Evaluation of Three Modified TBA Methods for Measuring Lipid Oxidation in Chicken Meat

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Modified aqueous extraction (AE) and lipid extraction (LE) methods of TBA analysis were compared with a commonly used distillation method (DM) for monitoring lipid oxidation in fresh, refrigerated, and frozen stored chicken meat. BHT was added before the homogenization step in all three methods. We found that 0.15% BHT in DM and 0.10% BHT per weight of lipids in the AE procedure were sufficient to prevent sample autoxidation. During cold storage conditions, TBA numbers obtained by the DM were approximately 1.35 times higher and 1.30 times lower compared to AE and LE procedures, respectively. These differences between methods remained constant over 6 days of refrigerated storage and also over 6 months of frozen storage, as demonstrated by regression analyses. High correlation coefficients ($r \geq 0.98$) were found in cross comparisons of the three methods. Results indicate that AE is an acceptable and convenient method for determining TBA values. When more details of lipid susceptibility to oxidation are required, the LE procedure for TBA assay is recommended.

The reaction of malonodialdehyde (MDA) with 2-thiobarbituric acid (TBA) has been widely used for measuring the extent of oxidative deterioration of lipids in muscle foods (Gray, 1978; Melton, 1983). The TBA test has been performed (a) directly on the food product followed by extraction of the colored complex (Turner et al., 1954; Sinnhuber and Yu, 1958; Uchiyama and Mihara, 1978; Pokorny et al., 1985), (b) on a portion of the steam distillate of a food sample (Tarladgis et al., 1960; Igene et al., 1979; Yamauchi et al., 1982; Ke et al., 1984), (c) on aqueous or acid extractions of food samples (Tarladgis et al., 1964; Witte et al., 1970; Vyncke, 1975; Sinnhuber and Yu, 1977; Salih et al., 1987), and (d) on lipids extracted from the food samples (Younathan and Watts, 1960; Pikul et al., 1983). The TBA test expresses lipid oxidation in milligrams of MDA per kilogram of sample (first three methods, a–c) and milligrams of MDA per unit of lipids (fourth method, d). The sample MDA is quantified by its reaction with TBA to form a colored complex with maximum absorbance near 532 nm. High-performance liquid chromatography has been used to isolate the specific TBA–MDA complex from other TBA-reactive substances (TBARS) that may also contribute to absorbance at 532 nm (Kakuda et al., 1981; Bird et al., 1983; Csallany et al., 1984). Although distillation is widely regarded as the standard method for TBA analysis, it is the most cumbersome. The purpose of this report is to compare these two new extraction methods with a commonly used modified distillation method to evaluate their technical validity.

MATERIALS AND METHODS

Materials. Whole breast muscles from 30 chickens were deboned manually 24 h after slaughter and processing at a commercial meat facility. The muscles were then ground through a 2-mm plate and mixed well. One part was used immediately for fresh analysis, and the remaining meat was divided into two equal portions and wrapped in 150-g aluminum foil packets. One portion was stored in a refrigerator at 1 °C and analyzed after 2, 4, and 6 days, while the other portion was kept in a freezer at −18 °C and analyzed after 2, 4, and 6 months. Samples were collected into a total of 35 aluminum foil packets each containing 150 g of meat, and the contents of each packet were used in all three methods of TBA analysis. Five separate packets were used for fresh analysis as well as for each time of the six refrigerated and frozen storage analyses. Breast meat was found to contain 1.24% extractable lipid composed of 55.7% phospholipids, 37.1% triacylglycerols, and 4.8% total cholesterol.

Distillation Method. The TBA distillation method was performed as described by Tarladgis et al. (1960) except that butylated hydroxytoluene (BHT) was added before homogenization. Ground chicken meat (10 g) was homogenized with 49.25 mL of distilled water and 0.75 mL of BHT dissolved in ethanol, at 4000 rpm for 2 min. The TBA number was calculated by multiplying the absorbance values at 532 nm by a constant coefficient K. This value was calculated from standard curves and known dilutions as follows:

$$K_{\text{distiln}} = S/A \times 72.063 \times 10^7/C \times 100/P$$

where S is the standard concentration (range from 1×10^{-8} to 8×10^{-8} mol) of 1,1,3,3-tetramethoxypropane (TMP) in 5 mL of distillate, A is the absorbance of the standard, 72.063 is the molecular weight of malonodialdehyde, C is the weight of the sample, and P is the percent of recovery. The average recovery from different concentrations of TMP (1×10^{-8} to 8×10^{-8} mol in 5 mL) obtained in 50 mL of distillate was 90.7%. With 10-g meat samples and 90.7% recovery, the average K_d value was 6.5.

Aqueous Extraction Method. The extraction TBA procedure was performed as described by Witte et al. (1970) except that perchloric acid was used in place of trichloroacetic acid as recommended by Salih et al. (1987) and BHT was added (Pikul et al., 1983). Since perchloric acid is explosive, appropriate safety precautions were observed. Ground chicken meat (10 g) was homogenized with 34.25 mL of cold (4 °C) extracting solution containing 4% perchloric acid and 0.75 mL of BHT in ethanol, at 4000 rpm for 2 min. The blended sample was filtered through Whatman No. 1 filter paper into a 50-mL Erlenmeyer flask and washed with 5 mL of distilled water. The filtrate was adjusted to 50 mL by adding 4% perchloric acid. Next, 5-mL aliquots of the filtrate were transferred to separate test tubes (1.5 × 20 cm) and mixed with 5 mL of 0.02 M TBA in distilled water. One test tube was incubated at room temperature in the dark for 15 h, while the other was heated in a boiling water bath for 1 h and then cooled for 10 min with cold tap water. The absorbance was determined at 532 nm against a blank containing 5 mL of perchloric acid (4%) and 5 mL of TBA reagent.

The TBA value used to express the results of the modified extraction method was calculated by multiplying the absorbance by a constant coefficient K, which was calculated from standard curves and known dilutions, as

$$K_{\text{extractn}} = S/A \times 72.063 \times 10^6/E \times 100/P$$

where S is the standard concentration (range from 1×10^{-8} to 8×10^{-8} mol) of 1,1,3,3-tetramethoxypropane (TMP) in 5 mL of filtrate, A is the absorbance of the standard, 72.063 is the mo-

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Table I. Calculation of TBA Number in Lipid Extraction Method

concn of MDA, μg/g lipid	lipid concn, % wt tissue	TBA no., mg MDA/kg meat
49.1	1.34	0.66
58.6	1.26	0.74
75.2	1.37	1.03
94.2	1.18	1.11
105.6	1.12	1.18
111.2	1.15	1.28

Table II. Absorbance Values at 532 nm for a Distillation Method of TBA Assay in the Presence of BHT

concn of BHT,[a] % total lipid	sample storage condition[b]	
	refrig (6 days)	frozen (6 months)
0.00	0.301 ± 0.012[c]	0.578 ± 0.016[d]
0.01	0.253 ± 0.011[b]	0.465 ± 0.014[c]
0.05	0.209 ± 0.012[a]	0.387 ± 0.012[b]
0.10	0.208 ± 0.010[a]	0.331 ± 0.010[a]
0.15	0.203 ± 0.008[a]	0.326 ± 0.010[a]

[a] BHT was added to 10 g of ground meat sample before homogenization. [b] Data are presented as mean ± standard deviation of six samples. Mean values within each column that do not have the same superscript letter are significantly different ($P < 0.05$, two-tailed t-test).

lecular weight of malonodialdehyde, E is the sample weight equivalent, and P is the percent of recovery.

The equivalent (E) for 10 g of sample was 1 when a 5-mL aliquot of the original 50-mL filtrate was analyzed. The average recovery of the standard TMP added was 94.0%. With a 10-g sample, and recovery of 94.0%, the average K_e value was 5.5.

Lipid Extraction Method. TBA assay of fat extracted from chicken meat was performed by the methods described previously (Pikul et al., 1983). For determining the concentration of MDA in lipid extracts from meat samples, absorbance values at 532 nm are converted from a standard (TMP) curve to micrograms of MDA per gram of lipids. TBA numbers were calculated by multiplying the micrograms of MDA per gram of lipid by the percentage of lipids in meat samples as shown in Table I. Total lipid extracted from tissues was used for the determination of triacylglycerols by the methods of Foster and Dunn (1973), total cholesterol by modified methods of Glick et al. (1964), and phosphorus by the methods of Eng and Noble (1968), which was multiplied by the factor 25.5 to estimate total phospholipids.

Statistical Analysis. Statistical analysis of BHT addition in distillation and aqueous extraction methods was performed by two-tailed Student's t-test (Steel and Torrie, 1960). Inferences and statistics related to linear regression and correlation were determined according to Dowdy and Wearden (1983).

RESULTS AND DISCUSSION

Effect of BHT Addition in the Distillation Method. In order to determine the optimal concentration of BHT necessary to prevent autoxidation during the steam distillation step to release MDA from meat samples, several concentrations of BHT were tested using 6 days refrigerated and 6 months frozen stored meat (Table II). The data show that TBA reaction absorbance is stable at 0.05–0.15% BHT per weight of lipid in refrigerator stored meat and at 0.10–0.15% BHT per weight of lipid in frozen stored meat. The table shows that at the lower concentration of BHT the absorbance increased, which indicates incomplete protection against autoxidation during steam distillation. Breast muscles held in refrigerated and frozen storage and analyzed without BHT protection show 1.5 and 1.8 times higher absorbance values, respectively, compared to the same samples with BHT added.

The original distillation method did not include antioxidants during the distillation step. However, some investigators have used antioxidants such as propyl gallate (PG), butylated hydroxyanisole (BHA), and *tert*-butylhydroquinone (TBHQ) or chelating agents such as ethylenediamine tetraacetic acid (EDTA) during the distillation step of the TBA assay (Moerck and Ball, 1974; Rhee, 1978; Rhee and Ziprin, 1981; Crackel et al., 1988). Yamauchi et al. (1982) used 0.3% addition of BHT to meat samples before homogenization and distillation for TBA assay. Our results demonstrate that the addition of 0.15% BHT per weight of lipids from chicken breast meat is sufficient to control sample autoxidation even in the samples kept in frozen storage for a long time. Crackel et al. (1988) found that the addition of 0.01% TBHQ to samples prior to homogenization significantly reduced the TBA numbers of fish, chicken breast, and thigh meat but not for beef.

Table III. Absorbance Values at 532 nm for Aqueous Extraction Method of TBA Assay in the Presence of BHT[a]

concn of BHT,[b] % total lipid	reaction conditions[c]	
	boiling (1 h)	incubn (room temp, 15 h)
0.00	0.521 ± 0.012[c]	0.373 ± 0.013[c]
0.01	0.481 ± 0.012[b]	0.356 ± 0.009[b]
0.05	0.457 ± 0.008[a]	0.333 ± 0.010[a]
0.10	0.453 ± 0.007[a]	0.329 ± 0.008[a]

[a] Meat samples frozen 6 months. [b] BHT was added to 10 g of ground meat samples before homogenization. [c] Data are presented as mean ± standard deviation of six samples. Mean values within each column that do not have the same superscript letter are significantly different ($P < 0.05$, two-tailed t-test).

Effect of BHT Addition in the Aqueous Extraction Method. In order to determine the optimal condition for the development of TBA–MDA color complex, we tested 1-h boiling compared to 15-h incubation at room temperature with identical meat samples. We found the significant differences between these two procedures as indicated in Table III. The absorbance values obtained after boiling were 1.3–1.4 times higher compared to those after incubation at room temperature for chicken meat stored frozen for 6 months. This is contrary to results presented by Salih et al. (1987) who found no significant differences in TBA number between incubation at room temperature for 15–17 h or boiling for 30 min to develop the color complex in raw and cooked turkey.

To determine whether autoxidation occurred during the blending of samples with perchloric acid, before the boiling or incubation step, different concentrations of BHT were added before blending in the aqueous extraction TBA method. The absorbance values decreased by an average 13.2% in boiling procedure and 11.8% in an incubation procedure when 0.10% BHT per mass of lipid was used. This work agrees with our previous findings (Pikul et al., 1983) and results presented by Rhee (1978) and Salih et al. (1987) and indicate that blending with perchloric acid can accelerate lipid oxidation and increase the TBA number of chicken meat unless an antioxidant is used.

Influence of 1 °C Storage on TBA Values. The effect of refrigerated storage of breast muscles on TBA values obtained by three different methods is presented in Figure 1. During the storage conditions, we found a wide range of lipid oxidation with TBA numbers from 0.33–0.58 in fresh meat to 1.10–1.65 after 6 days. There was a continuous increase in TBA values during storage, and values after 6 days of storage were 2.9–3.3 and 2.1–2.4 times higher than those obtained in fresh meat and after 2 days of storage, respectively. Mean TBA values determined by lipid extraction procedure were approximately 1.3 and 1.7 larger as those determined by the distillation and aqueous extraction methods, respectively. The TBA number obtained by the distillation method were approximately 1.3 times higher than those of the aqueous

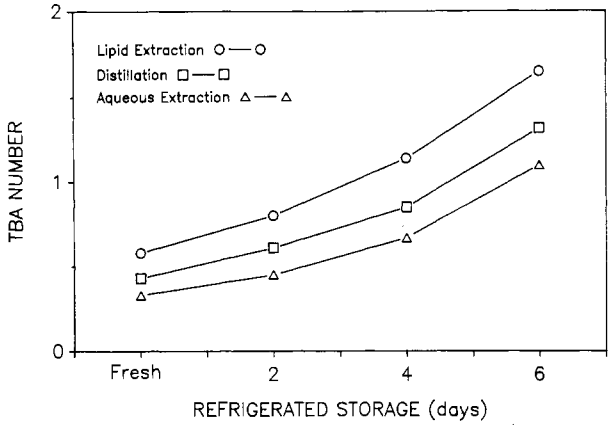

Figure 1. Effect of refrigerated storage on TBA numbers of chicken meat obtained by three modified methods of analysis.

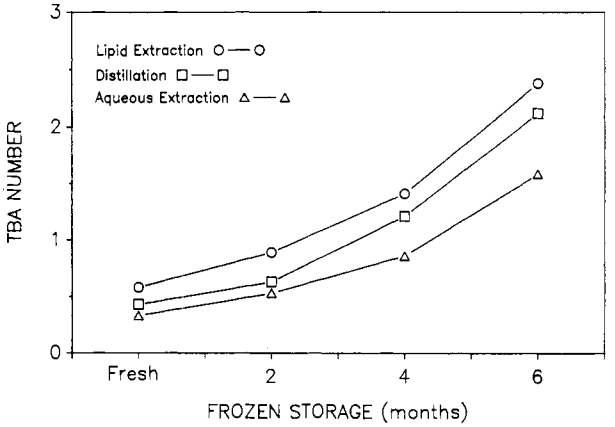

Figure 2. Effect of frozen storage on TBA numbers of chicken meat obtained by three modified methods of analysis.

Table IV. Statistical Comparison among Three Modified TBA Methods[a]

comparison	lin regressn, eq	lin correln coeff (r)	Fisher transformn (z)
	Refrigerated Storage		
AE × DM	$y = 0.0922 + 1.117x$	0.983	2.395
LE × DM	$y = -0.0427 + 0.8100x$	0.985	2.448
AE × LE	$y = 0.1840 + 1.352x$	0.978	2.260
	Frozen Storage		
AE × DM	$y = 0.0203 + 1.354x$	0.983	2.379
LE × DM	$y = -0.1552 + 0.958x$	0.987	2.505
AE × LE	$y = 0.1538 + 1.412x$	0.986	2.485

[a] Abbreviations: aqueous extraction method, AE; distillation method, DM; lipid extraction method, LE.

extraction method. Very high correlations ($r \geq 0.98$) were found among the three TBA methods during refrigerated storage as presented in Table IV.

Influence of −18 °C Storage on TBA Values. Changes in mean TBA values during frozen storage of breast muscles determined by three different procedures are presented in Figure 2. The frozen storage conditions produced a wide range of TBA numbers from 0.33–0.58 in fresh meat to 1.58–2.38 after 6 months. We found a continuous increase in TBA numbers during frozen storage, and values after 6 months were 4.1–4.9 times higher than those obtained in fresh muscle.

However, we found differences among TBA values obtained by three different methods. TBA values determined by a distillation procedure were approximately 1.3 times lower compared to the lipid extraction method and 1.4 times higher than those determined by the aqueous extraction procedure. The linear regression and correlation statistics from the comparisons among the three TBA methods applied to frozen storage samples are presented in Table IV.

Comparison of Three Modified TBA Methods. Six linear correlation coefficients obtained from comparisons with both refrigerated and frozen samples were converted to their corresponding z values by Fisher transformation as presented in Table IV. The z values from three TBA methods obtained from refrigerated samples were compared against the corresponding z values obtained from frozen samples to obtain three sets of z statistics (AE × DM, 0.656; LE × DM, 0.046; AE × LE, 0.660) to test the null hypothesis. In all three comparisons, the z statistic was <1.96; therefore, there were no significant differences between correlation coefficients obtained from refrigerated vs frozen storage treatment.

It was found that during refrigerated and frozen storage conditions TBA numbers obtained by the distillation

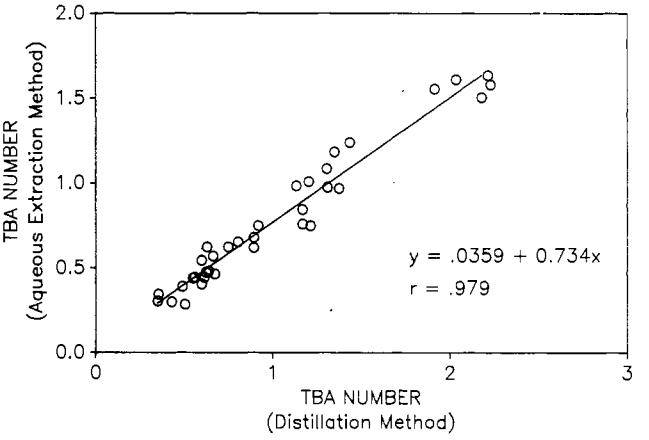

Figure 3. Relationship between TBA numbers of chicken meat determined by aqueous extraction and distillation methods.

method were approximately 1.3–1.4 times higher than those of the aqueous extraction procedure. These results are not comparable to those reported by Witte et al. (1970), Vyncke (1975), and Salih et al. (1987), who found that the TBA values determined by the distillation method of pork, beef, fish, and poultry meat were approximately 2.0–2.6 times larger than those determined by the extraction procedure. In those studies, however, the TBA distillation method was performed as described by Tarladgis et al. (1960) without antioxidant. Heat during distillation is used to release the malonodialdehyde from fatty acid hydroperoxides, and its bound state with phospholipids, proteins, and amino acids, whereas perchloric acid is used to release malonodialdehyde in the aqueous extraction method. Heat used in the distillation procedure may speed the lipid oxidation process (Witte et al., 1970; Siu and Draper, 1978), thus resulting in higher TBA values (Pikul et al., 1983). However, Siu and Draper (1978) found no differences between TBA values determined by distillation and TCA extraction methods when antioxidants or EDTA was added to meat samples.

A very high correlation coefficient ($r = 0.979$) was found between the TBA values obtained by comparison of aqueous extraction and distillation methods (Figure 3). Witte et al. (1970) and Salih et al. (1987) reported that the correlation between TBA values determined by the extraction and the distillation methods for pork and poultry meat was represented by r values of 0.85 and 0.91, respectively. In these papers the TBA distillation method was performed without antioxidant additions.

The correlation coefficient ($r = 0.983$) and regression line of the comparison between the TBA values obtained by

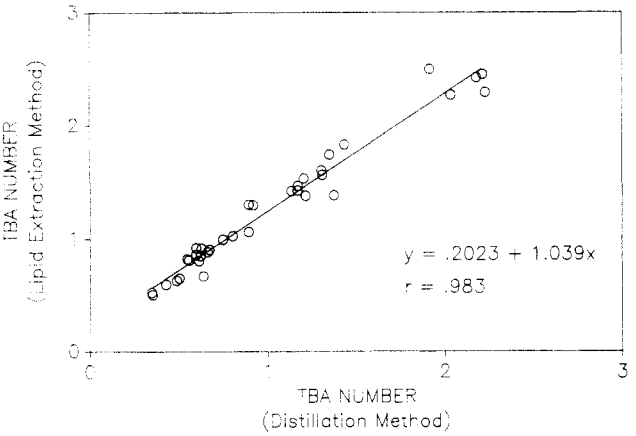

Figure 4. Relationship between TBA numbers of chicken meat determined by lipid extraction and distillation methods.

lipid extraction and distillation methods are presented in Figure 4. There is no published study comparing the TBA numbers determined by distillation and lipid extraction methods. However, some investigators compared the TBA values of food samples obtained by the distillation method with Turner's method (Tarladgis et al., 1960), water extraction method (Tarladgis et al., 1964), and modified fluorometric and ultraviolet spectrophotometry methods (Juhasz et al., 1983).

Recommended Procedures. The most widely used and standard method for measuring TBARS in meat is the distillation method of Tarladgis et al. (1960) with some modifications (Igene et al., 1979; Yamauchi et al., 1982; Ke et al., 1984; Crackel et al., 1988). The minimum recommendation is that BHT or other appropriate antioxidant should be added during the distillation procedure.

We found that TBA numbers obtained by the modified aqueous extraction method are lower but highly correlated with TBA numbers obtained by distillation methods with 0.15% BHT addition. The modified aqueous extraction method is faster and easier to perform than the distillation method. We recommend this procedure for use in situations where a large number of samples need to be analyzed quickly. The aqueous extraction method appears to be an acceptable alternative to the distillation TBA methods for poultry meat held in refrigerated and frozen storage. This method is also recommended by Salih et al. (1987) for monitoring lipid oxidation in cooked poultry meat.

Distillation and aqueous extraction methods express lipid oxidation in milligrams of MDA per kilogram of meat (TBA number), which depends not only on the MDA content per weight of lipid but also on the total lipid concentration in meat samples. When more details about the susceptibility of different kinds of lipids to oxidation during storage or heating is of interest, it is necessary to measure the changes of concentration of MDA per weight of lipids. In this case, we recommend the lipid extraction procedure for TBA assay because the TBA numbers obtained using this method are highly correlated with TBA numbers obtained by the distillation method. Total lipid extracted from tissue may also be used for the determination of triacylglycerols, phospholipids, and total cholesterol concentrations.

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Recoveries of Chlorinated Hydrocarbon Pesticides from Fat Using Florisil and Silica Sep-Paks

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Florisil and silica Sep-Paks were evaluated as a means of separating chlorinated hydrocarbon pesticides from fat; GC with electron-capture detection was used to measure residues spiked at the 0.005–0.5-μg level into 0.1 g of soybean oil. Fourteen single-peak residues and two multipeak residues, technical chlordane and Arochlor 1260, were tested. Results with several solvents showed that 1% diethyl ether in hexane used with the Florisil Sep-Pak gave the best overall recovery. Interferences in the electron-capture chromatograms are discussed.

Sep-Pak cartridges and other commercially available disposable columns offer several advantages over open-column chromatography for the analyst: speed of separation, low volume of solvent required, small amount of sample needed, and ability to separate or concentrate trace levels of compounds (Winterlin et al., 1981; Gorder and Dahm, 1981). Both bulk Florisil (Luke et al., 1984; Stein and Narang, 1984) and Florisil cartridges have been used to separate a variety of organic pollutants, including PCB (Lerman et al., 1982), PBB (Hu et al., 1982), the chlorinated hydrocarbons HCB and aldrin (Chiang, et al., 1987), and PCP and its derivatives (Mundy and Machin, 1981). In addition, silica cartridges have been used to separate PCB cogeners in oil (Steichen et al., 1982), carbofuran in soil (Gorder and Dahm, 1981), and aldicarb in potato extracts (Cochrane and Lanouette, 1981). As many countries, including the United States, still allow free or restricted use of some chlorinated pesticides, it is of interest to search for simpler multiresidue methods than existing ones (EPA, 1980). In most studies of pollutant recoveries from fat, the subject of separation efficiency is usually not discussed, and where it is, the fat passing into the sample eluate has been measured gravimetrically (Ansari and Hendrix, 1985). However, the use of Sep-Pak cartridges to separate phospholipids from neutral lipids and fatty acids has been reported (Hamilton and Comai, 1984).

In the present study we tested the recovery of 16 chlorinated pesticides, including 14 single-peak residues and two multipeak residues, technical chlordane and Arochlor 1260 PCB, using a variety of solvents on both Florisil and silica Sep-Paks. The regime used is rapid and involves only prerinsing, sample loading, elution, evaporation, reconstitution, and injection. The first five steps require about 20 min. In addition, the efficiency of each cleanup was measured by hydrolyzing the fat passing into the Sep-Pak eluate into methyl esters and then chromatographing and summing the areas of the hydrolysate peaks. Partially hydrogenated soybean oil was chosen for recovery studies because it is a uniform fat, containing >99% triglyceride, <0.1% moisture, and <0.1% free fatty acid content. Furthermore, the fatty acid composition of soybean oil is relatively simple, with typically >99% of fatty acids in the C_{16}–C_{18} range.

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MATERIALS AND METHODS

Apparatus. Perkin-Elmer Sigma 4 gas chromatographs with Ni^{63} electron capture detectors were used for pesticide recoveries. Ancillary equipment included a Perkin-Elmer AS-100B autosampler, a Dynatech GC-311H autosampler, and 1-mV recorders. The glass column used was 2 m × 4 mm (i.d.) and packed with 3.5% SE30/5.25% OV210 on 100/120-mesh Gas Chrom Q (custom packed). Columns used for confirmation were a 2 m × 4 mm (i.d.) column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120-mesh Supelcoport (Supelco, Inc., Bellefonte, PA); a 2 m × 4 mm (i.d.) column packed with 3% SP-2100 on 100/120-mesh Supelcoport (Supelco, Inc.); and a 30 m × 0.32 mm (i.d.) DB-5 capillary column with 1-μm film thickness (J & W Scientific, Folsom, CA). Injector and detector temperatures for all pesticide columns were 300 °C. Oven temperatures were 200 °C for the SE30/OV210, 225 °C for the SP-2250/SP-2401, and 195 °C for the SP-2100 and DB-5 columns. Nitrogen at 30 cm^3/min was used for both carrier and makeup with packed columns. Carrier flow for the DB-5 was 1 cm^3/min of hydrogen with 30 cm^3/min nitrogen makeup to the detector. DB-5 was operated in the split mode at a split ratio of 1:20.

A Hewlett-Packard 5890 GC with FID was used for fatty acid methyl ester (FAME) profiles. Ancillary equipment was a Hewlett-Packard 3393A integrator and 7673 A autosampler. The column used was a 30 m × 0.53 mm (i.d.) DB-225 column at 1 μm film thickness (J & W Scientific). Carrier and makeup gas flow rates were 6 and 25 cm^3/min helium, respectively. Temperatures: injector, 200 °C; detector, 220 °C; column, 180 °C for 13 min, to 210 °C at 5 °C/min, then hold for 20 min.

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