Analysis of Flavor Quality and Residual Solvent of Soy Protein Products[1]

E.T. RAYNER, J.I. WADSWORTH, M.G. LEGENDRE, and **H.P. DUPUY,** Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,
PO Box 19687, New Orleans, Louisiana 70179

ABSTRACT

A simple, direct, gas chromatographic technique is described for eluting and resolving residual solvent and flavor-related volatile components from soy products such as flour and protein isolates. No prior enrichment of volatiles is necessary. A sample, together with a small amount of water, is secured in a glass liner and placed in the heated injection port of a gas chromatograph. The volatiles are rapidly steam distilled from the sample by the heat, moisture, and flow of carrier gas and are adsorbed on the chromatographic column in situ. Residual solvent and other volatiles adsorbed on the column are resolved by temperature-programmed gas chromatography and identified by combined gas chromatography-mass spectrometry. The correlation between taste panel flavor score and concentration of volatile components is significant at the 1% level.

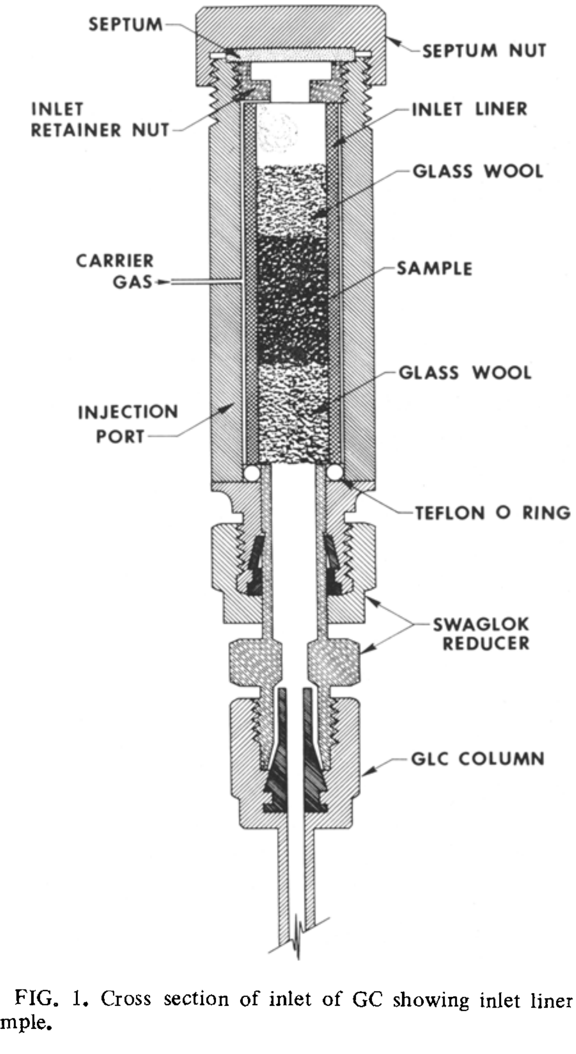

FIG. 1. Cross section of inlet of GC showing inlet liner with sample.

INTRODUCTION

The process of evaluating the flavor quality of food products is complex (1). Establishing effective taste panels for flavor testing is time consuming, quite costly (2,3), and ultimately is limited by the tasters' subjectivity. The need to develop effective procedures for detecting and identifying undesirable flavors in soy products is essential (4). Flavor is one of the main factors limiting the wider use of soybean products in foods (5). An effective direct gas chromatograph (GC) procedure for examining the volatiles in salad oils and shortenings was described in 1973 (6). The method is simple, rapid, sensitive, and requires no prior enrichment of volatiles. It has been shown to be effective in correlating the volatiles present in vegetable oils with the oil flavor scores (7). Recently, in conjunction with mass spectrometry (MS), the method was applied to assess the flavor quality of vegetable oils directly (8). The present work was undertaken to determine the utility of the direct GC-MS method for evaluating flavor quality in such solid products as soy flour, corn-soy foof blends, and soy protein isolates. The technique produces a profile of volatiles that can be used to estimate flavor quality and also to measure the residual solvent content of such products.

EXPERIMENTAL PROCEDURES

Sample Preparation for Gas Chromatography

The general assembly for sample preparation is illustrated in Figure 1 and basically consists of the following: A 3-3/8 in. length of 3/8 in. OD borosilicate glass tubing was lightly packed with a ½ in.-long plug of volatile-free glass wool. A 50- to 200-mg sample was placed on top of the glass wool, capped with another small plug of glass wool, and 100 μl of water was added on top. A clearance of ½ in. was allowed at the bottom of the liner. The septum nut, septum, and retainer nut of the GC were removed, and the liner containing the sample was inserted in the inlet of the GC on top of a Teflon O-ring. When the retainer nut was tightened above the upper rim of the liner, a seal was formed between the base of the inlet and the lower rim of the liner. On closing the inlet system with the septum and septum nut, the carrier gas was forced to flow upward and then down through the sample. Volatiles were readily removed from the sample as the carrier gas swept through the heated liner (120 C) and were absorbed on the top portion of the column, which was maintained at 30 C during the initial hold period. For the corn-soy food blends and soy protein isolates, volatiles were removed from the samples during a period of 30 min. For the soy flours, which contained considerable amounts of residual solvents, a 50 min elution time was used. The liner containing the spent sample was removed from the inlet, the integrator and programer were turned on immediately, and the temperature was raised to 60 C within 5 min. Temperature programming was then begun; when complete, the temperature was maintained on final hold to elute and resolve all of the volatiles absorbed on the column. The oven was then cooled to 30 C in preparation for the next sample.

Sample Preparation for Mass Spectrometry

A silicone membrane separator was used to interface the

---

[1] Presented at the AOCS Meeting, New York, May 1977.

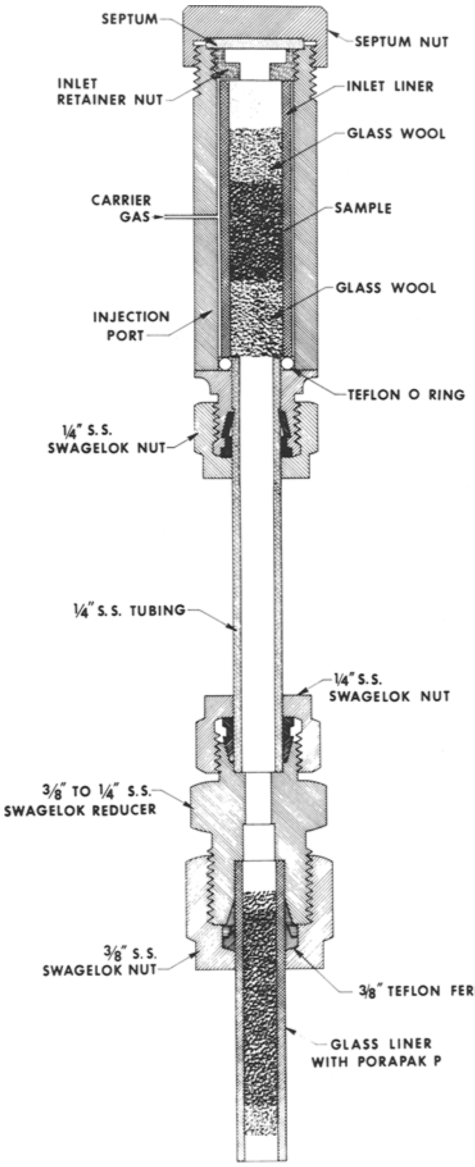

FIG. 2. Inlet liner assembly with Porapak P moisture trap for GC-MS analysis.

GC with the MS. The quantity of volatiles that permeates this membrane and enters the MS varies considerably, depending on the polarity of the compound and its temperature at the membrane. When small peaks from a profile of volatiles are to be identified by MS, it is sometimes desirable to increase the sample size to adequately supply the MS with detectable quantities of the volatiles. Sample sizes may be varied over a range of 50 to 200 mg to achieve the desired response. In making the MS determinations, a slightly modified technique is used for eluting volatiles from the sample because added water in the sample is basically incompatible with MS operation. The modification (9), consists of sweeping the sample's volatiles onto another sample glass liner, which is attached on the bottom of the GC inlet with a 3/8 in Swage-lok adapter and Teflon ferrules, as shown in Figure 2. This extra liner is composed of a volatile-free glass wool "sandwich" containing 0.4 g of Porapak P. With the conditions described for GC, the volatiles are eluted from the sample liner onto the Porapak P liner over a period of 30 min. During this elution period, the Porapak P assembly should be wrapped with a damp towel to cool it. By this technique the sample volatiles are trapped on the Porapak P polymer, and the water, originally needed for elution of volatiles from the sample, is dissipated into the air. The Porapak P liner is then inserted into the GC inlet at 160 C to desorb the volatiles, and the oven is temperature programmed for routine GC-MS analysis. It is essential that the liner be inserted in a position reverse to that used for adsorbing the volatiles on the Porapak P liner, as this enhances desorption by placing the trapped volatiles in immediate proximity to the GC column. If it is not convenient to use the Porapak P liner immediately, it may be stoppered with Teflon-covered corks, placed in a Teflon-lined screw cap vial, and stored in a freezer for a few days until ready for use.

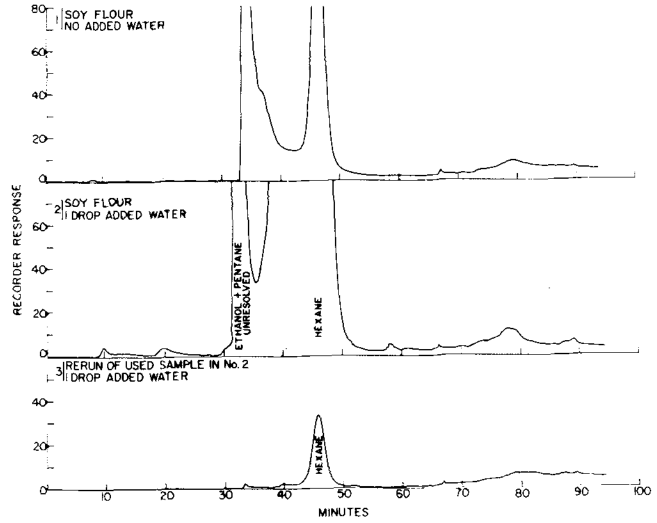

FIG. 3. Profile of volatiles for soy flour, showing the effect of water in releasing volatile components from a sample.

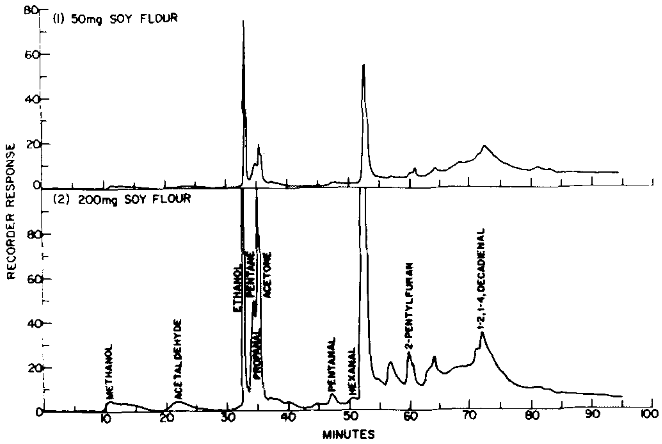

FIG. 4. Profiles of volatiles for soy flour, showing increased sensitivity with increased sample size.

Materials

The following materials were used in these studies: Porapak P (a styrene-divinylbenzene-based porous polymer), 80-100 mesh, from Waters Associates, Framingham, MA; Tenax GC (a 2,6-diphenyl-p-phenylene oxide-based porous polymer) 60-80 mesh, poly MPE (poly-m-phenoxylene) from Applied Science Laboratories, State College, PA; 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; experimental soy flours, corn-soy food blends, and soy protein isolates were used.

Gas Chromatography

The following conditions were used to obtain profiles of

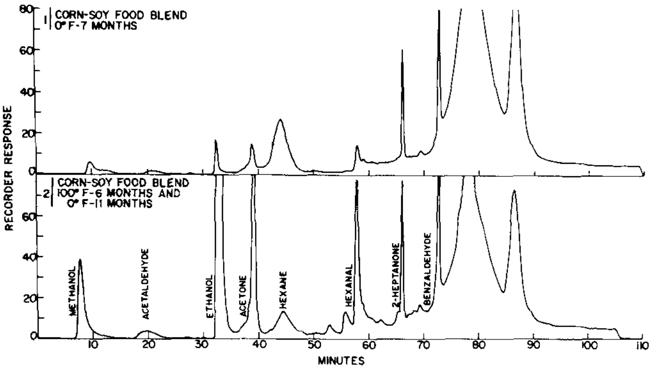

FIG. 5. Profile of volatiles for corn-soy food blend, showing the effect of storage at elevated temperature.

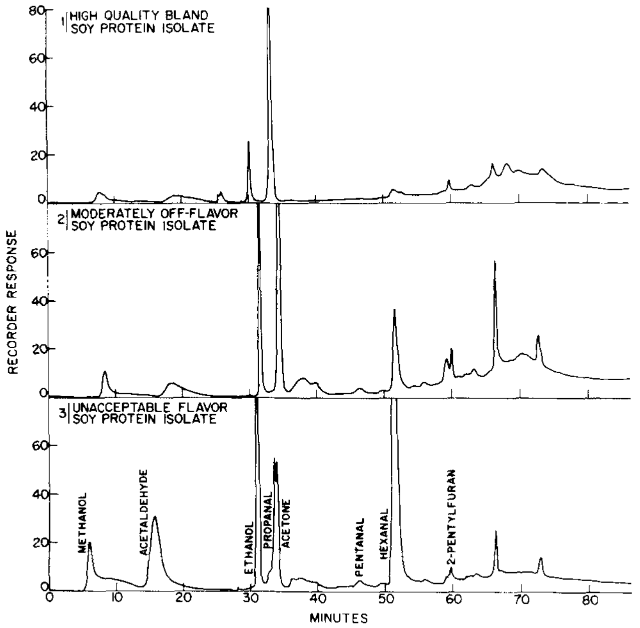

FIG. 6. Profile of volatiles for soy protein isolate, showing the patterns obtained for high quality, moderately off-flavor, and unacceptable flavor products.

volatiles of the soy products examined.

Instrument: Tractor MT-220 GC with dual independent hydrogen flame detectors, a Westronics MT22 recorder, and a Hewlett-Packard Integrator, model 3380 A. Stainless-steel U-tube, 1/8 in. OD, 9 ft long, packed with Tenax GC that had been coated with 8% Poly MPE. This adsorbent was used for the corn-soy food blends and soy protein isolates, and the volatiles were stripped from the sample for 30 min. For the soy flours, the dual compensating columns were packed with Porapak P adsorbent, and the volatiles were eluted from the sample for 50 min.

Flow rates: Nitrogen carrier gas, 60 ml/min in each column; hydrogen, 60 ml/min to each flame; air, 1.2 ft^3/hr (fuel and scavenger gas for both flames).

Temperatures: Inlet temperature was 120 C, detector at 270 C. Column oven temperature was raised from 30 C to 60 C in 5 min, then programmed to rise 3 C/min for 45 min. Final hold was at 195 C until column was clear.

Special seal: A Teflon O-ring was positioned at the bottom of the inlet of the GC.

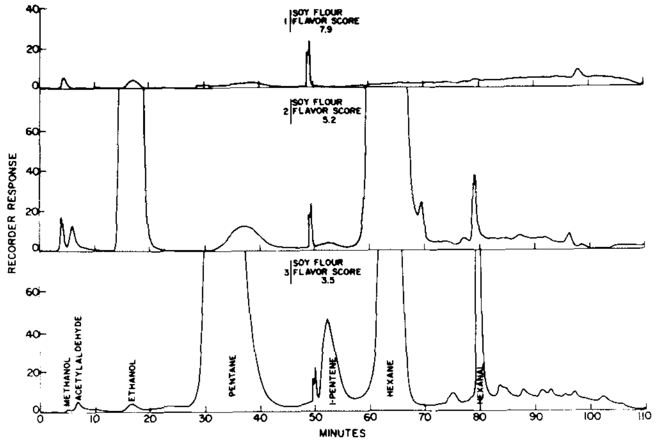

FIG. 7. Profile of volatiles for soy flour, showing the patterns associated with flavor scores.

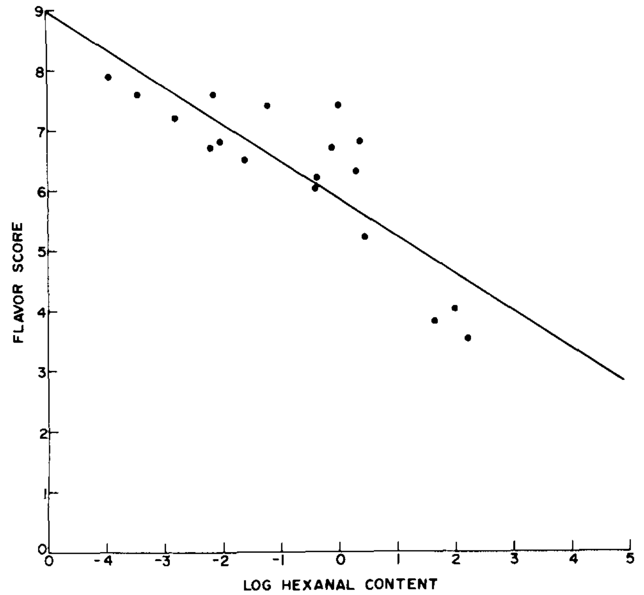

FIG. 8. Linear regression plots of flavor score on log of hexanal content. Flavor score=5.9 - 0.623 (log hexanal).

Attenuation: 10 x 8.

Mass Spectrometry

Instruments: Tracor Model 222 GC interfaced with a Hewlett Packard (Quadrapole) spectrometer, model 5930 A.

Ionization potential: 70 eV.

Scan range: 21 to 300.

Data processing: INCOS 2000 mass spectrometer data system.

GC conditions: For GC-MS analysis, the same conditions were used as those for GC described earlier, except that the inlet temperature was 160 C and the carrier gas was helium.

RESULTS AND DISCUSSION

The analysis of soy protein products for flavor quality or residual solvent by direct GC requires the presence of water. This is demonstrated in Figure 3, where analyses for residual solvent content of soy flours are given. There is a hexane peak present in chromatogram 1; however, when this peak is compared with the peak for hexane in chromatogram 2, where 1 drop of water was added to the sample prior to analysis, it is apparent that the hexane peak of chromatogram 1 is not an accurate indication of the amount of solvent present. Chromatogram 3 in the series confirms that essentially all of the hexane was effectively released in the procedure where water was used, since a rerun of sample 2, using still more water, reveals only a negligible amount of remaining hexane.

Because soy protein products are intentionally formulated to be bland, the volatiles present in he higher quality products will be fewer and in low concentrations. A highly sensitive method, therefore, is needed to obtain a profile of volatiles for assessing flavor quality of these products. The versatility and sensitivity of the direct GC method of analysis for this purpose is clearly shown in Figure 4. Chromatograms 1 and 2 represent profiles of volatiles from identical samples of soy flour. In chromatogram 1, a 50-mg sample was used, and in chromatogram 2, the sample was increased. The magnitude of the peaks obtained from the larger sample, as compared to the smaller one, is pronounced. This is especially important for certain key peak flavor indicators such as hexanal. It is also evident that the sample size of high quality soy products may be increased to facilitate identification by the direct GC-MS system described earlier.

One criterion for determing the validity of instrumental data in flavor analysis consists of analyzing a properly stored sample of known high quality product, and comparing the results with data from the same product analyzed after storage under conditions known to induce flavor deterioration. Such a comparison is shown in Figure 5. Chromatogram 1 shows the profile of volatiles obtained from a good quality corn-soy food blend stored at O F (-18 C) for 7 months. In general, the peaks observed are of moderate intensity. After storage at 100 F (38 C) for 6 months and 0 F (-18 C) for 11 months, however, dramatic changes are noted in the profile of valatiles. Methanol, ethanol, acetone, and hexanal show pronounced increases that suggest flavor deterioration. This is especially true for hexanal, which is a key peak indicator of flavor deterioration in edible oils (8).

Another system for assessing the validity of instrumental flavor measurements comprises analyzing products that have been flavor-scored by trained flavor panelists and comparing the instrumental data with the panelists' observations. Figure 6 illustrates this process for soy protein isolates. Chromatogram 1 represents a high quality, bland isolate. The profile of volatiles for this sample shows few peaks in very low concentration. Chromatogram 2 was obtained from a protein isolate described as moderately off-flavor. In the profile of volatiles obtained for this product, a significant increase is noted in the number and concentration of volatile peaks. Following the same trend, in chromatogram 3 the profile of volatiles shows numerous peaks of high concentration, in particular, the development of acetaldehyde and hexanal peaks. These were essentially absent in chromatogram 1. The protein isolate that produced chromatogram 3 was rated by flavor panelists as unacceptable. The direct GC method of analysis thus provides graphic, tangible, and quantitative evidence of flavor constituents. Trained flavor panelists can detect flavor changes only in a subjective manner and with limited sensitivity.

Another striking example of the ability to relate instrumental data obtained by direct GC analysis to the assigned flavor scores of a product is shown in Figure 7. Here the profiles of volatiles of three soy flours, (flavor scored by trained panelists on a flavor scale of 1 to 10, where 1 is strong and 10 is bland) were obtained by direct GC. The flour in chromatogram 1 had a high flavor score of 7.9, and there are no significant peaks in the profile of volatiles for this product. In chromatogram 2, the soy flour was flavor-scored at 5.2, and the instrumental data on this sample confirms the presence of some hexanal and very large concentrations of residual ethanol and hexane used in processing. The direct GC mehtod of analysis is particularly advantageous because residual solvents in a sample can be measured simultaneously with the analysis for flavor quality. This facet of the method is clearly shown in chromatogram 3. Here the soy flour was flavor scored at 3.5, indicating a product of poor flavor quality. This judgment is substantiated by the profile of volatiles, which indicates that a significant amount of hexanal is present along with substantially large quantities of residual pentane and hexane used in processing. The concurrent analysis of a product for flavor constituents and residual solvent should prove invaluable to the processor of soy products, who must meet requirements in both areas.

TABLE I

Subjective and Objective Analyses of Soy Flours

Taste panel score	GC-predicted score[a]
7.9	8.3
7.6	8.0
7.6	7.2
7.4	5.9
7.4	6.6
7.2	7.6
6.8	7.1
6.8	5.6
6.7	7.2
6.7	5.9
6.5	6.8
6.3	5.7
6.2	6.1
6.0	6.1
5.2	5.6
4.0	4.6
3.8	4.8
3.5	4.5

[a] Derived from the regression equation of flavor scores on log of hexanal content.

To evaluate the above procedures on a quantitative basis, a regression analysis was calculated for 18 flours that had been flavor scored by a taste panel and also analyzed by direct GC. Based on past experience (7,8), the log of the hexanal peak was selected to correlate with the taste panel results. Figure 8 shows the experimental data and the regression line for the simple linear regression of the flavor score on the log of the hexanal content. Although there is a slight indication of curvature in the experimental data, the correlation coefficient for the regression was -0.84, which is highly significant (1% level). The standard error of the regression was 0.75 flavor score units, which compares favorably with the standard error of typical taste panels. Table I shows a direct comparison between the taste panel flavor scores and those derived from the regression equation. The deviation in flavor scores between the panel and the direct GC varied between 0.1 and 1.5 units for the flours.

In its optimum application, the direct GC/MS system of analysis provides the food processor with an accurate means to specify precise standards for his raw materials. Further, the effect of processing variables on flavor quality during production can be readily monitored, and shelf life or stability of the finished product can be reliably assessed by the objective accuracy of instrumentation.

ACKNOWLEDGMENTS

The authors are grateful to G.N. Bookwalter and R.A. Anderson, who provided the corn-soy food blends, and to D.H. Honig, K.A. Warner, and J.J. Rackis, who supplied the flavor-scored soy flours, and to G.I. Pittman and J.H. Conkerton, who drew the figures.

REFERENCES

1. Am. Soc. for Test. Mater., Designation E-480-73:1 (1973).
2. Larmond, E., Food Technol. 27(11):28 (1973).
3. Martin, S.L., Ibid. 27(11):22 (1973).
4. Honig, D.H., K. Warner, and J.J. Rackis, J. Food Sci. 41:642 (1976).
5. Kalbrener, J.E., A.C. Eldridge, H.A. Moser, and W.J. Wolf, Cereal Chem. 48:595 (1971).
6. Dupuy, H.P., S.P. Fore, and L.A. Goldblatt, JAOCS 50:340 (1973).
7. Dupuy, H.P., E.T. Rayner, and J.I. Wadsworth, Ibid. 53:628 (1976).
8. Dupuy, H.P., E.T. Rayner, J.I. Wadsworth, and M.G. Legendre, Ibid. 54:445 (1977).
9. Fore, S.P., M.G. Legendre, and G.S. Fisher, 50th Annual Fall Meeting AOCS, Chicago, Sept. 1976; JAOCS 53:455A, Abstract No. 27 (1976).

[Received July 8, 1977]