Characterization of Used Frying Oils. Part 2: Comparison of Olestra and Triglyceride

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In this study, the analytical scheme presented in our previous paper [Gardner, D. R., R. A. Sanders, D. E. Henry, D. H. Tallmadge and H. W. Wharton, *J. Am. Oil Chem. Soc. 69*:499 (1992)] was used to provide a detailed qualitative comparison between a heated olestra and a heated triglyceride that had been used to fry potatoes. The purpose was to determine if unique components are created in olestra when it is exposed to typical frying conditions. Prior to their analysis, the heated and unheated olestra and triglyceride were converted to their corresponding fatty acid methyl esters (FAMEs) by transesterification. The FAMEs obtained were separated by degree of polarity by means of adsorption chromatography and solid-phase extraction. High-resolution capillary gas and liquid chromatography were used to profile isolated fractions, and detailed comparisons of these profiles were carried out in an effort to disclose components only present in the heated olestra. Spectroscopic data confirmed that by the methods employed, the only detectable qualitative differences between heated triglyceride and heated olestra were attributable to components also observed in unheated olestra. These species are generated during the manufacture of olestra and are not uniquely created by its use as a frying oil. In those chromatographic fractions containing altered fatty acids, no components were observed to be generated at levels ≥5 ppm upon heating olestra that were not also generated upon heating triglyceride.

KEY WORDS: Fatty acid methyl esters, gas chromatography, heated fat, heated fry oil, high-performance liquid chromatography, mass spectrometry, olestra, sucrose polyesters (SPE).

Olestra is the proposed common or usual name for the mixture of octa-, hepta- and hexaesters of sucrose formed with long-chain fatty acids. Olestras have chemical and physical properties similar to triglycerides (1), and functionally they can be used for many of the same food preparation applications as triglyceride-based oils. However, unlike triglycerides, olestra is not metabolized (2) and contributes no calories to the diet.

Under thermal oxidative conditions, typical of those found in frying of food, olestra should undergo the same type of chemical reactions as triglyceride-based oils because the fatty acids in each are similar. Previously, it was observed (3) that the formation of olestra polymers occurs *via* linkage of the long-chain fatty acids, in a manner identical to that observed in triglycerides. However, polymers represent only a fraction of the total products formed upon heating. The purpose of this work was to extend the examination of heated olestra to include a much broader range of components resulting from heating.

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It is well established that many hundreds of different compounds are created during thermal oxidation of triglycerides. To isolate and identify all those compounds created in heated olestra would be largely repetitive of that work already completed for triglycerides. Therefore, the approach taken here was to focus on only those unique components, if any, that are formed during the heating of olestra. This was accomplished through the combined use of sample fractionation, followed by high-resolution chromatographic profiling of the isolated components. Differences between profiles of heated olestra *vs.* those of a heated triglyceride sample were investigated. Matching the starting fatty acid compositions of the olestra and the triglyceride insured that any differences observed were truly unique to olestra and not artifacts resulting from differing fatty acids in the two oils (4,5).

In the first part of this work (6), we presented the details of the fractionation procedures. Here we present the use of this analytical scheme to fractionate samples of olestra, triglyceride, heated olestra and heated triglyceride. Five isolated fractions (I, II, IIIA, IIIB and IV) from each sample were profiled by high-resolution capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC). The digitized chromatographic profiles were enlarged and overlaid by computer to allow highly detailed comparisons of the components found in each of the samples. This allowed potentially unique components to be located in the heated olestra profiles. The chromatographic differences observed were explored further with additional chromatographic and/or spectroscopic methods to verify or dismiss the presence of any unique components.

EXPERIMENTAL PROCEDURES

Sample history. Refined, bleached and deodorized soybean oil was partially hydrogenated to an iodine value of 89. Olestra (76.3% octaester, 23.3% heptaester, 0.2% hexaester and 0.2% pentaester) was synthesized from sucrose and fatty acid methyl esters (FAME) (derived from the soybean oil stock) as described by Volpenhein (7). Both the olestra and soybean oil were heated and used to fry potatoes as described in the first part of this study (6). Fatty acid composition (8), peroxide value (9), % free fatty acid (10) and % polymer (3) data for the unheated and heated samples are presented in Table 1.

Separation methodology. Separation methodologies including sample methylation, silica gel chromatography and solid-phase extraction were executed as described previously (6). Starting sample weights are provided in Table 2.

Separation and identification of fraction components. Instrumental conditions and sample preparation for capillary GC and GC/mass spectrometry (MS) also have been described previously (6). Two-dimensional GC analyses were carried out with the same instrumentation and conditions for the first column as were cited above for GC/MS. Chromatographic sections cut from the first

TABLE 1

Fatty Acid Composition (relative %) and Intact Analyses of Triglyceride and Olestra—Before and After Heating

Fatty acid	Triglyceride (unheated)	Olestra (unheated)	Triglyceride (heated)	Olestra (heated)
C16	10.4	9.7	10.7	10.2
C16:1	0.1	0.1	0.1	0.1
C18	5.4	5.9	5.4	6.0
C18:1	61.7	62.3	62.0	62.4
C18:2	19.9	19.8	18.4	18.1
C18:3	0.4	0.5	0.4	0.4
C20	0.3	0.3	0.4	0.3
C20:1	0.3	0.3	0.3	0.3
C22	0.6	0.6	0.8	0.7
C22:1	—	—	0.1	0.2
C24	0.2	—	0.4	0.2
C24:1	—	—	0.2	0.2
% Free fatty acid	0.04	0.03	0.49	0.35
Peroxide value	1.06	1.04	4.98	3.27
% Polymer	<0.1	0.9	6.4	13.1

TABLE 2

Weights of Fractions Obtained from Separation of Fatty Acid Methyl Esters from Triglyceride (TG) and Olestra (OA)—Before and After Heating

	I	II	IIIA	IIIB	IV	Recovery
Unheated TG (1.996 g)	1.810	0.124	0.020	0.009	0.012	
(% of total)	(90.7%)	(6.2%)	(1.0%)	(0.5%)	(0.6%)	(98.9%)
Unheated OA (2.003 g)	1.877	0.132	0.006	0.005	0.009	
(% of total)	(93.9%)	(6.6%)	(0.3%)	(0.3%)	(0.5%)	(101.5%)
Heated TG (2.009 g)	1.721	0.114	0.062	0.047	0.017	
(% of total)	(85.7%)	(5.7%)	(3.1%)	(2.3%)	(0.8%)	(97.6%)
Heated OA (2.008 g)	1.806	0.107	0.060	0.047	0.035	
(% of total)	(89.9%)	(5.3%)	(3.0%)	(2.3%)	(1.7%)	(102.2%)

column's eluent were reanalyzed on a DB-1 (J&W Scientific, Folsom, CA), 15 m × 0.32-mm i.d. (0.1 micron film) capillary column, which was held at 65°C for 15 min (from injection), then programmed at 5°C/min to 270°C. Head pressures for the first and second columns were held at 9 psi and 5.5 psi, respectively. The outlet of the second column was connected to a flame ionization detector to develop the separations discussed. It was subsequently connected to the mass spectrometer to provide information on the peak identities.

High-performance liquid chromatography (HPLC). Chromatograms were obtained with a Hewlett-Packard 1090L liquid chromatograph (Hewlett-Packard, Avondale, PA). Column eluent was detected with an ACS Model 750:14 evaporative light scattering detector (Polymer Laboratories, Stow, OH) operated at 40°C and a nebulizer pressure of 40 psi nitrogen. Samples were diluted in methylene chloride ($MeCl_2$) such that 500 μg of total sample was injected into the chromatograph. Normal-phase separations of Fraction IIIA were achieved with an Ultrasphere silica column (5 μm, 250 mm × 4.6 mm) (Beckman, San Ramon, CA) maintained at 40°C with a flow rate of 1 mL/min. The following solvent program was used: 99:1 $MeCl_2$/acetonitrile (ACN) (hold 1 min), with incrementally increased acetonitrile of 0.5%/min for 14.5 min. Reverse-phase separations of Fraction IIIB were achieved in two Lichrocart ODS columns (3 μm, 250 mm × 4.6 mm) (EM Science, Cherry Hill, NJ) connected in series and maintained at 40°C with a flow rate of 1 mL/min. The following solvent program was used: Linear gradient from 80:20 ACN/$MeCl_2$ to 70:30 (0.5%/min), linear gradient from 70:30 to 30:70 ACN/$MeCl_2$ (1.6%/min), solvent ramped to 100% $MeCl_2$ in 0.1 min and held for 10 min (run time 40 min). Reverse-phase separation of Fraction IV has been described previously (6).

Chromatographic data collection, integration and plotting. Analog data output from gas and liquid chromatographic detectors was transmitted through Hewlett-Packard (HP) 18542A analog-to-digital converters to a Hewlett Packard 1000 Series A900 computer. These data were integrated with HP Laboratory Automation System (LAS/3350A, Rev.D.01) software. Chromatographic plots were expanded, overlaid and formatted by means of an HP CPLOT/3350A (Rev.C.00.02) software plotting package.

RESULTS AND DISCUSSION

The analytical methodology applied in this study offers a significant advantage over others that have been reported for analyzing complex oil-based systems. Namely, it allows analytical investigation of nearly all of the components contained within heated glyceride and nonglyceride-based frying oils (6). For those chromatographic fractions containing altered fatty acids, it was estimated from internal standards that components with concentrations as low as 5 ppm could be detected in the heated olestra. The data collected did not indicate that there were any components, within the limits of detection stated, that were generated as a result of frying foods in olestra that were not also generated in triglyceride. Although some differences in component levels were observed, no attempt was made at determining their significance because this fell beyond the scope of the current work.

Fractionation. The weights and recoveries of the materials derived from the fractionation of the FAMEs from olestra and triglyceride are given in Table 2. With little exception, the data for olestra closely parallel those for the triglyceride. The greater weights in unheated triglyceride Fractions IIIA, IIIB and IV compared to unheated olestra are due to the presence of naturally occurring components (*e.g.,* sterols), products of incomplete methylation (monoglycerides and diglycerides), and some carry-over of unaltered methyl esters. High-resolution chromatographic studies of the individual fractions are reported below.

Fractions I and II. Fractions I and II are comprised primarily of unaltered FAMEs (6). Profiles of these fractions are reflective of the fatty acid composition displayed in Table 1 and were not affected by heating. No species unique to the heated olestra were detected. One class of modified fatty acids that was known to be present in this fraction, cyclic fatty acid monomers (CFAMs), was measured by a separate procedure (11). The CFAMs generated in the heated olestra were qualitatively and quantitatively comparable to those found in the heated triglyceride (Ianelli and Bross, personal communication, 1990). The increase upon heating was about 700 ppm for both triglyceride and olestra.

Fraction IIIA. Most altered methyl esters that are present in heated oils are found in Fraction III (6). A Sep-Pak (Waters Associates, Milford, MA) separation of this fraction yields Fraction IIIA, which primarily contains monomeric oxidized FAMEs, and Fraction IIIB, which contains mainly dimer FAMEs. From the data shown in Table 2, it appears that comparable amounts of material were generated.

GC profiles of Fraction IIIA were obtained from nonpolar (DB-5) and polar (DB-WAX) capillary columns. For the samples studied, the polar column provided a better separation of the classes of compounds found to be typical of Fraction IIIA materials. Profiles from this column are shown in Figure 1 for heated triglyceride, heated olestra and unheated olestra. They have been scaled to allow relative comparison of the component levels as they were found to exist in the bulk methylated samples. These plots and the data in Table 2 clearly show that most of the material in this fraction of heated oils are products formed during the frying operation. Major components in the IIIA fraction of heated olestra also were present at similar levels in the heated triglyceride. Detailed qualitative comparisons of the minor components in these chromatograms were effectively carried out by enlarging and overlaying the chromatograms.

Of the more than one hundred components detected, only three of those found in heated olestra were not also found in the chromatograms of either heated triglyceride or unheated olestra. These were subsequently revealed in the heated triglyceride by GC/MS analyses, as described below. The chromatographic regions containing these species are shown in more detail in Figure 2, where the components in question appear as small inflections in the chromatographic traces. Repeating the analytical fractionation and chromatographic profiling procedures revealed that these subtle inflections were reproducible. As a consequence, each was subjected to further investigation.

One method commonly used to determine if multiple components exist in poorly resolved chromatographic regions is to employ mass spectrometry for selectively monitoring ions unique to a species of interest. By using isobutane chemical ionization (CI), GC/MS analyses conducted on Fraction IIIA showed that the heated olestra Component IIIA-1 (Fig. 2A) was linked to a base ion of *m/z* 187. At the retention time where the purported unique component eluted in the heated olestra fraction, a low-level component containing the 187 ion also was observed in the same fraction of heated triglyceride. These findings indicated that the component was not unique to heated olestra, as the chromatographic data had implied.

To more fully substantiate the findings from the GC/MS experiments, a two-dimensional GC scheme was used to isolate and re-analyze, on a nonpolar stationary phase, the components eluting from the DB-WAX column between the C10:1 and C11:1 aldehydes (Fig. 2A). The chromatograms that were obtained are shown in Figure 3, with the component containing the *m/z* 187 ion labeled. Figure 4 shows the isobutane CI spectra of the *m/z* 187 peaks found in heated olestra and heated triglyceride. Virtually identical electron ionization (EI) and ammonia CI spectra were obtained from these peaks, providing convincing evidence that this component was not unique to the heated olestra. This same component was later found to be responsible for the only difference disclosed by the nonpolar (DB-5) GC characterizations of Fraction IIIA. Its exact identity has not yet been firmly established.

In addition to Component IIIA-1, Components IIIA-2 and IIIA-3 (Fig. 2A and B) were each analyzed by GC/MS. Spectral data for each of these three components were compared with data collected on the same chromatographic regions of heated triglyceride. In each case, the results were the same. Isobutane CI, ammonia CI and EI spectra of the heated triglyceride sample all contained the same ions as were observed for heated olestra. The chromatographic differences were due to the differences in the levels of the components and not the types of components. These components exhibited mass spectra identical to those measured for larger components in the neighboring region and are believed to be isomeric forms of these neighbors.

To account for nonvolatile species, which would not be analyzable by the GC methodologies employed, both normal and reverse-phase HPLC analyses were carried out on Fraction IIIA. Like the GC analyses, HPLC determinations showed that the best separation of components in the IIIA fractions could be achieved with a polar column.

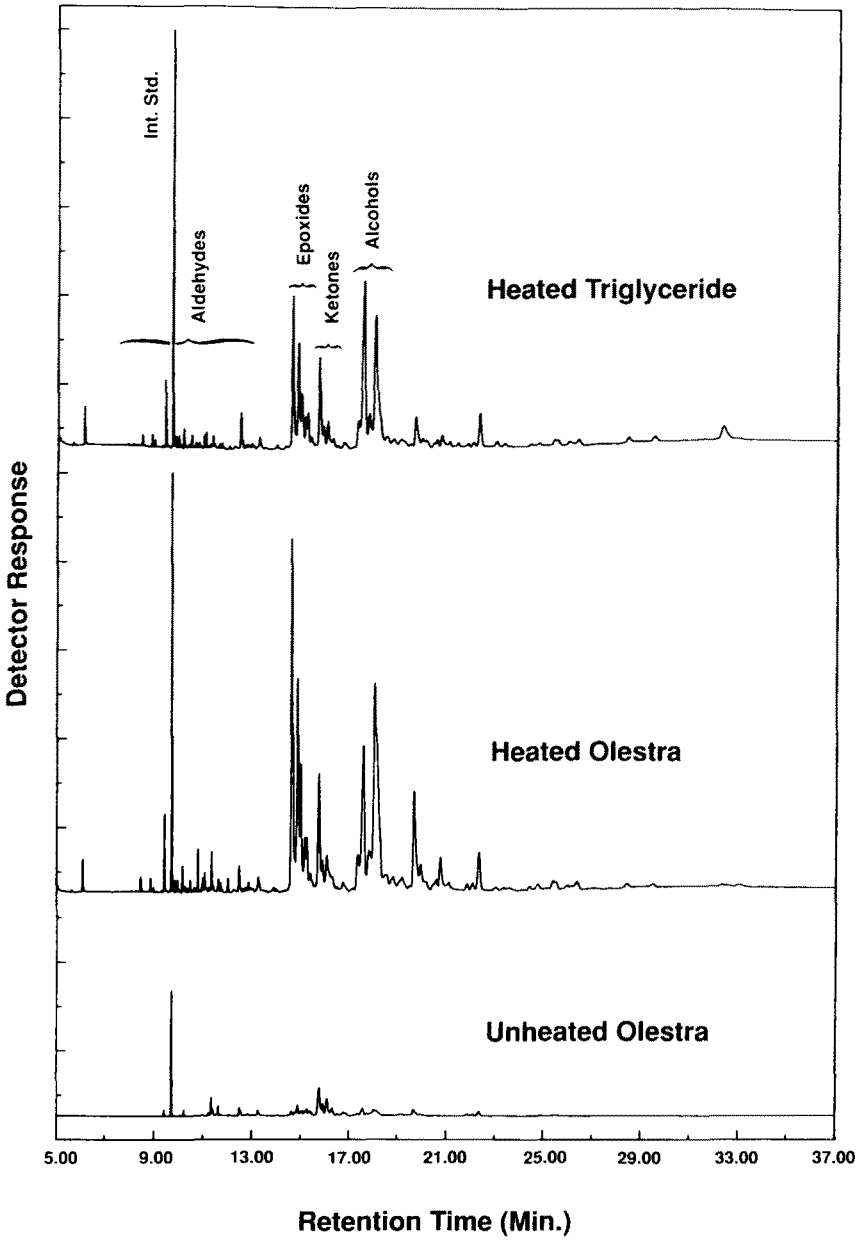

FIG. 1. GC characterizations of Fraction IIIA on a DB-WAX column.

The normal-phase separations of IIIA are shown in Figure 5. Aside from a small amount of detector noise (at approximately 5 min into the heated olestra run), the HPLC profiles did not reveal any features in heated olestra that were not also found in either the heated triglyceride or unheated olestra samples.

Fraction IIIB. GC profiles of Fraction IIIB are compared in Figure 6. Using expanded chromatogram overlays, component-by-component examination of these characterizations did not reveal any species that occurred only in the heated olestra. Despite eluting as a complex series of incompletely resolved components, individual dimer species were still able to be compared and were observed to have been formed at comparable levels. The apparently large quantitative differences between several of the other features in the chromatograms of heated olestra and heated triglyceride were not unexpected. Unaltered FAMEs, which elute very early in the analysis, are more concentrated in the IIIB fraction of heated triglyceride, and result from some variation in the fractionation. Sterols are present at relatively higher levels in the heated triglyceride because they are largely removed during the manufacture of olestra. Higher diglyceride levels in the triglyceride are due to incomplete methylation. Other components found in the heated olestra, but not heated triglyceride, also were observed in the unheated olestra (Fig. 6). However, no attempt was made to identify these because they were not formed upon heating.

Unlike the HPLC profiles obtained for the IIIA fractions, reverse-phase analyses provided better resolution

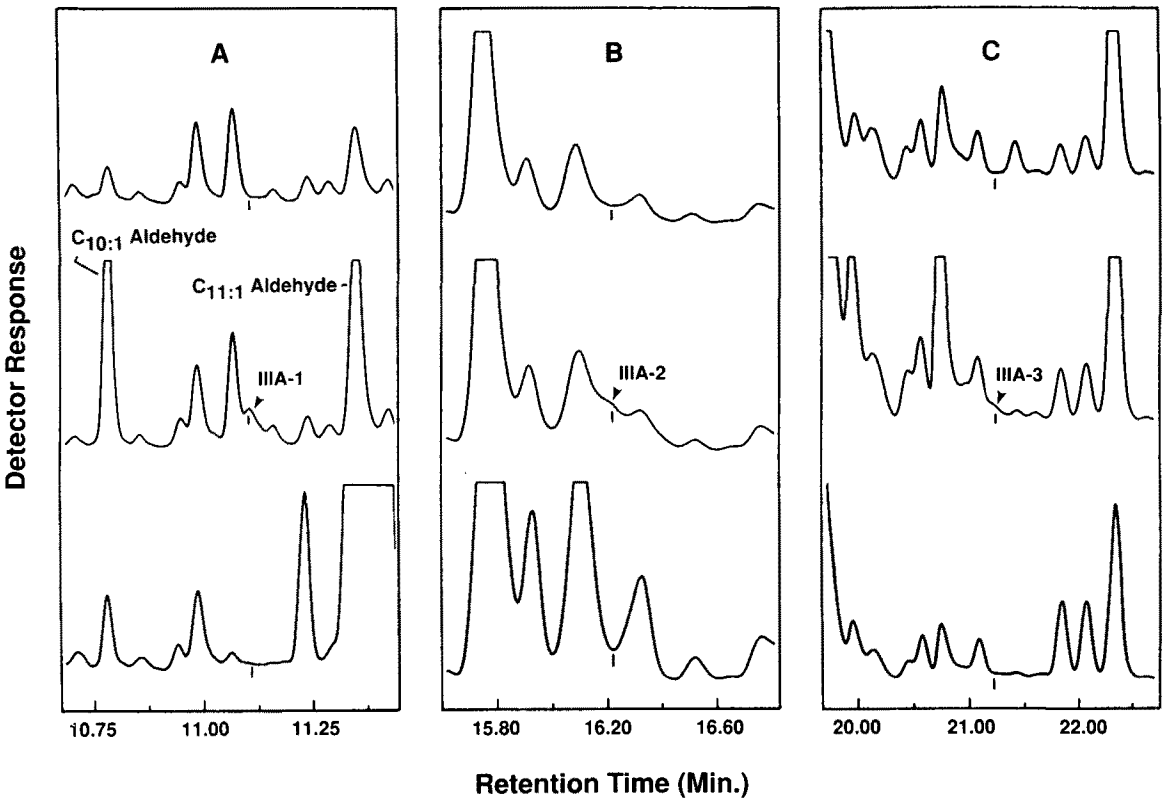

FIG. 2. Expanded GC profiles of Fraction IIIA showing Components IIIA-1 (A), IIIA-2 (B) and IIIA-3 (C) in heated olestra. Profiles are of heated triglyceride (top), heated olestra (middle) and unheated olestra (bottom).

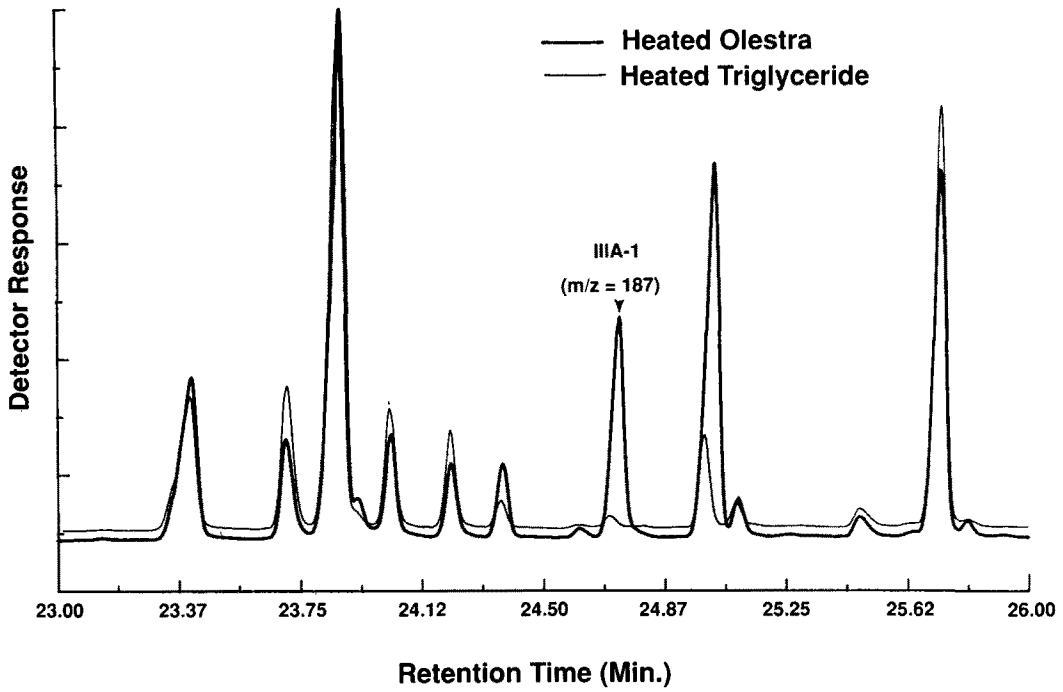

FIG. 3. DB-5 characterization of two-dimensional GC cut of region between C10:1 and C11:1 aldehydes containing component IIIA-1 (Fig. 2A).

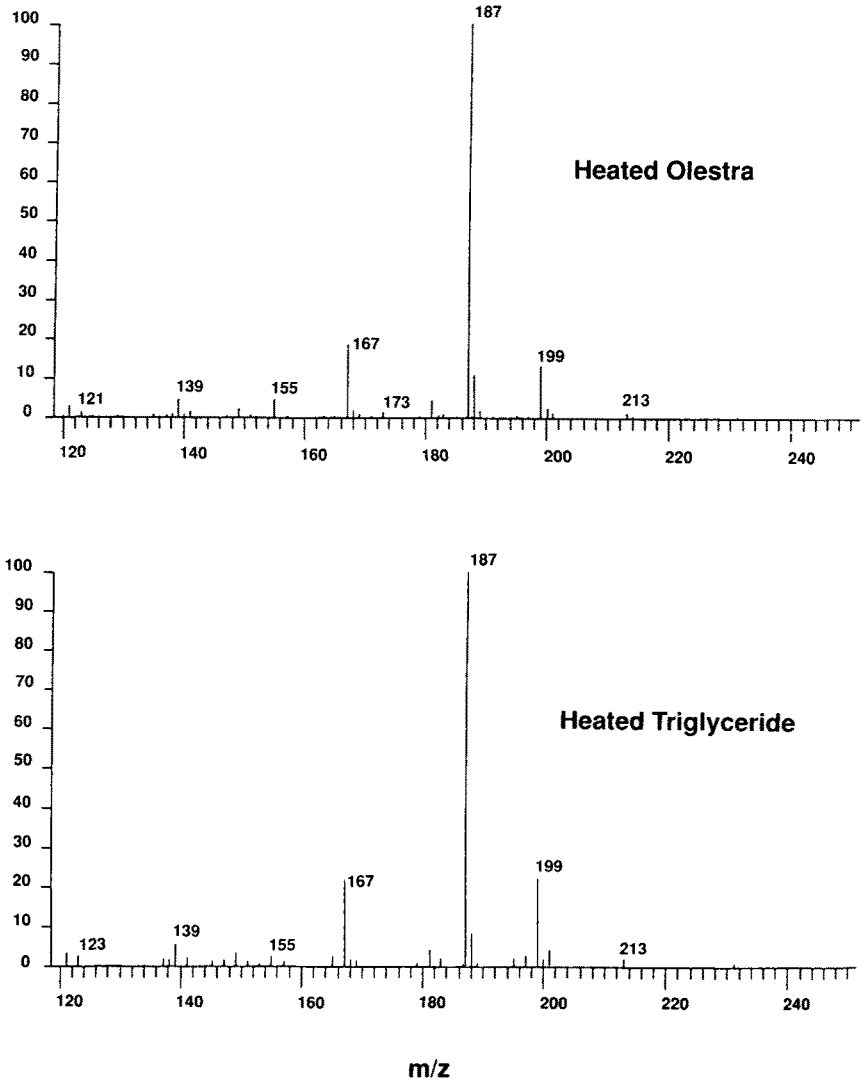

FIG. 4. Isobutane CI mass spectra of the *m/z* 187 peaks in the chromatograms of Figure 3.

of the components of the IIIB fractions than did the normal-phase scheme, and they are shown in Figure 7. The most striking differences between the heated olestra and heated triglyceride HPLC profiles were intense features found in the profile of heated triglyceride that were largely absent in that of heated olestra. These observations are consistent with the GC findings, where such species were identified as unaltered FAMEs, sterols and diglycerides. No chemical species unique to the heated olestra were disclosed from either the normal or the reverse-phase HPLC results.

Fraction IV. Fraction IV consists largely of the oil components resulting from incomplete sample methylation, *i.e.,* partial glycerides and lower (mono- and di-) sucrose esters. They are highly polar in nature and tend to be nonvolatile. Profiling of these components is best performed by HPLC.

Reverse-phase HPLC characterizations of Fraction IV derived from heated olestra, heated triglyceride and unheated olestra are shown in Figure 8. The components in the triglyceride chromatogram were characterized previously by spectroscopic means in our earlier work (6). The olestra profiles were found to contain many components eluting at times that were identical to those measured for heated triglyceride species. Those components not also found in heated triglyceride were observed in unheated olestra, and were not related to the frying operation.

The comparisons presented for Fraction IV, along with those data presented for Fractions I, II, IIIA and IIIB, demonstrate that esterified fatty acids of olestra and triglyceride undergo similar chemical reactions when heated under conditions typical of frying. Because no qualitative differences were observed for the components generated in olestra and triglyceride, the reactions that occur must therefore take place in manners largely independent of the backbone (sucrose or glycerine) to which the fatty acids are attached.

ACKNOWLEDGMENTS

Fractionations of the samples in these studies were carried out by J. Baumgardner. Sample characterizations were conducted by

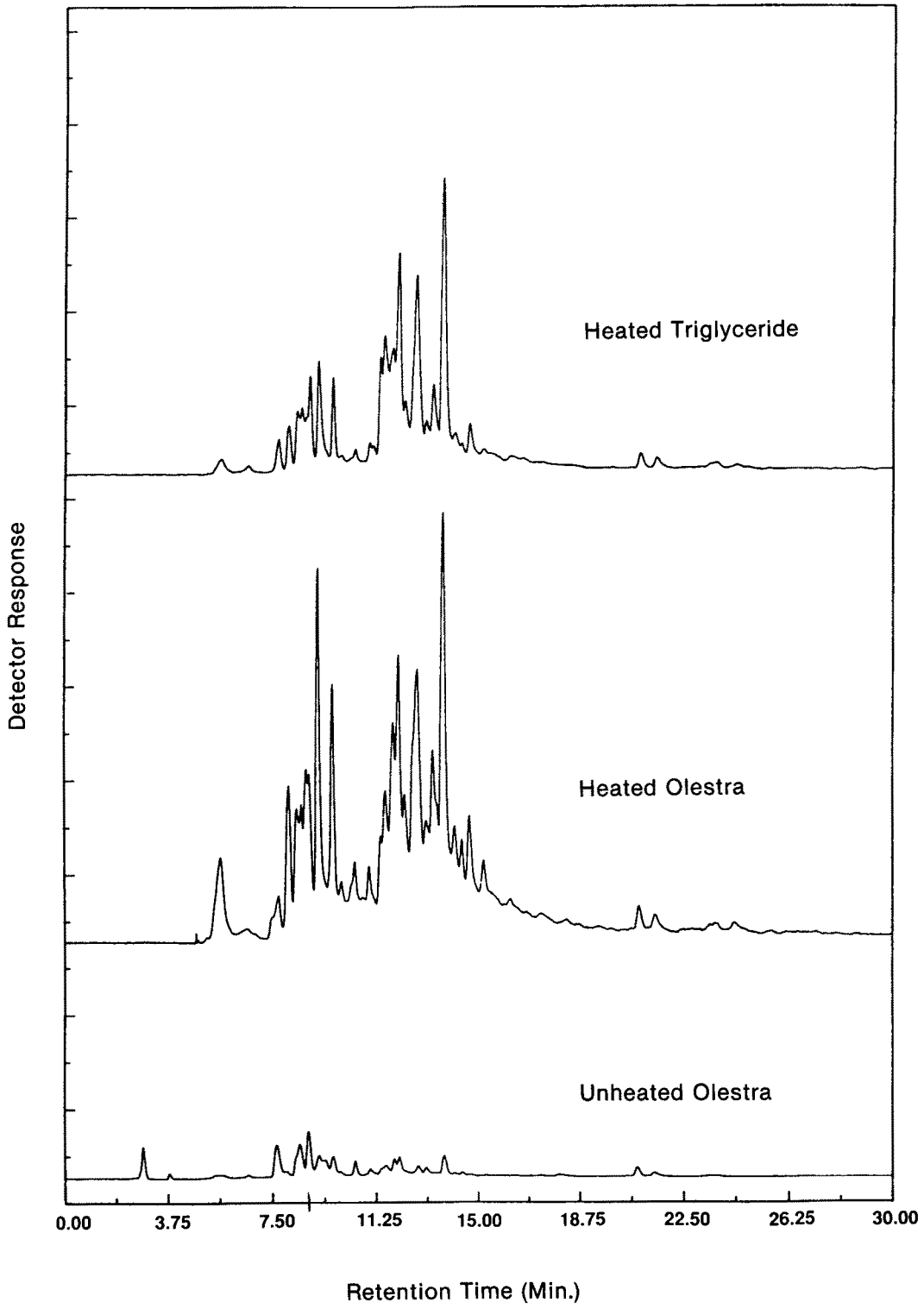

FIG. 5. Normal-phase HPLC profiles of Fraction IIIA.

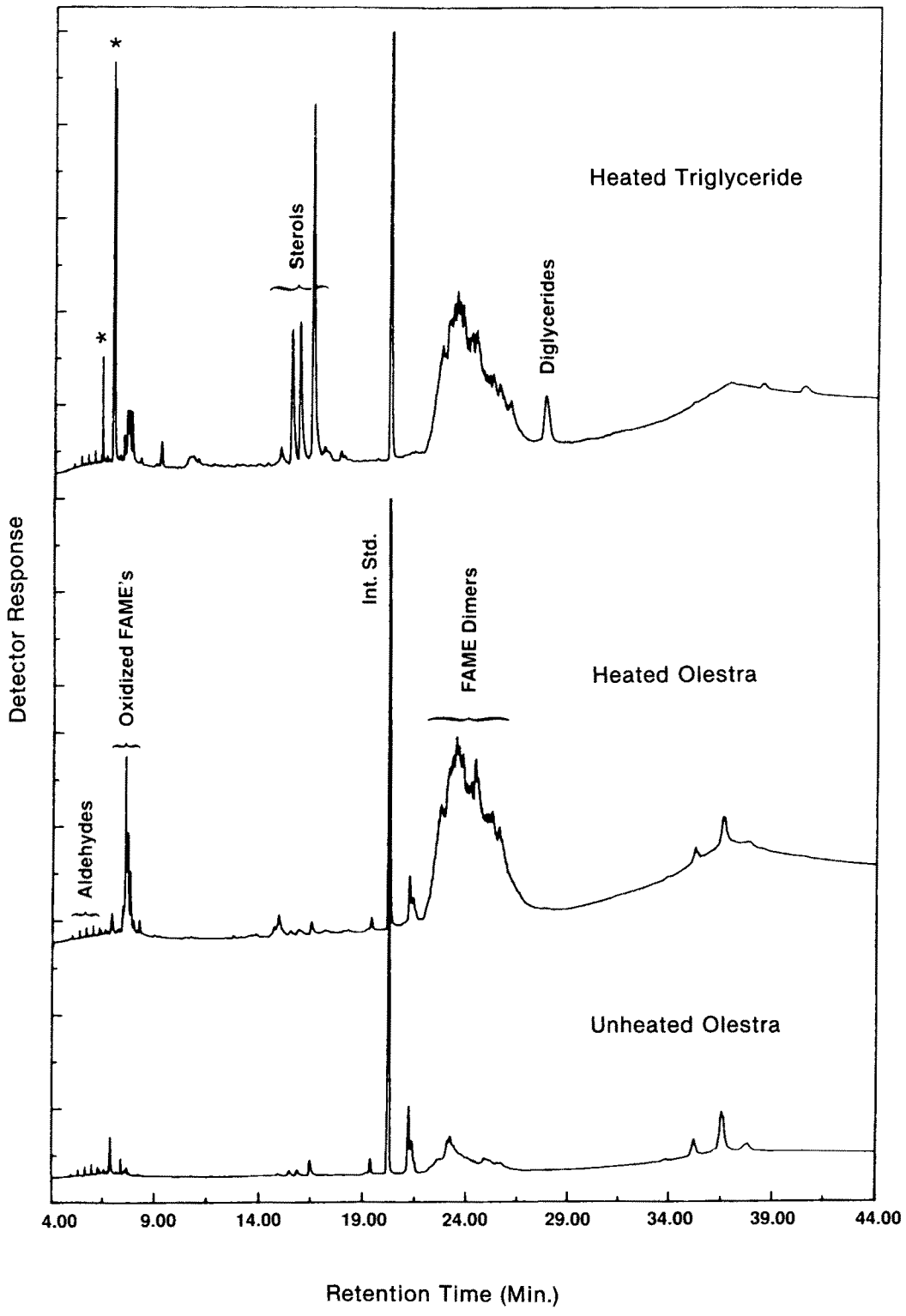

FIG. 6. GC characterizations of Fraction IIIB on a DB-5 column. (Asterisks identify unaltered FAME components.)

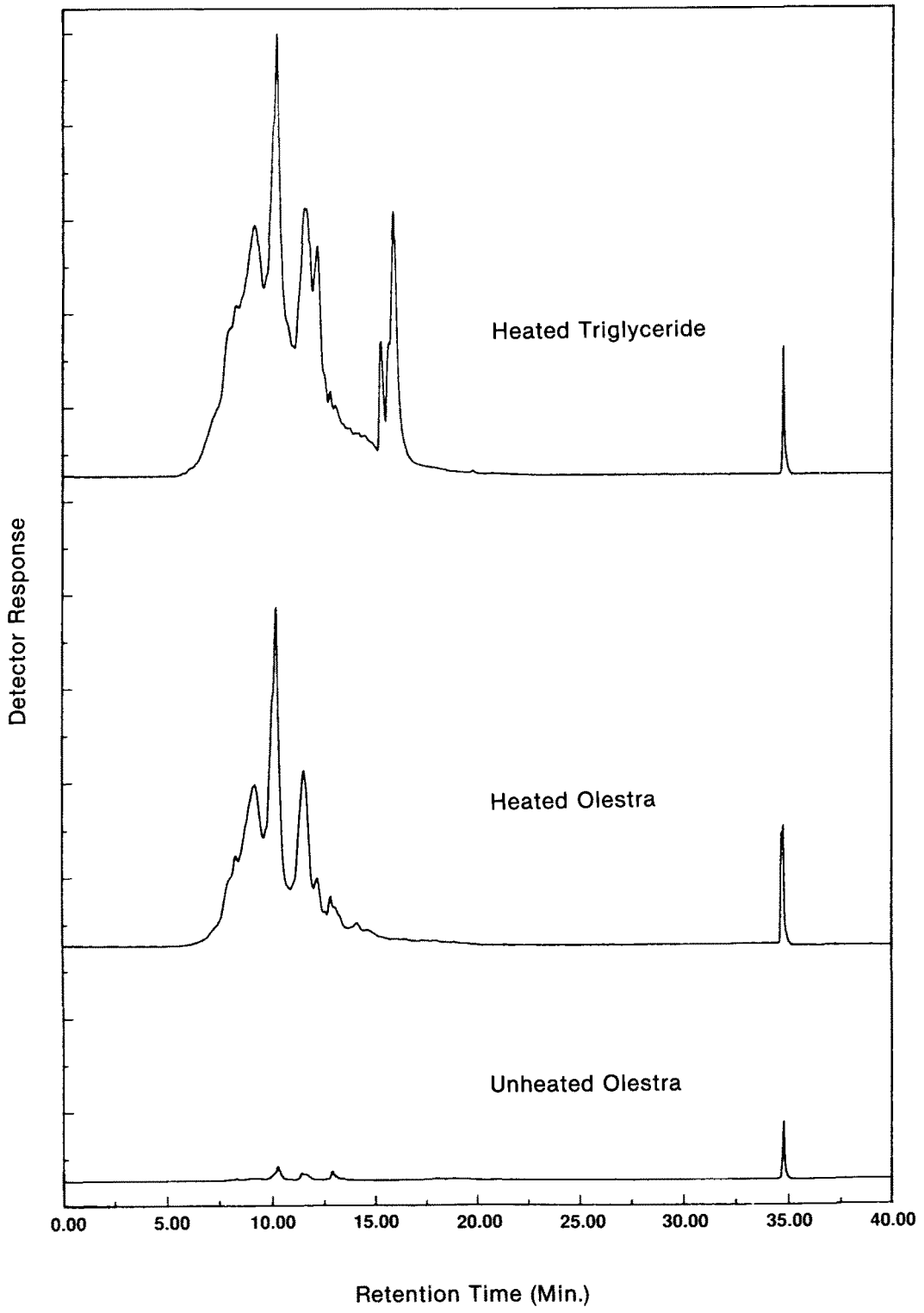

FIG. 7. Reverse-phase HPLC profiles of Fraction IIIB.

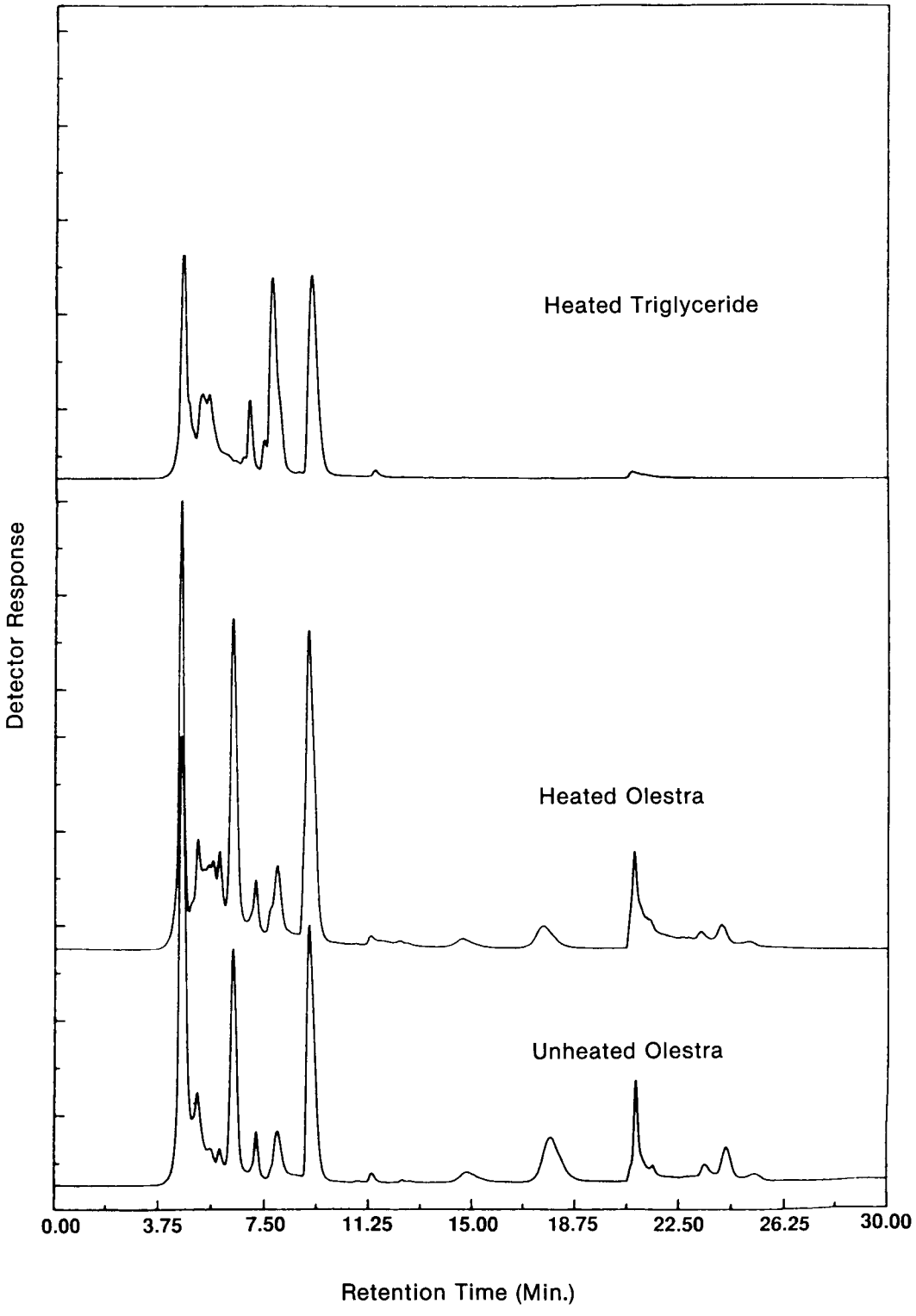

FIG. 8. Reverse-phase HPLC profiles of Fraction IV. (Unheated olestra profile has been enlarged for better comparison and represents a relative concentration four times larger than actual size.)

C. Damo (GC), T. Morsch (GC/MS, two-dimensional GC), D. Ewald and P. Hudson (HPLC).

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[Received November 21, 1991; accepted April 22, 1992]