Gas Chromatographic Analyses of Fatty Acids on Laboratory-Prepared Fused Silica Silar 10C Capillary Columns

Richard F. Arrendale,* Glenn W. Chapman, and Orestes T. Chortyk

Plant-derived, long-chain fatty acids were readily analyzed by glass capillary gas chromatography (GC-2) as their fatty acid methyl esters (FAME) on columns coated with the very polar cyanopropyl silicone liquid stationary phase, Silar 10C. Until now, the laboratory preparation of Silar 10C capillary columns made of the chemically inert, highly flexible fused silica capillary tubing has not been reported. We have successfully adapted our laboratory methodology for the preparation of fused silica GC-2 columns to the preparation of fused silica Silar 10C capillary columns. As a result, good, stable, low surface activity fused silica Silar 10C capillary columns were produced. We have demonstrated the applicability of these columns for the GC-2 analyses of fatty acids from cooking oils, forage grasses, and tobacco smoke. The laboratory preparation of these Silar 10C capillary columns and their analytical utility are discussed.

The advantages of fused silica (FS) capillary columns have been frequently enumerated. FS tubing is a very thin-walled, almost pure SiO_2 tubing that is coated on the outside with a protective layer of polymer, resulting in a strong but flexible column (Dandeneau et al., 1979; Dandeneau and Zerenner, 1979). FS tubing is by far the most chemically inert material for the preparation of capillary columns. However, the chemically inert FS tubing has a very low energy surface, which is not readily wettable by very polar liquid stationary phases, such as Silar 10C (Lipsky et al., 1980). Consequently, the surface energy of the FS tubing must be increased to allow uniform adhesion of a smooth, stable film of this very polar liquid phase. To accomplish this, we applied our recently developed method for the laboratory preparation of FS polar capillary columns (Arrendale et al., 1983). In this technique, the critical step involved the application of a nonextractable layer of Superox-4, a high molecular weight (4×10^6) polar polymer (Arrendale et al., 1981, 1983). This treatment produced two desirable effects: (1) the deactivation of residual surface activity and (2) an increase in surface energy. The resulting Superox-4 treated surface possessed a high-energy polar character (Arrendale et al., 1983). We have prepared FS columns of Silar 10C by completing the following two steps. The next step in this process was the application of a nonextractable intermediate layer of Silar 10C to the Superox-4-treated FS surface. This treatment produced a still higher energy, wettable surface; the last step was the static coating of the column with Silar 10C (Figure 1).

We have applied these laboratory-prepared Silar 10C columns to analyses of FAME of cooking oils, forage grasses, and tobacco smoke. Silar phases are cyanopropyl silicones that are the most polar of the silicone phases available and also possess high thermal stability (275 °C). Consequently, a Silar 10C column is an excellent phase for the GC-2 analyses of FAME, as it retains unsaturated compounds and has selectivity for the separation of cis–trans isomers of unsaturated FAME. In this study we have compared the distribution of fatty acids from laboratory-refined or commercially refined oils (soybean, sunflower, cottonseed, and safflower) and of fatty acids from Coastal Bermuda grass, fescue grass, and tobacco smoke. Practical details for the preparation of fused silica Silar 10C capillary columns are discussed and GC-2 analyses of FAME from the above sources are presented.

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EXPERIMENTAL SECTION

Materials. All solvents used were "distilled-in-glass" grade (Burdick and Jackson Laboratories, Muskegon, MI) and were distilled in glass prior to use. FS capillary tubing was obtained from Hewlett-Packard (Avondale, PA) or Alltech (Deerfield, IL), Silar 10C from Applied Science (State College, PA), and Superox-4 from Alltech (Deefield, IL). FAME standards were obtained from Applied Science (State College, PA) or Analabs (North Haven, CT) and were used as received. Boron trichloride–methanol (10% w/v) was obtained from Applied Science (State College, PA). All connections to vacuum sources, helium sources, and coating solution reservoirs were made with heat-shrinkable Teflon TFE-FEP tubing (Alltech, Deerfield, IL).

Capillary Column Preparation–Intermediate Phase Application. The first two steps of column preparation ("Capillary Tubing Preparation" and "Superox-4 Pretreatment and Deactivation") in Figure 1 are given in detail elsewhere (Arrendale et al., 1981). Generally, 25 m × 0.25 mm i.d. columns were prepared. The Superox-4-treated FS tubing was then rinsed with a 0.5% solution of Silar 10C (5 mg/mL) in methylene chloride by pulling a plug of the solution (to fill about 10% of the capillary tubing length) into the tubing with vacuum and then pushing the plug through the tubing with nitrogen pressure, at a rate of about 1 cm/s. The tubing was dried under nitrogen flow, placed in an oven, and heated at 300 °C for 1 h. During the heating step, air was excluded with a low flow of high-purity nitrogen. Best results were obtained when this application of intermediate phase was repeated.

Static Coating. The treated FS tubing was statically coated with a 0.5–1.0% solution of Silar 10C (5–10 mg/mL of methylene chloride) by using a modified version of the Bouche and Verzele (1968) static coating method (Arrendale et al., 1983). After being coated each column was installed in a gas chromatograph and conditioned and tested, as previously described (Arrendale et al., 1981, 1983).

Sample Preparation. Laboratory-extracted fescue, Coastal Bermuda grass, and crude oils from whole soybeans and once-refined commercial soybean, sunflower,

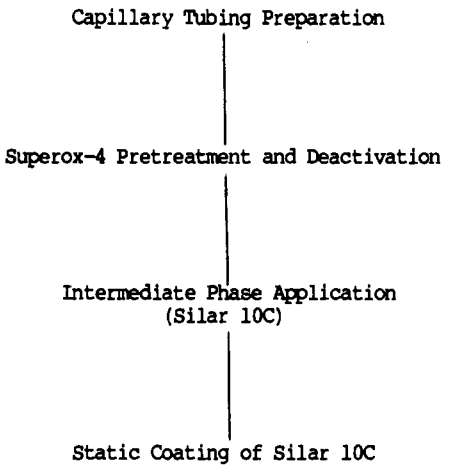

Figure 1. Preparation scheme for fused silica (FS) Silar 10C wall-coated open tubular (WCOT) columns, using our Superox-4 pretreatment and deactivation procedure.

cottonseed, and safflower oils were used to prepare their FAME. About 50–60 mg of each oil or 100 mg of each grass lipid extract was saponified in a 15 × 150 mm culture tube with 2.0 mL of 0.5 N NaOH–CH_3OH for 8 min at 83 °C (Temp Blok module heater). Fatty acids were then esterified with 3.0 mL of 10% boron trichloride (BCl_3)–CH_3OH for 5–15 min at 83 °C. FAME were extracted with hexane (10 mL). The hexane solution was washed twice with distilled H_2O and filtered through anhydrous sodium sulfate (Chapman and Robertson, 1977, 1980).

The fatty acids from tobacco smoke were isolated in a subfraction (Fraction F-61) as previously described (Chamberlain et al., 1975; Walters et al., 1978). About 65 mg of F-61 was esterified with 10 mL of 10% BCl_3–CH_3OH for 1 h at 76 °C, in a sealed flask. The reaction mixture was transferred to a 125-mL separation funnel and 25 mL of distilled H_2O was added. FAME were extracted with hexane (2 × 25 mL) and the hexane solution was filtered into a volumetric flask. A portion of the FAME of tobacco smoke was transferred to a tapered test tube and concentrated with dry nitrogen.

Capillary GC Analysis. The FAME were analyzed by GC-2 using a Hewlett-Packard 5720 research chromatograph equipped with a laboratory-constructed capillary inlet system (Severson et al., 1980). Peak area integrations were obtained with a Hewlett-Packard 3353 laboratory automation system. Peak identifications were confirmed by capillary GC–MS on a Hewlett-Packard 5985B GC–MS system.

RESULTS AND DISCUSSION

The successful application of our Superox-4 pretreatment and deactivation procedure in the preparation of FS Silar 10C capillary columns is exemplified by the chromatogram of a standard activity mixture (Figure 2). Visual comparison of peak height and shape for the polar components of the standard activity mixture with those of the nonpolar paraffinic hydrocarbons (C_{12}, C_{13}) indicates low capillary column surface activity (Arrendale et al., 1981, 1983). Column efficiencies were made about half those obtainable with moderately polar phase columns. For example, FS Silar 10C columns (25 m × 0.25 mm i.d.) possess 500–1000 effective theoretical plates (N) per meter (based on the C_{22} FAME with a partition ratio $k = 6$) while a Carbowax 20M or Superox-4 column with similar dimensions would be expected to possess over 2000 effective theoretical plates (N) per meter. However, the major plant FAME contained in a standard mixture were easily separated and even separation of the cis–trans isomers of the

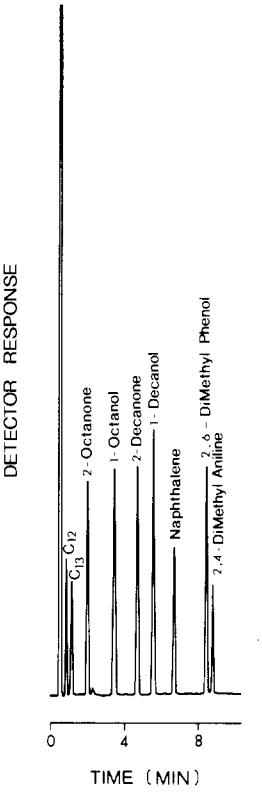

Figure 2. Chromatogram of activity standard mixture on a FS Silar 10C WCOT column produced with the Superox-4 pretreatment and deactivation procedure. Conditions: 80–250 °C at 8 °C/min; 28 cm/s H_2 flow; split injection mode; FID; 10 m × 0.2 mm i.d.

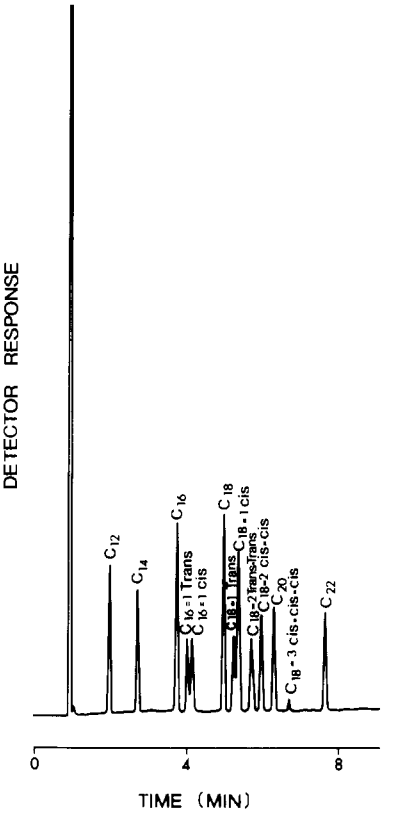

Figure 3. Separation of a standard mixture of fatty acid methyl esters (FAME) on a FS Silar 10C WCOT column. Conditions: 150–240 °C at 4 °C/min; 28 cm/s H_2 flow; split injection mode; FID; 25 m × 0.25 mm i.d.

unsaturated FAME was achieved (Figure 3). Reproducibility of column preparation with this methodology was

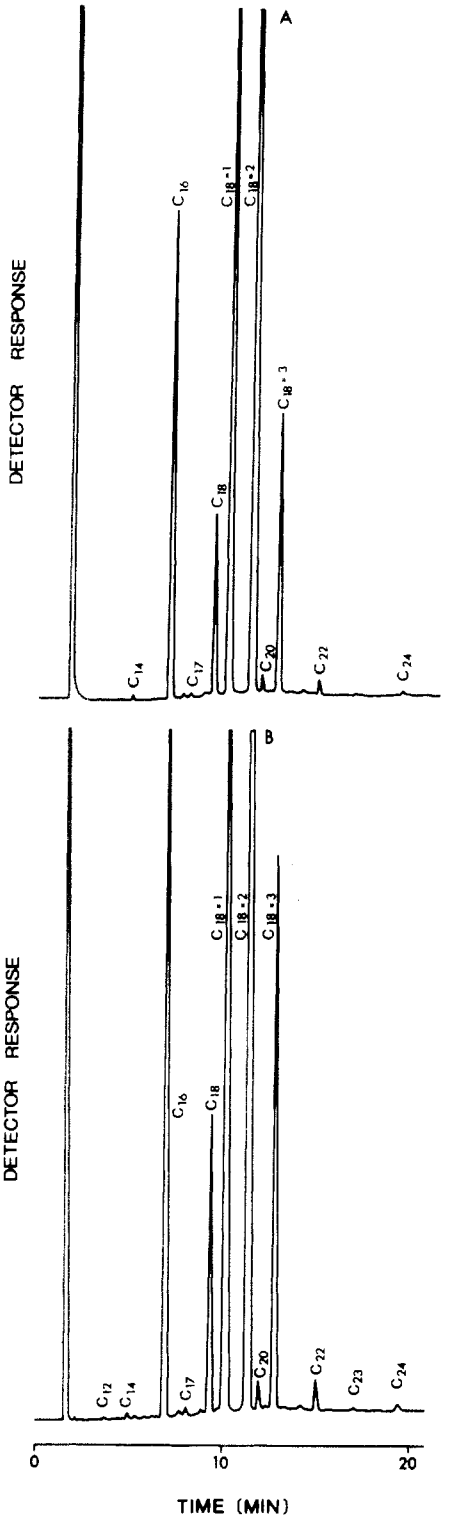

Figure 4. Separation of FAME from commercially refined (A) and laboratory-refined (B) soybean oils on a FS Silar 10C WCOT capillary column. Conditions: 150–240 °C at 4 °C/min; 28 cm/s H_2 flow; split injection mode; FID; 25 m × 0.25 mm i.d.

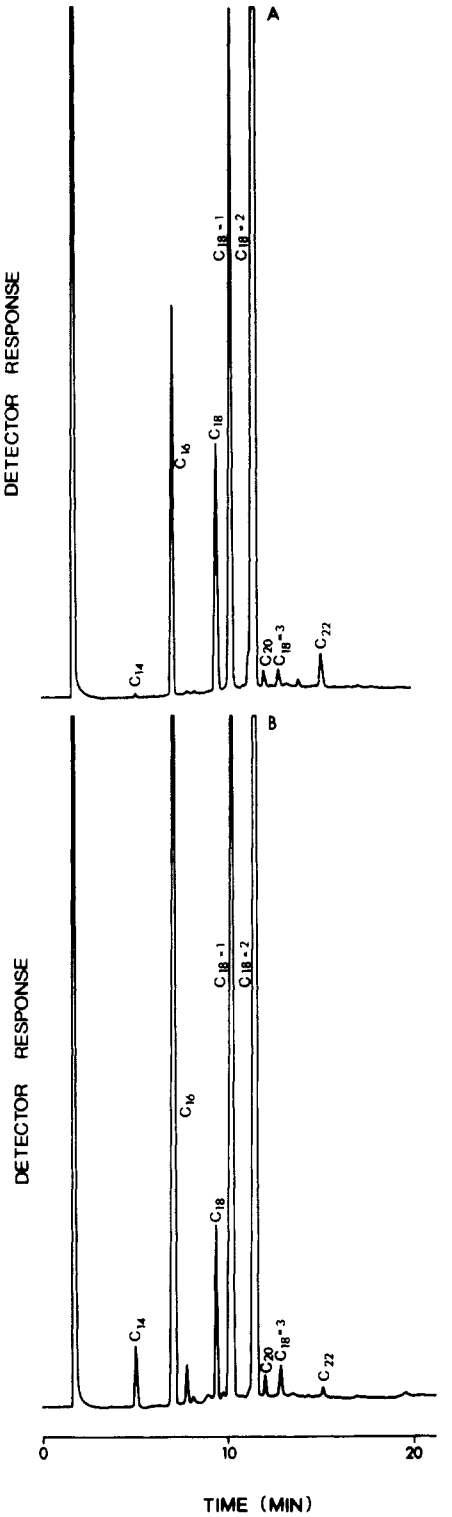

Figure 5. Separation of FAME from commercially refined sunflower oil (A) and cottonseed oil (B) on a FS Silar 10C WCOT column. Conditions: 150–200 °C at 4 °C/min; 30 cm/s H_2 flow; split injection mode; FID; 25 m × 0.25 mm i.d.

very good. Silar 10C capillary columns were successfully prepared on 0.20, 0.25, and 0.30 mm i.d. fused silica tubing in lengths from 10 to 40 m. Although column efficiencies were low, liquid-phase film stability was good, as indicated by column lifetimes of greater than 1 year with little or no change in column characteristics after 1000 analyses using the split injection technique. To our knowledge, no other procedure for the preparation of fused silica Silar 10C capillary column has been published.

The fatty acids from commercially refined and laboratory-refined soybean oil were compared, and their separation (as FAME) on a FS Silar 10C column is shown in Figure 4. The reproducibility of response for a standard mixture of FAME (C_{12}–C_{22}) under these conditions (Figure 4) averaged less than 3% relative standard deviation (RSD) for five repetitive analyses (C_{18} FAME was used as the internal standard). The deviation of detector response from unity for this standard mixture of FAME also averaged less than 3%; therefore, unitary response was assumed. The distribution, based on peak areas of fatty acids from each sample, was calculated, and these data

Table I. Distribution of Fatty Acids[a] in Oil Samples

sample type	% distribution							
	C_{14}	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}	C_{22}
laboratory-refined soybean oil	0.08	11.99	3.81	20.21	55.37	8.37	0.38	0.50
commercially refined soybean oil	0.09	11.44	4.30	23.66	52.69	7.05	0.39	0.39
commercially refined cottonseed oil	0.85	24.12	2.58	18.23	53.24	0.54	0.28	0.16
commercially refined sunflower oil	0.04	6.98	4.31	14.43	73.00	0.30	0.26	0.69
commercially refined safflower oil	0.05	6.45	2.73	12.87	77.69	0.05	0.06	0.10

[a] Analyzed as fatty acid methyl esters.

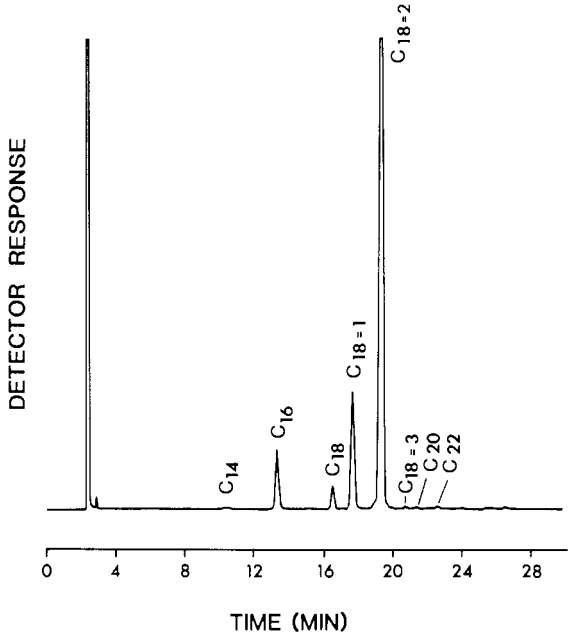

Figure 6. Separation of FAME from safflower oil on a FS Silar 10C WCOT columns. Conditions: 140–240 °C at 4 °C/min; 25 cm/s H_2 flow; split injection mode; FID; 25 m × 0.25 mm i.d.

showed no significant difference in the fatty acids distribution of commercially refined and laboratory-refined soybean oil (Table I). The fatty acids of commercially refined cottonseed, sunflower, and safflower oils were also examined (Figures 5 and 6; Table I). These data show that safflower oil is lowest in saturated fatty acids (9%) and highest in unsaturated fatty acids (90%) among these four oils. It also contains high levels of linoleic acid ($C_{18:2}$) and low levels of linoleic acid ($C_{18:3}$).

Forage grasses like fescue and Coastal Bermuda produce higher levels of linolenic acid ($C_{18:3}$) compared to linoleic acid ($C_{18:2}$). Figure 7 shows the separation of the FAME from Coastal Bermuda and fescue grasses using a laboratory prepared FS Silar 10C capillary column.

A chromatogram of the FAME isolated from a cigarette smoke fraction and separated on a FS Silar 10C capillary column is shown in Figure 8. Tobacco leaf contains long-chain fatty acids (C_{12}–C_{34}) as well as a variety of short-chain (C_2–C_{11}) carboxylic acids that are transferred to tobacco smoke during the smoking process (Davis, 1976; Severson et al., 1978). Studies have shown that fatty acids contributed to the flavor and aroma of cigarette smoke (Davis, 1976) and 5–15% of the major fatty acids are transferred to cigarette smoke during the smoking process (Severson et al., 1978).

CONCLUSION

Our Superox-4 pretreatment and deactivation technique was successfully adapted for the preparation of FS Silar 10C capillary columns. These highly polar columns exhibited low surface activity and good liquid-phase film stability, allowing long column lifetimes. The laboratory

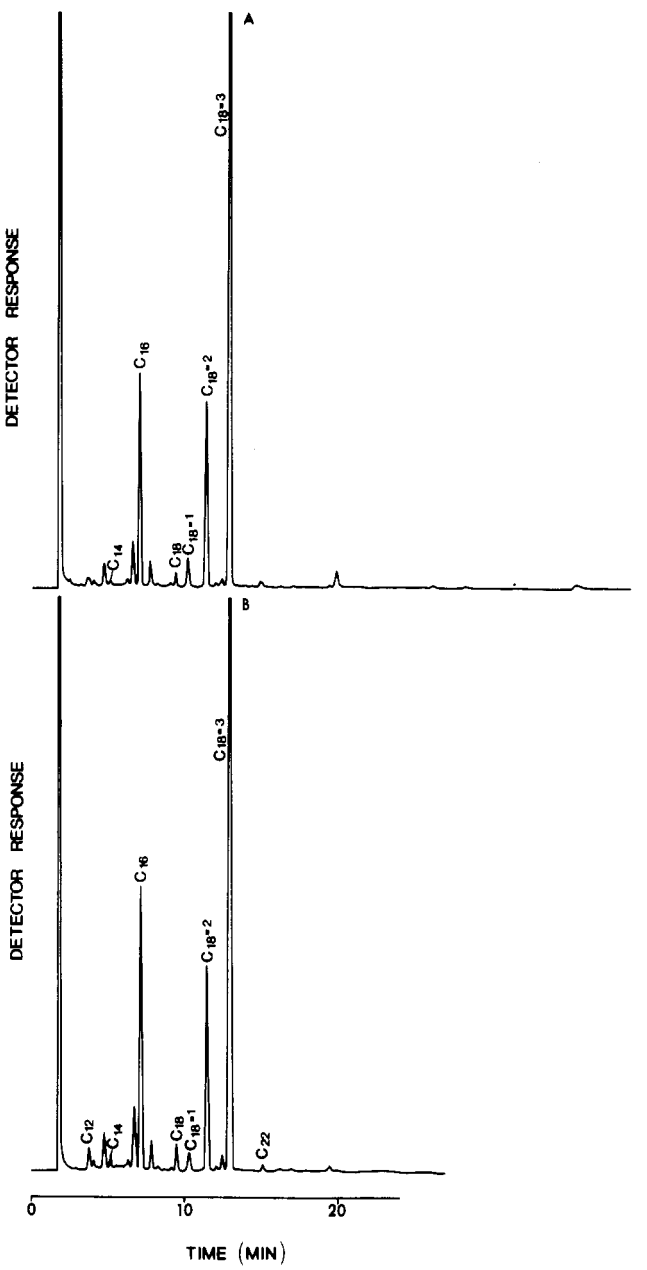

Figure 7. Separation of FAME from fescue grass (A) and Coastal Bermuda grass (B) on a FS Silar 10C WCOT column. Conditions: 150–200 °C at 4 °C/min; 30 cm/s H_2 flow; split injection mode; FID; 25 × 0.25 mm i.d.

preparation technique was simple, rapid, and reproducible. Silar 10C columns were successfully prepared with fused silica tubing of various dimensions. Fatty acids (FAME) from a variety of plant sources were rapidly and successfully separated on these cyanopropyl silicone phase columns. Silar 10C can now be coated on the chemically inert, highly flexible fused silica capillary tubing, and GC-2 columns of good quality can now be prepared in the lab-

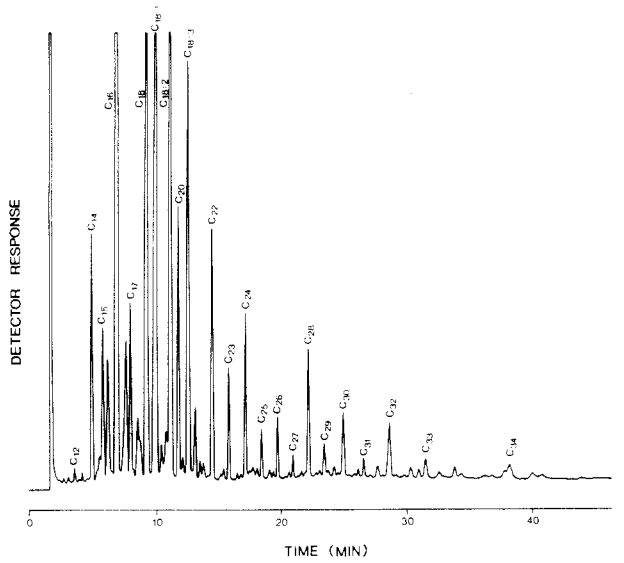

Figure 8. Separation of FAME from cigarette smoke on a FS Silar 10C WCOT column. Conditions: 150–240 °C at 4 °C/min; 30 cm/s H_2 flow; split injection mode; FID; 25 m × 0.25 mm i.d.

oratory.

Registry No. C_{14}, 544-63-8; C_{16}, 57-10-3; C_{18}, 57-11-4; $C_{18:1}$, 27104-13-8; $C_{18:2}$, 60-33-3; $C_{18:3}$, 463-40-1; C_{20}, 506-30-9; C_{22}, 112-85-6; C_{12}, 143-07-7; C_{17}, 506-12-7; C_{23}, 2433-96-7; C_{24}, 557-59-5; C_{15}, 1002-84-2; C_{25}, 506-38-7; C_{26}, 506-46-7; C_{27}, 7138-40-1; C_{28}, 506-48-9; C_{29}, 4250-38-8; C_{30}, 506-50-3; C_{31}, 38232-01-8; C_{32}, 3625-52-3; C_{33}, 38232-03-0; C_{34}, 38232-04-1; Superox-4, 25322-68-3; vitreous silica, 60676-86-0.

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Elimination of Sample Autoxidation by Butylated Hydroxytoluene Additions before Thiobarbituric Acid Assay for Malonaldehyde in Fat from Chicken Meat

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This study tested the influence of the antioxidant butylated hydroxytoluene (BHT) on the thiobarbituric acid (TBA) assay for malonaldehyde in fat extracted from chicken breast and leg meat. It was found that 125 μg of BHT/mg of fat was required to prevent autoxidation during the heating step of the TBA assay. In addition, autoxidation of sample lipid during extraction can be prevented by the addition of 0.01% BHT to chloroform–methanol (1:2) reagent. For samples extracted with BHT, the addition of 75 μg of BHT/mg of fat before heating was sufficient to prevent sample autoxidation. Meat samples analyzed without any BHT additions yielded 6 times higher malonaldehyde concentrations compared to samples that received BHT during extraction and 75 μg of BHT/mg of fat during the TBA assay. It is concluded that antioxidant protection is necessary during TBA assays to prevent sample autoxidation and consequent artifactually high analytical results.

There are many variations of the thiobarbituric acid (TBA) assay for malonaldehyde (MA). However, all variations are similar in that they each have a critical heating step (usually 100 °C for 30–60 min) that is necessary for releasing MA from lipid hydroperoxides. This heating in the presence of air may promote sample autoxidation and lead to artifactually high assay values.

For the analysis of MA in chicken and other meats, the tissue distillation–TBA assay method of Tarladgis et al. (1960) is most commonly used. This original method did not include antioxidants during the distillation step, and neither do many meat distillation assays in current use. However, some investigators have used antioxidants such as propyl gallate, EDTA, and BHA during the distillation step of the TBA assay (Rhee, 1978; Rhee and Ziprin, 1981; Moerck and Ball, 1974). Yamauchi et al. (1982) used 0.3% addition of BHT to meat samples before homogenization and distillation for TBA assays. This recent use of antioxidants in tissue distillation indicates that sample aut-

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