A Preconcentration and Subsequent Gas Liquid Chromatographic Analysis Method for Trace Volatiles[1]

J.A. SINGLETON and **H.E. PATTEE**, USDA Mid-Atlantic Area, Southern Region, USDA, AR, Depts. of Botany and Biological and Agricultural Engineering North Carolina State University, Raleigh, NC 27650.

ABSTRACT

A glass column containing a porous polymer was used to concentrate headspace volatiles from enzymatically mediated reactions and inserted directly into the injection port of a gas liquid chromatography (GLC) for elution and separation of adsorbed volatiles. The polymer column was placed in an entrainment system attached to a water aspirator at 30 psi to collect volatiles produced by the enzymatic reaction. A useful chromatogram was obtained from 1 g of raw material by this method. Volatiles collected in this manner could be stored on the polymer matrix at ambient temperatures without deleterious effects for subsequent GLC analysis. Multiple columns of the same or different trapping material could also be used in the entrainment system.

INTRODUCTION

Several different sampling methods have been used in vapor analysis, including recycling vapor and trapping volatiles in cryogenic traps (1), sweeping the sample with nitrogen and trapping the volatiles in cold traps (2), low temperature-high vacuum distillation and use of differential cryogenic trapping (3) and direct vapor analysis (4,5). Each of these methods has its advantages, depending on the nature of the sample.

Porous polymers have been used in certain chromatographic analyses as columns for gas liquid chromatography (GLC) since their introduction by Hollis (6). Most of these polymers are styrene ethylvinyl benzene or divinyl benzene cross-linked type polymers, and differ primarily in pore size and surface area. Physical characteristics and unique adsorptive and desorptive properties make these polymers ideal for trapping volatile compounds.

Preconcentration of headspace volatiles by polymer trapping prior to GLC analysis is now developing into one of the most widely used techniques available to analytical and flavors chemists. Jennings et al. (7) described a polymer trapping technique for the enrichment of headspace vapors over beer and wine, and Withycombe and Lindsay (8) used a similar procedure to trap volatiles from beer. Jennings and Filsoof (9) used porous polymer traps to concentrate headspace vapors from canned pork meat, cantaloupe, leaded gasoline, peaches, tobacco smoke and wine. In the studies cited, the volatiles were swept onto the traps with nitrogen, then desorbed to cryogenic traps for GLC analysis.

A polymer trapping and subsequent GLC analysis method was used for preconcentrating volatiles from enzymatic reactions that could be easily analyzed by GLC. The polymer preconcentration method reported in this study features simplicity of equipment design, ease of operation, elimination of column development, storage of trapped volatiles at ambient temperatures on the polymer matrix and the elimination of transferring trapped polymer isolates to cryogenic traps prior to elution. This method has been found to be as reproducible as headspace sampling with a syringe when preestablished collection times are adhered to for successive analysis.

EXPERIMENTAL

Apparatus for Trapping Volatiles

A glass liner (83 mm long × 4 mm wide) for the injector port of a gas chromatograph was packed with Porapak Q (80-100 mesh, Waters Associates) as shown in Figure 1 and used to trap the volatiles. The polymer material was sandwiched between 2 silinized glass wood plugs and held in place by 2 rubber septum washers.

The injector port of the gas chromatograph was modified by drilling out the front lip of the port to allow insertion of the polymer trap. This modification did not interfere with the normal operation of the gas chromatograph. Thus, for conditioning or sample analysis the trap was inserted and the rubber septum of the injector port replaced and secured with the septum retainer nut. Polymer traps were conditioned for 1 hr at 200 C with a carrier gas flow of 25 ml/min.

The complete assembly of equipment for trapping headspace volatiles is shown in Figure 2. A 1-ℓ round-bottom flask fitted with a vacuum stopcock was the reaction flask. A glass tee inserted into the entrainment system after the trap was used to vent the system to the atmosphere; and

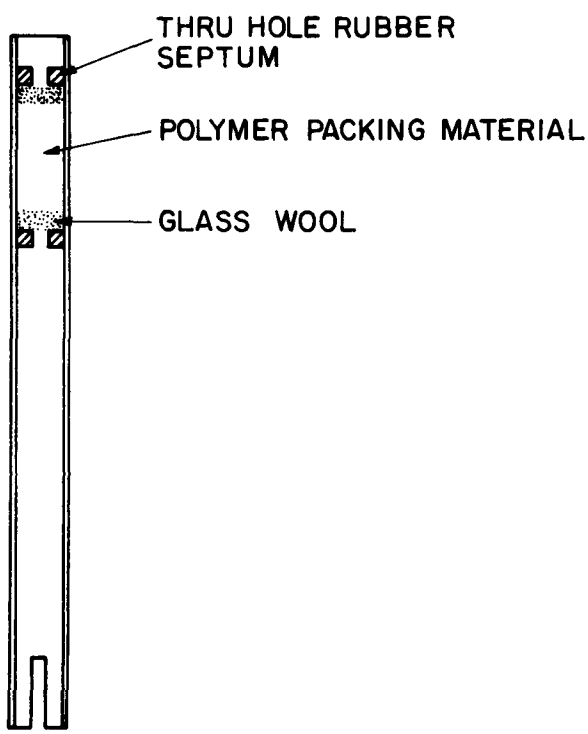

FIG. 1. Injector insert-polymer trap.

[1] Paper Number 5523 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, North Carolina 27650.

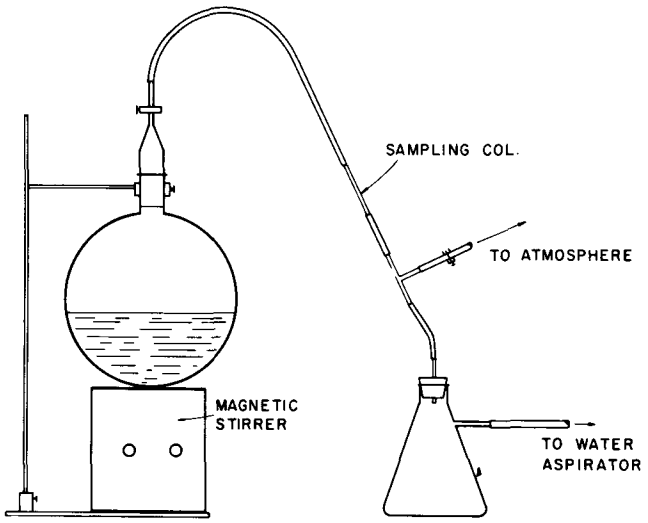

FIG. 2. Apparatus for polymer trapping of headspace volatiles.

the entire system was connected to a water aspirator maintained at ca. 30 psi.

Sample Preparation and Volatile Collection

The sources of volatiles studied were peanut and soybean homogenates prepared as described by Singleton et al. (4). The homogenate sample (100 g) was placed in the reaction flask and stirred continuously at ambient temperatures with the stopcock closed. After 20 min, the stopcock was opened and the headspace vapors pulled onto the polymer trap by water aspiration for 10 min. Headspace volatiles adsorbed on the polymeric material were ready for GLC analysis without further preparation.

Storage of Collected Volatiles

Collected volatiles were often left adsorbed on the polymeric matrix and stored in brown glass bottles at ambient temperatures. Usually the volatiles were left stored for ca. 70 hr before they were analyzed by GLC.

Gas Chromatography

All analyses were performed on a Series 2700 Varian Gas Chromatograph equipped with dual flame ionization detectors. The modified injector port was held at 200 C, which is the isothermal temperature limit of Porapak Q. Separation of the volatiles was achieved on a Chromosorb 102 column (1/8 in. x 6 ft) by programming 125 C to 200 C at 1 C/min with a carrier gas flow of 25 ml/min.

RESULTS AND DISCUSSION

Secondary volatile reaction products are formed from the hydroperoxides produced by the action of lipoxygenase on polyunsaturated acids with a *cis,cis*-1,4 pentadiene configuration. Both the enzyme and substrate are present in peanut and soybean homogenates. Figures 3A and 3B are the gas chromatograms of the volailtes from both types of homogenates. The 2 profiles show the presence of pentane, pentanal and hexanal, but differ markedly otherwise. In model systems with linoleic acid at neutral pH, soybean lipoxygenase produces C-13 and C-9 positional hydroperoxides in 1:1 ratio whereas peanut lipoxygenase produces them in a 4:1 ratio. The ratio of isomeric hydroperoxides, in turn, determine both qualitatively and quantitatively the end products produced (10). Thus, the difference shown in

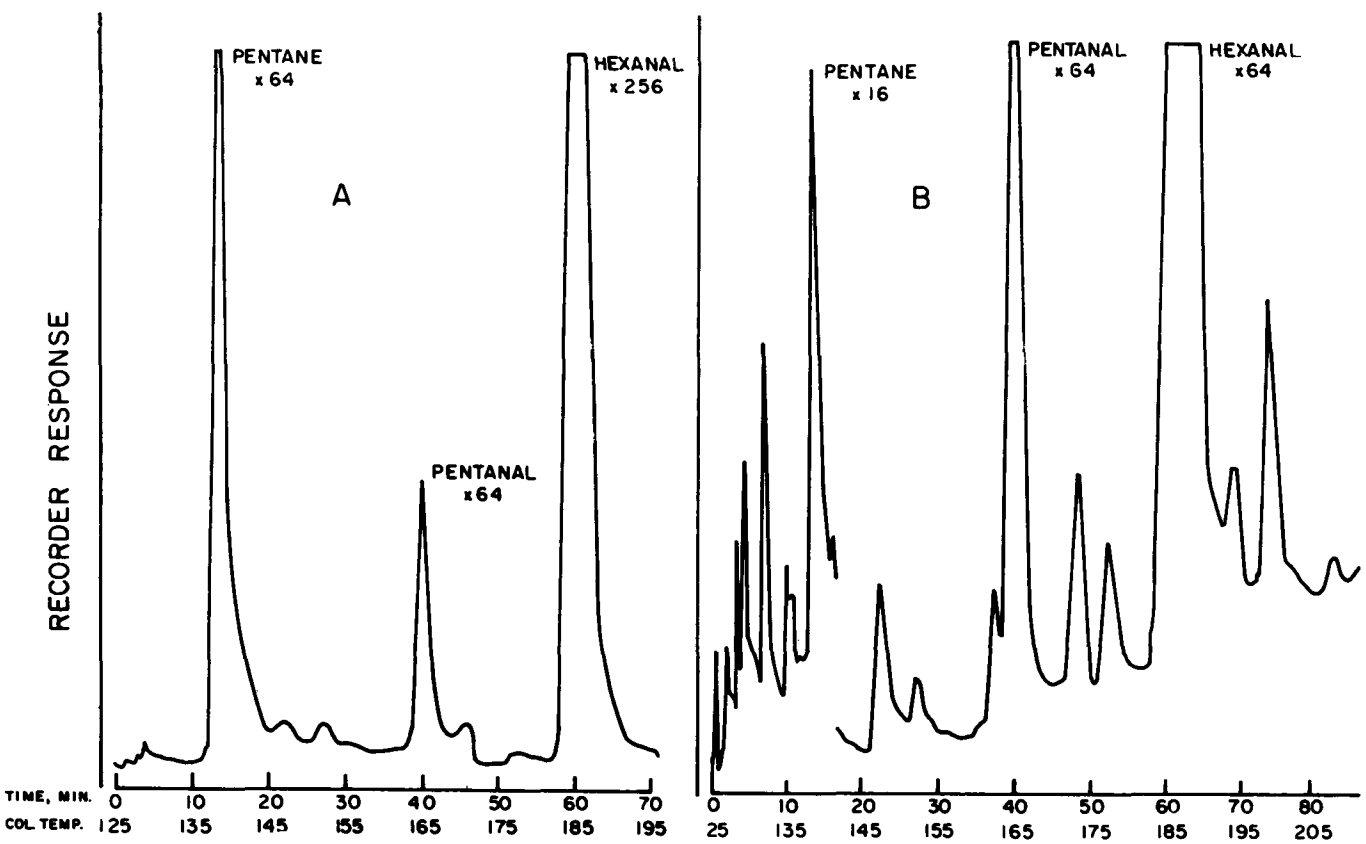

FIG. 3. Lipoxygenase-produced volatiles eluted from polymer traps: (A) peanut homogenate; (B) soybean homogenate.

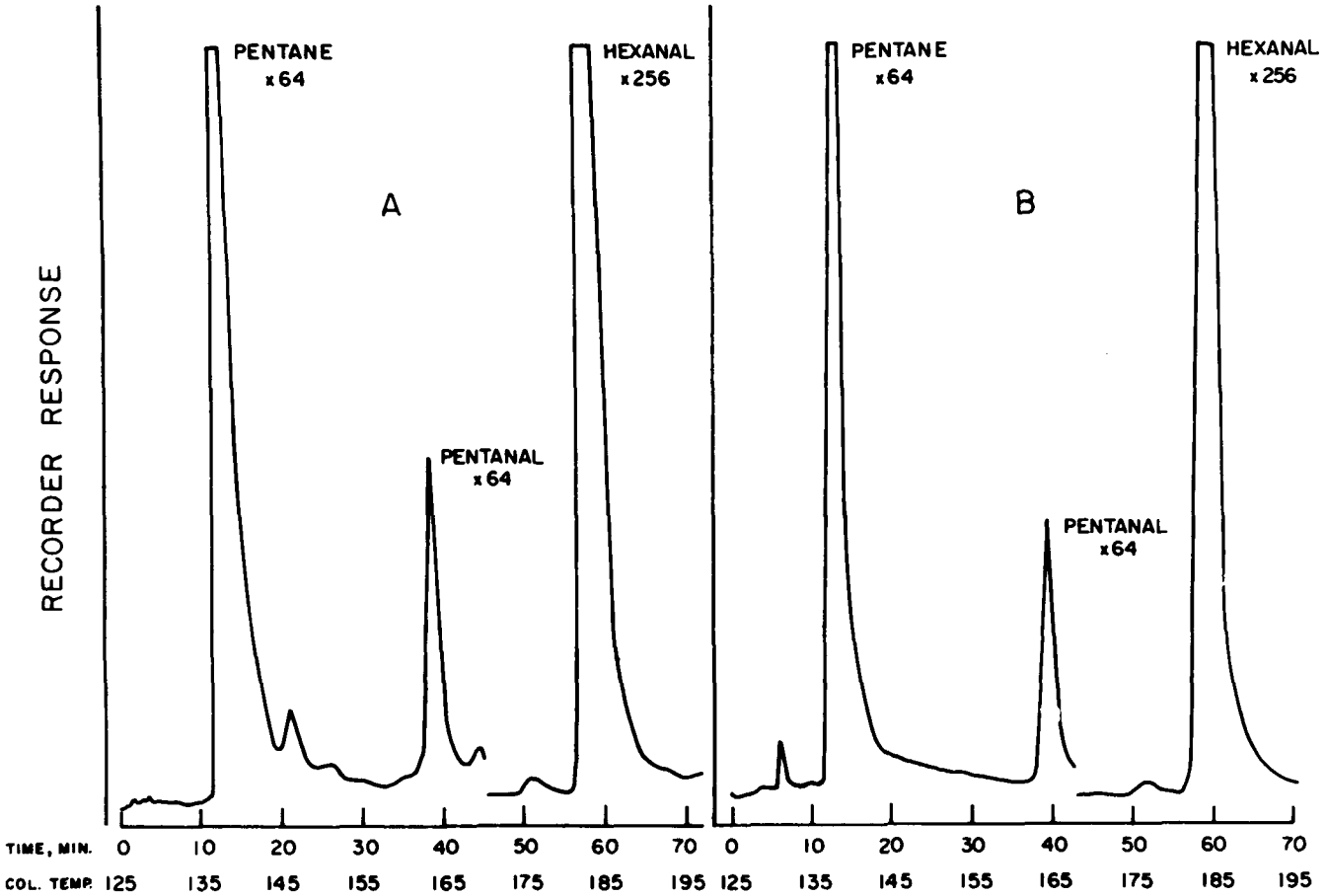

FIG. 4. Effect of storage on polymer-trapped volatiles: (A) chromatographed immediately after collection; (B) polymer trap stored for 70 hr at ambient temperature prior to GLC analysis.

Figure 3 likely reflects different ratios of hydroperoxides produced by the enzyme in the homogenates. The profiles shown are closely similar to corresponding profiles of volatiles that were isolated by low temperature, low pressure distillation and then transferred to a cryogenic trap prior to GLC and mass spectrometry (10). Polymer trapping, as described, requires no cryogenic trapping or transfer from one vessel to another. Therefore, these results attest to the usefulness of the injector insert polymer trapping method for preconcentrating headspace volatiles. Quantitation of chromatograms may be achieved quite easily by adding a known amount of internal standard to the reaction flask prior to collection on the polymer matrix.

Often it is desirable to store volatile isolates for later GLC analysis, especially when the number of samples to be analyzed is large or when the instrument malfunctions. Figure 4 shows the chromatograms of volatiles analyzed (A) immediately after entrapment on the polymer and (B) after storage of the volatiles on the polymer for 70 hr at ambient temperatures. These 2 chromatograms represent 2 different samples and the small peaks seen in each chromatogram are not significant. The similarity between the chromatograms is quite obvious. Therefore, storage of isolated volatiles on a polymeric matrix is quite possible without deleterious effects. The polymer injector insert method has several important advantages over previously reported methods for trapping volatiles with adsorbents: the volatiles need not be flushed from the adsorbent into cryogenic traps for analysis or storage, nor do the volatiles

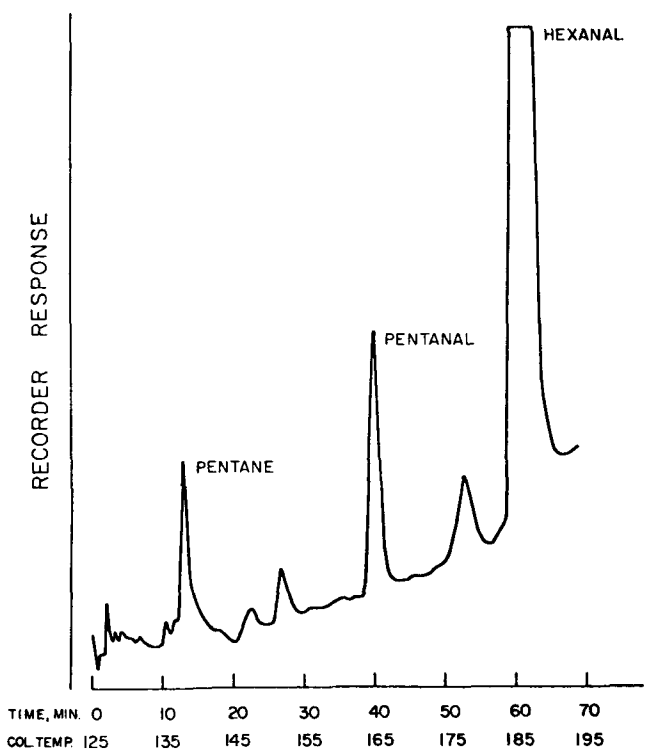

FIG. 5. Headspace volatiles from 1 g of peanuts.

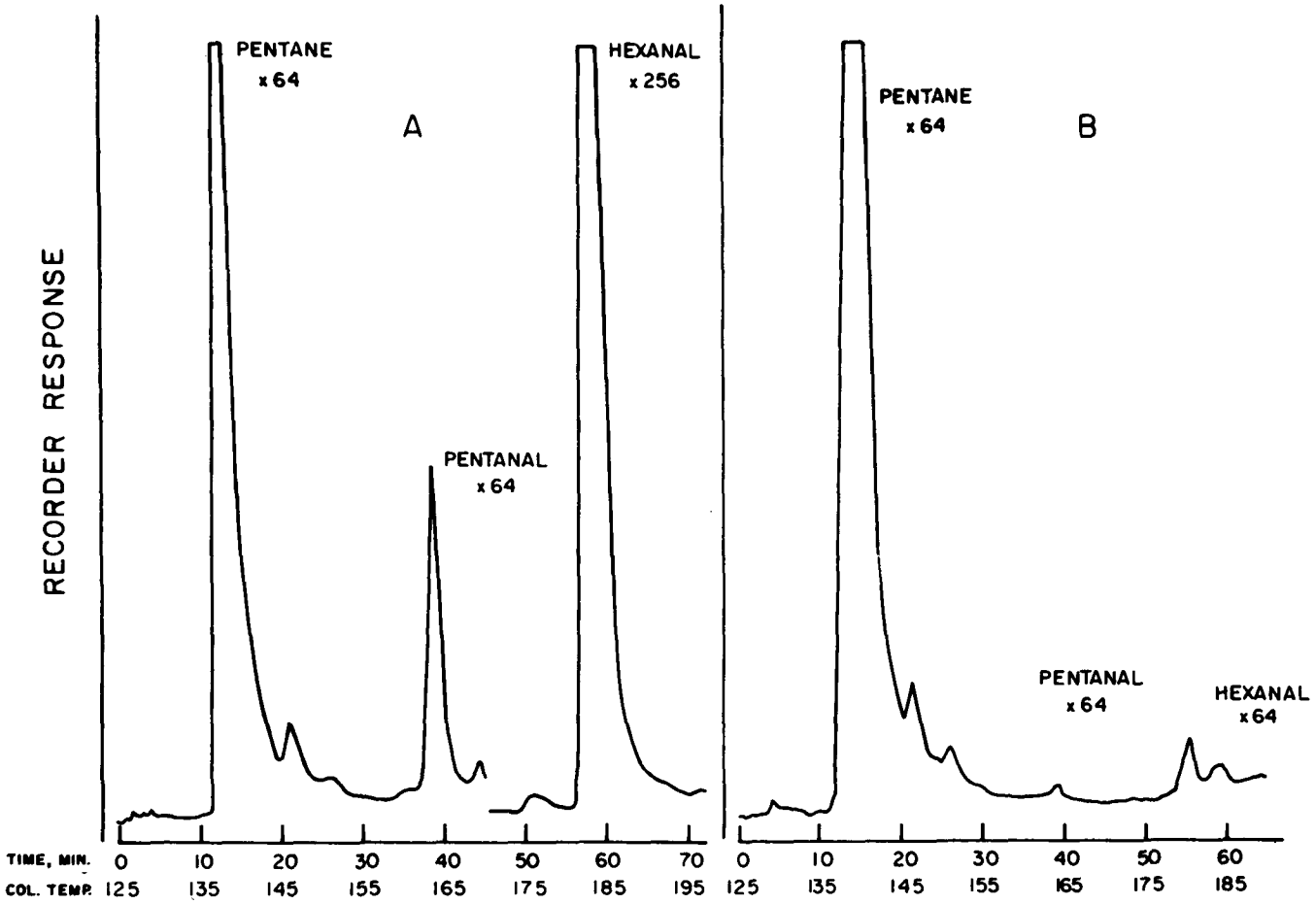

FIG. 6. Profiles of volatiles eluted from adjacent traps: (A) first polymer column; (B) second polymer column.

require storage at low temperatures; therefore, the risk of contamination and loss is reduced.

The amount of material available as a source of volatiles can be a limiting factor; therefore, the volatiles should be trapped as efficiently as possible. To test the efficiency of our trapping method, we macerated 1 g of material (1 peanut), and the volatiles were collected and analyzed as described in the experimental section. A very useful chromatogram (Figure 5) was obtained with prominant peaks for all the secondary reaction products.

The apparatus used to trap volatiles could readily accommodate more than one trap. Figures 6A and 6B are the GLC profiles of volatiles collected in 2 polymer traps placed in tadem. Pentane was collected in both traps due to volatility, polarity and concentration. The use of 2 traps was desirable because the adsorption of all the pentane in one trap would have made the chromatograph analysis difficult. Only traces of pentanal and hexanal were present in the second trap. For analysis of greater scope, a series of traps could be used; and each might contain a different adsorbent. This would add another dimension to polymer trapping in the analysis of multicomponent mixtures.

Polymer trapping of lipoxygenase-produced volatiles offers some definite advantages over solvent extraction techniques and the direct GLC procedure reported by St. Angelo et al. (1). By solvent extraction procedures, pentane, a major secondary reaction product, will be masked upon gas chromatographic analysis unless the chromatographic column, extractant solvent used, and the operating parameters are judiciously selected. The development of porous polymer preconcentration techniques has eliminated many of the problems associated with other methods for analyzing headspace components.

REFERENCES

1. Nawar, W.W., and I.S. Fagerson, Anal. Chem. 32:1534 (1960).
2. Hornstein, I., and P.E. Crowe, Ibid. 34:1354 (1962).
3. Pattee, H.E., J.A. Singleton and W.Y. Cobb, J. Food Sci. 34:625 (1969).
4. Singleton, J.A., H.E. Pattee and T.H. Sanders, Ibid. 40:386 (1969).
5. Singleton, J.A., H.E. Pattee and T.H. Sanders, Ibid. 41:148 (1976).
6. Hollis, O.L., Anal. Chem. 38:309 (1966).
7. Jennings, W.G., R. Wohleb and M.J. Lewis, J. Food Sci. 37:69 (1972).
8. D.A. Withycombe and R.C. Lindsay, Tech. Quarterly 9:XXVII (1973).
9. Jennings, W.G., and M.J. Filsoof, J. Agric. Food Chem. 25:440 (1977).
10. Leu, K., Lebensm-wiss. u Technol 7:98 (1974).
11. St. Angelo, A.J., H.P. Dupuy and R.L. Ory, Lipids 7:793 (1972).

[Received June 2, 1980]