Instrument Service, Inc., River Ridge, LA.

Set-up of Automated GC System

Holes of the appropriate size were bored into the inlet block to accept the auxiliary heater and thermocouple. The inlet block was then mounted on the heated valve-compartment cover with the block outlet close to the valve access hole. Electrical connections for the auxiliary heater and thermocouple were made as defined in the instrument operating manual. The inlet block was connected to the valve in place of the gas sample loop supplied by the manufacturer, as shown in Figure 1. Stainless-steel tubing, 1/16 in. × 0.085 in., was used to make the connections.

PROCEDURE

Sample Preparation

The lower portion of a preconditioned sample tube was packed firmly with a glass-wool plug, followed by looser glass-wool plugs so that a ¼ in. clearance at the bottom and a 1 in. clearance at the top was left. A 500 mg portion of sample was weighed onto the glass wool and covered with a small plug of glass wool. The sample liner was placed into the inlet block with the sample positioned at the inlet and the end cap hand-tightened. Perforated septa sealed the sample tubes in the inlet block and provided for gas flow through the sample tube.

Automated Analysis

Once the sample tube was placed in the inlet block, the volatile profile analysis was initiated by depressing the START PROGRAM buttons on the GC terminal. The analysis sequence proceeded automatically in 2 steps: volatile isolation and GC oven temperature programming using a KEYSTROKE PROGRAM and RUN PROGRAM shown in Table I.

Volatile isolation was conducted by switching on the valve to allow carrier gas to flow through the sample tube and heat the inlet block (Aux 2) to 220 C. Volatiles were purged onto the GC column for 25 min, at which time the valve and inlet block heater were turned off. The GC temperature program was then initiated by the KEYSTROKE PROGRAM using a "dummy valve" command (step 30, Table I). During the GC temperature program, a RUN PROGRAM was used to reset valve 12 for the next run and provide documentation of the inlet block temperature and recorder attenuation of the chromatogram.

GC Conditions

Carrier (nitrogen) flow—50 mL/min; inlet purge (nitrogen) flow—5 mL/min; H_2 flow—40 mL/min; air flow—400 mL/min; valve air—80 psi; oven temperature profile—hold at 30 C during volatile isolation, then 17 C/min to 98 C, then 4 C/min to 240 C with a 5 min hold at the upper limit; valve temperature—180 C; inlet block temperature—220 C during volatile isolation.

System performance was monitored daily by analyzing the reference oil. Long-term trends in system performance were identified by charting retention times and peak areas of the spiked compounds: butanal, heptane, hexanal, decane, nonanal, and pentadecane. Retention times and peak areas gradually increased as the column aged.

RESULTS AND DISCUSSION

System Performance

Heating freshly deodorized oils at 160-170 C, as reported in the literature (7-9), did not generate measurable amounts of volatiles. Increasing the inlet block temperature to 220 C was necessary to obtain measurable concentrations of volatiles for freshly deodorized oils. An inlet block temperature of 220 C was used for all analyses. Method precision was determined from same-day replicate analyses of the reference oil. Coefficients of variation for the spiked compounds ranged from 3% to 10%.

Automatic operation capabilities of the GC system also permitted the monitoring and maintaining of overall performance. GC operations could now be conducted based on the time of day using clock programs. This option was successfully used to run daily system purges and blank runs overnight. The program found most useful was to purge the GC system by heating the inlet block to 220 C, the valve to 275 C and the oven to 250 C for 1 hr, then resetting these zones to their initial temperatures. A blank run initiated at 6:30 a.m. to provide verification that the GC system was not contaminated before the start of each day's analyses was particularly effective.

Experience subsequently identified proper packing of glass wool into the sample tube as the primary means of

TABLE I

Analysis Sequence: Control Programs

KEYSTROKE PROGRAM			RUN PROGRAM		
Step	Command	Function	Time	Command	Function
10	VALVE 1 ON	Start collection of volatiles.	0.04	VALVE 12 OFF	Reset valve to initial condition.
11	AUX 2 TEMP 200	Heat inlet block to release volatiles.	0.10	LIST AUX 2 TEMP	Document inlet block temperature.
20	WAIT 25	Collect volatiles for 25 min.	1.00	LIST ATTN 2↑	Document attenuation of chromatogram.
25	VALVE 1 OFF	Stop collection of volatiles.			
26	AUX 2 TEMP OFF	Inlet block heater OFF.			
30	START VALVE 12 ON	Start GC temperature program.			

maintaining satisfactory system performance. Poorly packed sample tubes allowed oil seepage that contaminated the transfer lines, resulting in high volatile concentrations in the blank run and required clean-up of the system by flushing the transfer lines with methanol. Removing the sample tube after volatile isolation and determination of the weight loss provided a means of detecting oil seepage. Normal weight losses ranged from 1 mg to 5 mg. Weight loss in excess of 5 mg was usually associated with oil seepage. Packing the first plug of glass wool firmly into the sample tube was the key to eliminating such seepage. Additional glass wool plugs were packed more loosely to provide even distribution of the oil without restricting carrier gas flow. Properly packed sample tubes eliminated contamination from oil seepage.

In-Plant Quality Control

Applicability of the automated technique for in-plant use was evaluated by analyzing in-process soybean oils including: (a) crude nondegummed; (b) degummed; (c) refined; (d) bleached; (e) deodorized. Trends in volatile concentrations are shown by representative chromatograms in Figures 2-6. Total area counts and estimated ppm volatiles are given in Table II. Volatile concentrations were calculated from the response of the spiked compounds in the reference oil.

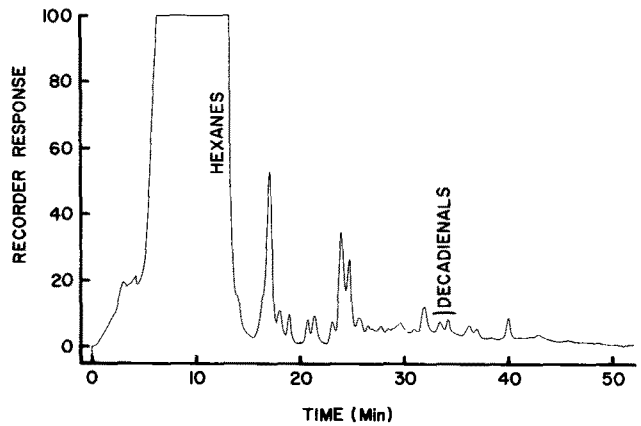

FIG. 2. Volatile profile of crude nondegummed soybean oil.

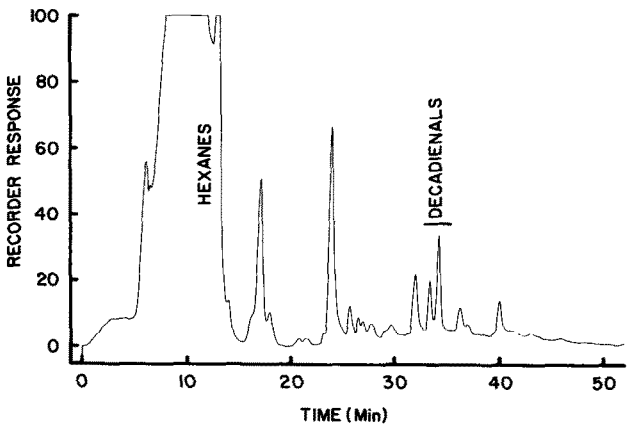

FIG. 3. Volatile profile of degummed soybean oil.

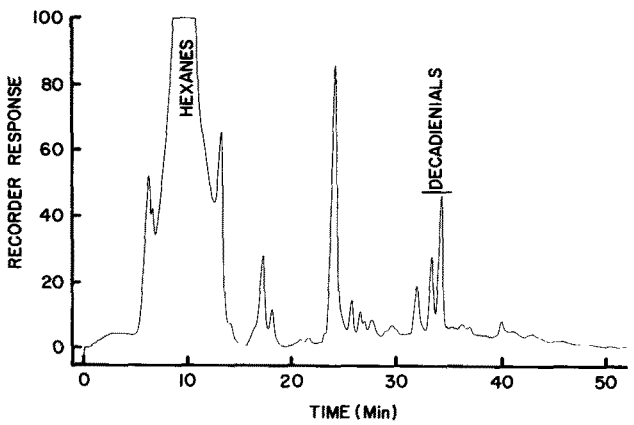

FIG. 4. Volatile profile of caustic refined soybean oil.

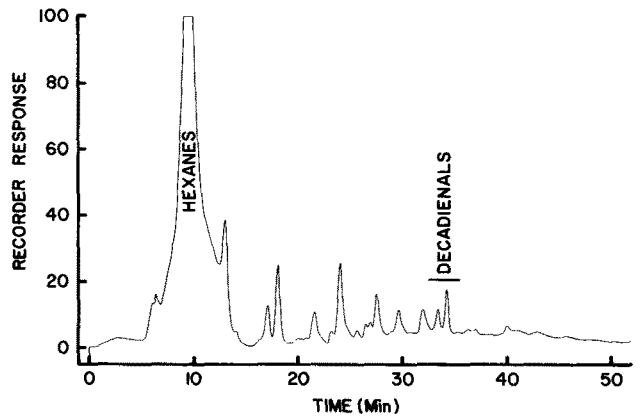

FIG. 5. Volatile profile of refined and bleached soybean oil.

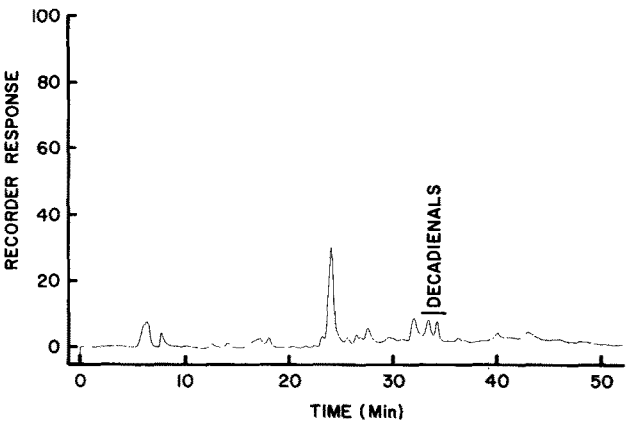

FIG. 6. Volatile profile of refined, bleached and deodorized soybean oil.

TABLE II

Estimated Volatiles Concentration

Sample	Total area counts ($\times 10^6$)	Estimated volatiles (ppm)
Crude SBO	16.1	1030
Degummed SBO	8.4	540
Refined SBO	7.2	460
Bleached SBO	3.5	225
Deodorized SBO	0.5	32

A review of the volatile profiles showed that: (a) total ppm volatiles of in-process oils were primarily dependent on the concentration of residual hexanes; (b) crude soybean oil contained relatively few volatiles other than hexanes; (c) during processing of the oil, concentrations of later eluting volatiles increased; (note the development of decadienals that have been correlated to soybean oil off-flavors (8)); (d) deodorization removed nearly all of the hexanes and significantly reduced the concentration of later eluting volatiles; (e) using higher sampling temperatures (i.e., 220 C vs 170 C) resulted in higher estimated total volatile concentrations. However, for monitoring consistency and trends, the repeatability of the method, not the absolute numbers, was the critical factor.

Automating VPA and implementing systematic performance checks greatly simplified the routine operation of the GC system. Direct operator involvement was reduced by ca. 25 min per sample, compared with the manual valve switching and temperature control approach initially used. Sample tubes were prepared in advance by the more experienced operators for later use by less experienced operators. Therefore, minimal training was required to obtain satisfactory VPA on a routine basis. VPA provides a hard copy fingerprint of oil volatiles. Flavor panels would have a difficult time qualifying the flavors of the undeodorized products, whereas this GC technique easily qualitated and quantitated volatile flavor compounds in both in-process oils and finished products. Qualitative information is most important for monitoring production because each process oil or product has a characteristic volatile profile. Changes in the profile, in terms of volatiles being present or absent, are just as important as absolute concentrations when correlating VPA results to processing conditions and end-product oil quality.

Routine operation of the automated VPA technique has shown that the potential for this technique goes beyond verifying flavor scoring of finished products. VPA can also be used to: (a) monitor the effects of processing conditions; (b) evaluate the effects of storage conditions; (c) reduce the number of flavor panels; (d) follow oil quality changes during shipment; (e) monitor the changes in oil quality of finished products, e.g. margarine, mayonnaise, salad oil and salad dressing. All of these potential uses can lead to better product performance and even improved productivity by defining conditions for making a product right the first time.

In summary, the automated system for VPA developed in our laboratory not only met our objectives for reducing manpower requirements in an in-plant operation, but was found to be: (a) easy to operate; (b) consistent in performance; (c) adaptable to different analytical conditions; (d) useful in determining in-process and finished oil quality in a manner that has great commercial value.

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♣Changes in Lipid Class and Fatty Acid Compositions During Maturation of *Hibiscus esculentus* and *Hibiscus cannabinus* Seeds

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ABSTRACT

The major lipid classes and their constituent fatty acids were analyzed in maturing seeds of *Hibiscus esculentus* and *H. cannabinus*. The seeds matured in 40 and 45 days, respectively. The active accumulation period was from the 13th to 25th and 15th to 30th day after flowering, respectively. While a continuous increase in the content of triacylglycerols (TAG) was noticed in *H. esculentus*, TAG was at its peak in *H. cannabinus* on the 20th day after flowering. The contents of polar lipids were high in the immature seeds but decreased during maturation. The major fatty acids in both species were palmitic, oleic and linoleic. Cyclopropane fatty acids were present only in TAG of both species throughout maturation period. Cyclopropene and epoxy acids appeared in TAG in traces at the final stages of seed maturation. Oleic and linoleic acids were preferentially esterified at the secondary positions of TAG. The contents of palmitic and stearic acids at the secondary positions were sharply reduced as TAG accumulated.

INTRODUCTION

Changes in fatty acids, both common and unusual, during seed maturation, have received much attention (1,2). We have been carrying out such studies on oilseeds containing unusual acids, e.g., cyclopropene (3), conjugated trienoic (4) and petroselinic (5) acids. The changes in major lipid classes and the constituent fatty acids of 2 species of Malvaceae, *Hibiscus esculentus* Linn. (Okra) and *H. cannabinus* Linn. (mesta, Kenaf, 'Ambadi') form the subject of the present investigation. *H. esculentus* (HE) seeds appear to be a potential source of oil and protein (6,7). *H. cannabinus* (HC), though at present grown in India mainly for fiber (8), is becoming an important commercial source of oil (9). Both seed oils contain linoleic (18:2), oleic (18:1) and palmitic (16:0) acids as major components as in cottonseed oil (*Gossypium* species of Malvaceae) and small amounts of cyclopropenoid (CFA) and epoxy fatty acids (6-12).

MATERIALS AND METHODS

The seeds of HE (var. Pusa Savani) were obtained from the