Novel Technique for the Analysis of Volatiles in Aqueous and Nonaqueous Systems[1]

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ABSTRACT

A simple, efficient, external inlet assembly is described for analyzing volatile components in raw and processed foods by direct gas chromatography and mass spectrometry. The device comprises three sections: a sample inlet, a condenser, and a six-port rotary valve. The versatility and effectiveness of this assembly is demonstrated by the analysis and identification of volatiles from diverse food products as salad oils, vinegar, and corn-soy food blends. The procedure is rapid, efficient, and offers the following desirable features: it is compatible with all commonly used chromatographs and can accomodate samples of different size; sample volatiles are obtained without use of prior enrichment techniques, at ambient or elevated temperatures; uniform heating enhances volatiles elution, thereby improving sensitivity; moisture and air are removed to facilitate mass spectral analysis; the closed nature of the system minimizes loss of low molecular weight volatiles during elution, thus producing a highly reliable profile of volatiles.

INTRODUCTION

The detection and identification of volatile components in food products has recently become the object of extensive research. Advancements in the fields of gas chromatography (GC) and mass spectrometry (MS) have significantly enhanced progress in this area. In 1971, Dupuy et al. (1) described a novel direct GC procedure for determining volatiles in vegetable oils, requiring no prior enrichment procedures. The technique was further refined, and in 1973 was applied to the direct gas chromatographic examination of volatiles in salad oils and shortenings (2). Williams and Applewhite (3) have reported high correlation of the flavor scores of vegetable oils with volatiles profile data. Jackson and Giacherio (4) also found excellent correlation between volatiles and taste panel flavor score of aged soybean oils with their versatile modification. Other works (5-9) have confirmed the validity of instrumental analysis as a reliable means of assessing food flavor, quality, and shelf life.

When combined GC and MS (GC/MS) determinations are performed, it is essential to remove water from the volatiles to avoid incompatabilities in the mass spectral process. The use of an adsorption/desorption method (10-12) overcomes the moisture problem, but this technique is somewhat complex, and in certain instances may result in loss of low molecular weight volatiles as moisture is vented to the atmosphere. This paper describes a simple, single-operation, closed inlet device which facilitates the removal of water from volatiles in GC/MS analysis. The system is compatible with all commonly used gas chromatographs and can be constructed to accomodate sample sizes according to need. The unique rechargeable condenser removes sample moisture, and the closed arrangement of the inlet minimizes the possibility of losing low molecular weight volatiles, thus enhancing the sensitivity and effectiveness of GC/MS determinations.

EXPERIMENTAL PROCEDURES

External Closed Inlet Device

Inlet assembly. The body of the inlet assembly is constructed of a 1/4 in. stainless steel (s.s.) pipe nipple of suitable length to accomodate the sample size anticipated in normal usage. Located at both ends is a 1/4 in. s.s. pipe cap which has been drilled and tapped to accomodate a 1/16 in. Swagelok fitting.

A perforated silicone septum is positioned at each end of the nipple between the pipe caps, covering both rims of the 3/8 in. o.d. glass tube liner containing the sample. At the top end, a 1/16 in. s.s. Quick-Connect fitting is attached to connect the inlet to a source of carrier gas. Figure 1 presents a schematic of the inlet assembly. In addition to the components shown, a silicone, rubber-embedded heating tape is wrapped around the outside of the assembly body to provide efficient and uniform heat as required.

Condenser assembly. The condenser consists of a 1/4 in. s.s. tube approximately 2 ½ in. long, containing a 60/80 mesh support coated with sodium sulfate. The contents are retained by a plug of glass wool at each end. A 1/8 in. copper coil surrounds the tubing to cool the condenser with a flow of air during elution of volatiles. Figure 1 shows the condenser assembly attached to the inlet by means of a ¼ in. s.s. Swagelok nut. In addition to the condenser components shown, a silicone, rubber-embedded heating tape is wrapped around the copper coil to supply heat to regenerate the sodium sulfate to an anhydrous state, as required.

Six-port rotary valve. The six-port rotary valve shown in Figure 1 was obtained from Carle Instruments, Fullerton, California, catalog item No. 5521. Located between the GC column and condenser assembly, this valve is used in one of two positions: the "inject" position or the "run/purge" position. The configuration of both positions is shown in Figure 1, and the specific function and use of each is discussed next.

GC and GC/MS Procedure

Analysis of anhydrous samples. When samples are free of water and do not require addition of water for efficient GC or combined GC/MS analysis, the following procedure is used. A sample of selected weight is placed in the glass GC liner and supported or retained in place, with plugs of volatile-free glass wool. The liner is inserted into the top of the inlet assembly and the pipe cap is hand-tightened. This positions the liner between the two perforated silicone septa, forming a seal at the rims on both ends. The helium carrier gas is then connected to the pipe cap via Quick-Connect fitting. Heat is applied to the inlet and the condenser assemblies by means of the silicone, rubber-embedded heating tapes. The rotary valve is set to the inject position, forcing GC carrier gas to flow through the sample in the heated inlet. The volatile components thus are stripped from the sample and pass through the heated condenser to the top of the relatively cool GC column, where they are adsorbed. A stripping period of 20-30 min is sufficient for most samples. When stripping of volatiles is complete, the rotary valve is set to the run/purge position. In this mode, the GC column is isolated from the inlet and condenser assemblies, and volatiles adsorbed at the top of

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the column can be temperature-programmed in the usual manner for GC or combined GC/MS analysis. As the volatile components are processed through the GC or GC/MS instruments, heat to the inlet assembly is turned off, the cooling inlet is opened, and the spent liner is removed and replaced with an empty glass liner which retains the passageway for a continuing flow of carrier gas. Maintaining the flow of gas to the atmosphere promotes cooling of the inlet and thoroughly flushes the condenser assembly in preparation for a subsequent sample. When cooling is complete, the empty liner in the inlet assembly is replaced with a new liner containing the next sample for analysis.

Analysis of samples containing moisture. Some samples for analysis are inherently aqueous (e.g., vinegar, mayonnaise, etc.) or they may be anhydrous but require the addition of several mg of water to promote efficient steam distillation of the volatiles. The procedure for samples containing moisture is as follows. The sample is prepared in a glass liner as described earlier under *"Analysis of anhydrous samples,"* and placed in the inlet assembly. The rotary valve is set to the inject position. Compressed air for cooling the condenser is turned on, and heat is applied to the inlet assembly. Thus, carrier gas flows through the heated inlet and elutes the sample's volatiles and moisture, which pass to the cool condenser. Moisture is trapped there by the sodium sulfate matrix while volatiles flow on to the top of the GC column, where they are adsorbed. When elution is complete, the rotary valve is changed to the run/purge position. The sample volatiles are then processed for GC or combined GC/MS analysis as described under *Analysis of anhydrous samples.* As the volatiles are being processed, heat to the inlet assembly is discontinued, and heat to the condenser is turned on. When the inlet assembly has cooled sufficiently, it is opened and an empty glass inlet liner is substituted for the spent liner to restore the flow of carrier gas. Heat to the condenser (ca. 130 C) is maintained for 15 min. The combination of heat and the sweep of carrier gas through the condenser regenerates the sodium sulfate to an anhydrous state in preparation for the next analysis. Compressed air for cooling is passed through the coils surrounding the condenser to promote a return to ambient temperature.

Materials

The following materials were used in these studies: Tenax GC (a 2,6-diphenyl-p-phenylene oxide-based porous polymer) 60/80 mesh, and Poly MPE (poly-m-phenoxylene) from Applied Science Laboratories, State College, PA.; Quick-Connect fittings, from Crawford Fitting Co. Solon, OH; potassium carbonate, anhydrous, from Fisher Scientific Co, Fair Lawn, NJ; Teflon O-rings (conditioned at 200 C for 2 hr) from Alltek Associates, Arlington Heights, IL; Pyrex glass wool (conditioned at 200 C for 16 hr) from Corning Glass Works, Corning, NY; An experimental corn-soy food blend was supplied by the Northern Regional Research Center, Peoria, IL; flavor-scored soybean oils were obtained from the A.O.C.S. Flavor and Nomenclature Committee; the rancid soybean oil was an experimental laboratory oil; and commercial red wine vinegar was purchased locally.

Instruments

Standard direct GC or combined GC/MS was performed with a Tracor Model 222 GC interfaced with a Hewlett-Packard (Quadrapole) spectrometer Model No. 5930-A. Ionization Potential of the spectrometer was 70 eV, and the scan range was 21 to 350. Data processing was accomplished with an INCOS 2000 mass spectrometer data system.

The adsorption-desorption GC technique used for the corn-soy food blends has been reported previously (10-12).

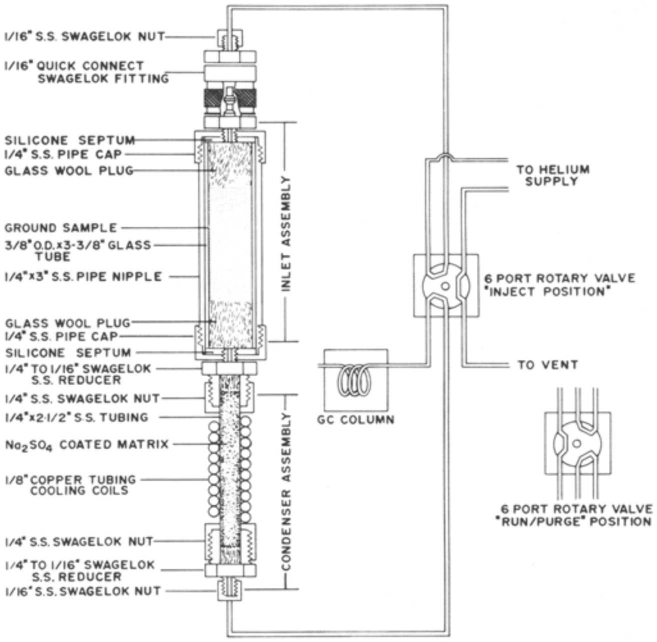

FIG. 1. Diagram of external closed inlet device, showing both configurations of the 6-port rotary valve.

The inlet assembly used has been described in detail in EXPERIMENTAL PROCEDURES.

GC Conditions

The following conditions were used for all three types of instrumental data obtained in this study.

Columns. Stainless steel U tubes, 1/8 in. o.d., 8 ft long, packed with Tenax GC that had been coated with 7% Poly MPE.

Flow rates. Helium carrier gas, 35 ml/min in each column; hydrogen 50 ml/min to each flame, and air 660 ml/min (fuel and scavenger gas for both flames).

Temperatures. Inlet temperatures: 160 C for the corn-soy food blend; room temperature and 120 C for the rancid soybean oil; 160 C for the flavor-scored soybean oils; and 130 C for the red wine vinegar. In all instances, the detector was at 270 C, and the column temperature was raised from 35 C to 80 C in 5 min, followed by temperature-programming 4 C/min to a final hold of 220 C until the column was clear.

Attenuation. 1 X 32.

Sample preparation. All samples were prepared as described under GC and GC/MS Procedure, *Analysis of anhydrous samples,* with the exception of the red wine vinegar. For this sample, 200 mg of anhydrous potassium carbonate was added to the glass inlet liner and "sandwiched" in place with plugs of glass wool. The red wine vinegar was absorbed on another wad of glass wool in the inlet liner and finally capped with additional glass wool. The liner containing the vinegar was placed in the GC inlet assembly in a manner to allow carrier gas to flow through the sample and then through the potassium carbonate, where acid components were trapped and prevented from reaching the GC column.

Sample size. Corn-soy food blend, 1000 mg; rancid soybean oil, 300 and 600 mg; flavor-scored soybean oils, 600 mg; and red wine vinegar, 100 mg.

RESULTS AND DISCUSSION

The analysis of volatiles by direct GC often requires the presence of water (10). In many products, moisture functions as a release agent that enhances the liberation of

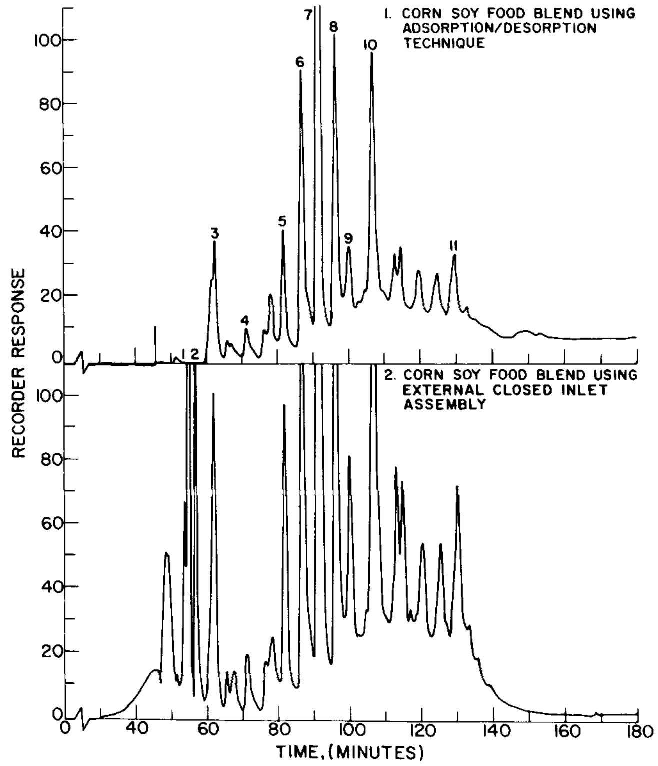

FIG. 2. Chromatograms of corn-soy food blends obtained by the adsorption-desorption technique and the closed inlet device. (1) acetaldehyde; (2) ethanol; (3) methylene chloride; (4) chloroform; (5) pentanal; (6) 1-pentanol; (7) hexanal, (8) 1-hexanol; (9) 2-heptanone; (10) 2-pentylfuran; (11) trans-2-,trans-4-decadienal.

volatile components related to flavor quality. The identification of these volatiles by combined GC/MS analysis contributes greatly to the overall knowledge of a product's aroma and flavor quality. Since water is incompatible with MS operations, the volatiles must be essentially moisture-free prior to such analyses. The unique closed inlet device described was designed specifically to efficiently elute volatiles from various food products and render them anhydrous when MS identification is indicated. Figure 2 shows two profiles of volatiles obtained from a corn-soy food blend that contained ca. 10% moisture. In chromatogram 1, the adsorption/desorption technique (10-12) was used. It reveals a marked absence of volatile components in the area preceeding the 60 min retention time. The compounds not present apparently are low molecular weight materials that presumably are lost when the volatiles are trapped on the polymer material used to remove moisture in the adsorption/desorption process. Two such compounds appear to be acetaldehyde and ethanol. These compounds occur in chromatogram 2, which was obtained from the same corn-soy food blend with the closed inlet device. It is also apparent from chromatogram 2 that method of analysis was more sensitive than for chromatogram 1, since more volatile components appear and each occurs with greater intensity. Methylene chloride, pentanal, 2-heptanone, and trans-2-,trans-4-decadienal are good examples of the increased sensitivity of the closed inlet device.

An essential factor in flavor analysis is the need to avoid sample treatment conditions that tend to alter the composition of the volatiles present. The closed inlet device appears particularly well suited to overcome this difficulty, as demonstrated in Figure 3. The two chromatograms shown were obtained by analyzing a rancid soybean oil with the closed inlet device. Chromatogram 1 shows the volatile materials that were eluted from 600 mg of oil at room temperature. Chromatogram 2 reveals the volatiles eluted from 300 mg of oil at 120 C. Generally, the chromatograms are similar, showing common volatile peaks, chiefly near the 13, 15, 19, 21, 22, and 27 min retention times. These components seem to represent those least affected by heat. The volatiles obtained by heating the oil are of greater intensity, and at least two major peaks are produced after the 30 min retention period that do not occur in the chromatogram of unheated oil. It appears that these peaks represent thermal decomposition products of hydroperoxides. It is significant that the external, closed inlet device can effectively produce a profile of volatiles at ambient temperature, free of such heat-generated components. The number and intensity of peaks eluted by ambient temperature analysis can be increased as required by use of larger sample sizes. Since the inlet assembly can be constructed to accomodate samples of varying quantities, this poses no special problem.

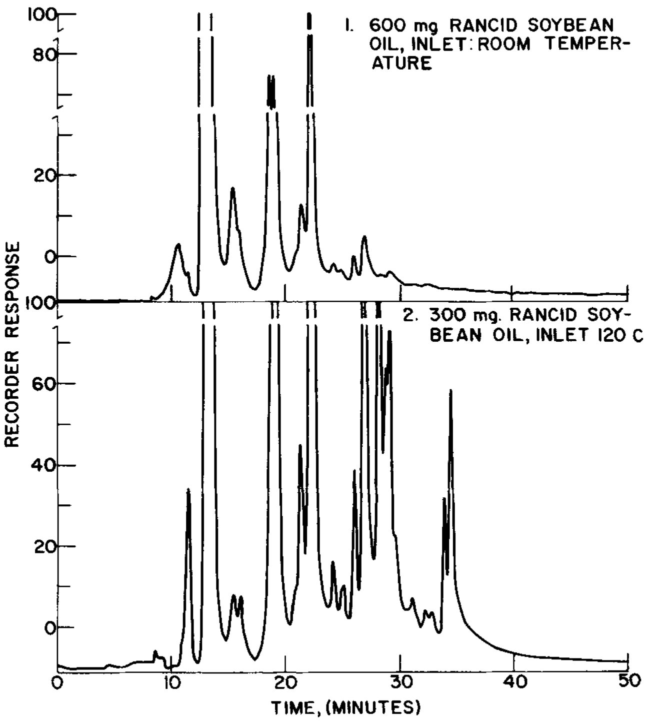

FIG. 3. Chromatograms of rancid soybean oil analyzed with the closed inlet device at room temperature, and at 120 C.

When volatiles are to be measured in a predominantly aqueous food product, the closed inlet device is particularly useful. Figure 4 shows the profile of volatiles obtained from 100 mg of red wine vinegar. In this instance, the condenser assembly efficiently absorbed the moisture present and permitted effective MS identification of the volatile components shown. Potassium carbonate, which had been placed in the inlet assembly as described earlier, removed the vinegar's acetic acid, which otherwise might have contaminated the GC column.

For routine analyses of flavor components, the unique closed inlet device appears to offer greater sensitivity and an improved profile of volatiles than that obtainable by direct GC with standard injection port. The enhanced sensitivity derives from more uniform heating, which is applied along the entire length of the sample tube by means of the silicone, rubber-embedded heating tape. Figure 5 shows a comparison of the analyses by the closed inlet device and standard direct GC of two soybean oils flavor-scored on a 1 to 10 basis. Chromatograms 1 and 2 were obtained from the analysis of a high quality soybean oil with a flavor score of 8. Both chromatograms are generally similar; however, chromatogram 2, produced by the closed inlet device,

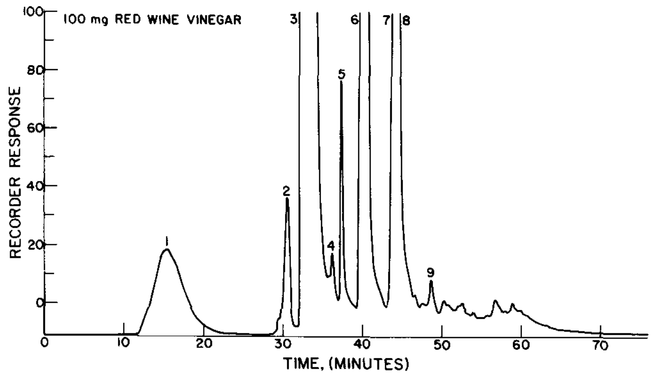

FIG. 4. Chromatogram of red wine vinegar analyzed with the closed inlet device: (1) methanol; (2) acetaldehyde; (3) ethanol; (4) acetone; (5) methyl acetate; (6) ethyl acetate; (7) 3-methyl-1-butanol; (8) 3-hydroxy-2-butanone; (9) iso-amyl acetate.

shows several additional components, and in most instances the volatiles are of greater intensity than those in chromatogram 1. Similarly, a comparison analysis of the poorer quality soybean oil, flavor-scored at 4, is shown in chromatograms 3 and 4. As observed with the higher quality oil, both chromatograms reveal a similar pattern, but chromatogram 4, produced by using the closed inlet device, reveals a greater number of volatile peaks, generally of higher intensity, than those shown in chromatogram 3.

The data suggests that the unique closed inlet device constitutes an efficient, rapid, and versatile method for conducting routine GC or combined GC/MS analyses on food products for the effective study of their flavor components.

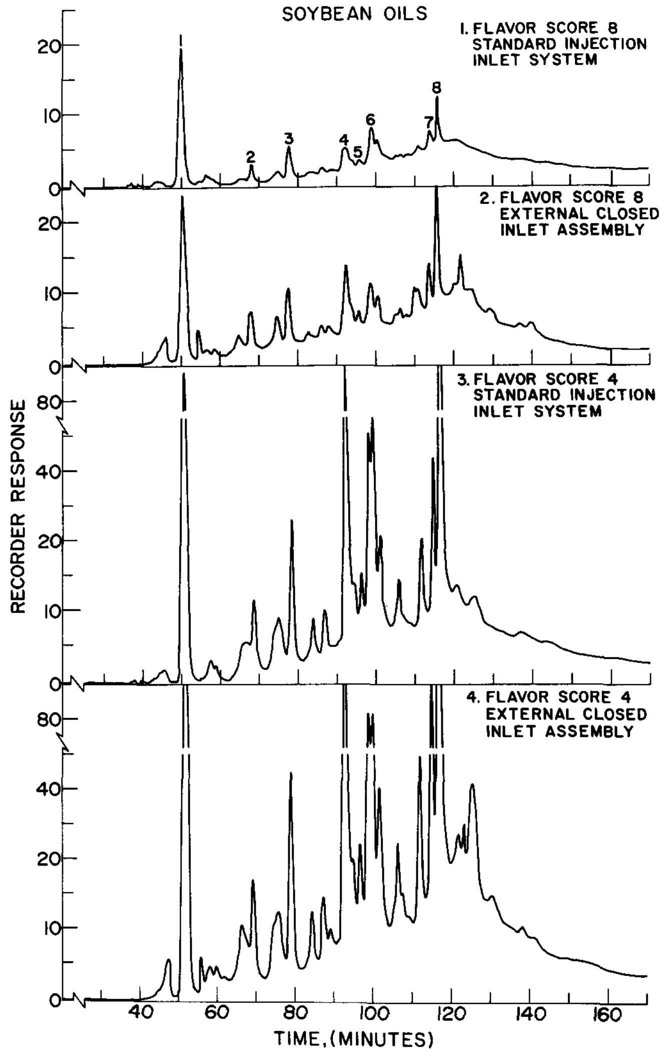

FIG. 5. Chromatograms of flavor-scored soybean oils obtained by analysis with the standard injection inlet system and with the closed inlet device: (1) pentane; (2) pentanal; (3) hexanal; (4) trans-2-heptenal; (5) 2-pentylfuran; (6) trans-2,trans-4-heptadienal; (7) trans-2,cis-4-decadienal; (8) trans-2,trans-4-decadienal.

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