therein.
2. Oda, R., M. Okano, S. Tokiura and F. Misumi, *Bull. Chem. Soc. Jpn. 35*:1219 (1962).
3. Wohl, R.A., and J. Cannie, *J. Org. Chem. 38*:1787 (1973).
4. Temnikov, T.I., and V.N. Yandovskii, *Zh. Org. Khim. 4*:178 (1968).
5. Hayashi, Y., S. Ueda and R. Oda, *J. Chem. Soc. Jpn. 90*:946 (1969).
6. Smith, J.R.L., R.O.C. Norman and R.M. Stillings, *J. Chem. Soc. Perkin Trans. I*,1200 (1975).
7. Maerker, G., E.T. Haeberer, E.T. Donahue and T.A. Foglia, *J. Heterocycl. Chem. 7*:563 (1970).
8. Gunstone, F.D., and F.R. Jacobsberg, *Chem. Phys. Lipids 9*:26 (1972).
9. Palameta, B., and M. Prostenik, *Tetrahedron 19*:1463 (1963).

[Received September 9, 1985]

A High Performance Size-Exclusion Chromatographic Method for Evaluating Heated Oils[1]

Pamela J. White* and **Yen-chin Wang**
Food and Nutrition Department, Iowa State University, Ames, IA 50011

High performance size-exclusion chromatography (HPSEC) was used to measure compounds with high-molecular weight (MW) formed during the heating of oil. Formation of the high-MW compounds is believed to be a reliable indicator of heat abuse in oils. The HPSEC method employs two μ-spherogel size-exclusion columns (500 and 1000 Å) in a series to separate the high-MW compounds which are detected at a wavelength of 234 nm by using a variable wavelength detector.

The method was examined in the following study. Two sources of soybean oil were heated under laboratory conditions at 182 ± 2 C for eight 7-hr days. Samples were taken periodically and tested by using HPSEC. Oil samples from two commercial deep-fat frying operations were similarly tested. In all cases, size, number and apparent MW of the compounds formed increased with increasing frying time.

The HPSEC procedure was compared with a method involving separation of polar and nonpolar components in a used frying fat by means of column chromatography on silica gel.

During deep-fat frying, volatile and nonvolatile decomposition products are formed from the oil. The volatile ones distill out during frying, but the nonvolatile products accumulate in the oil and form higher and higher molecular weight (MW) compounds as heating progresses. This changes the functional, nutritional and sensory properties of the fat or oil, resulting in a darkened color, an increased viscosity, a decreased smokepoint and increased foaming of the oil (1-4). This, in turn, is said to cause an increased absorption of oil into the food being fried and an overall decrease in food quality. The high-MW compounds also have been suggested by some researchers to be harmful when fed at high levels to rats (5-10).

The measurement of heat abuse in oils has proved to be a challenge. It is difficult to volatilize the high-MW compounds for gas chromatography purposes and to dissolve the abused oils in solvents for liquid chromatography. Traditionally, overall values such as free fatty acid (11,12), iodine value (13,14), nonurea-adduct forming esters (15,16) and viscosity (12,15) have been used to assess heat damage in frying oils. However, none of these methods has proved to be a good measure of heat abuse.

A column chromatography (CC) method that measures the polar materials in an oil does give a good indication of total oil abuse, but it is not specific (17). A quick method that measures the dielectric constant has been used to estimate frying oil degradation (18). Again, the dielectric constant method gives only one overall value. It would be difficult to adapt the procedure to real situations because the reading is influenced by many outside factors, such as water or fat extracted from the fried food (19-21). In addition, Graziano (18) reported that fresh oils differ in dielectric constants, so the instrument must be standardized each time an oil is tested. In the last 10 years, researchers have developed methods for measuring the high-MW fraction or portions of it. The high-MW compounds should be a more reliable indicator of heat abuse because of their low volatility and resultant increased stability (20,21).

Aitzetmüller published a series of articles about using liquid chromatography (LC) to estimate the artifacts in heated oils (22-24). Each was an improvement upon the previous method. He was able to measure the total polar artifacts by gradient elution and a moving wire detector (24). In 1978, however, Billek et al. (25) compared the LC method with several others and found Aitzetmüller's method to have poor reproducibility and to be very time consuming.

Several researchers have used gel permeation chromatography to study the formation of high-MW compounds in heated oil (26-28). The major drawbacks to these methods have been incomplete and lengthy (5-6 hr) separations.

Firestone (29) used gas liquid chromatography (GLC) to determine differences between oxidative and thermal dimers in heated oils. The dimers have been shown to

[1]This is Journal Paper No. J-11947 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. 2568.
*To whom correspondence should be addressed.

correlate well with heat abuse. Paradis and Nawar (20,21) also developed a GLC procedure to measure the amounts of dimeric triglycerides present in heated oils as a marker for heat abuse. In contrast, Guillaumin (30) used GLC to analyze cyclic monomers in heated fats. These GLC methods do not measure the formation of all high-MW compounds so may leave out some important heat changes. Waltking and coworkers (31) used GLC to quantify the total polymers in heat-abused oil by using a complex formula relating the peak area of an internal standard to the amount of noneluted material. As with the CC method, this gives one overall value for heat abuse that is related to polarity of the compounds that form during heating.

In recent work, Perrin et al. (32) examined the triglyceride polymers from heated oils by means of size-exclusion chromatography and a refractive index detector. They reported partial separations of triglycerides, dimeric triglycerides and polymers. Perrin's work suggests that it is possible to measure heat abuse in oils by examining groups of high-MW compounds that are formed. A way to reduce the total number of compounds being measured, yet give more specific data indicating total heat abuse of the oil, is to measure selectively those compounds that are polar by using an ultraviolet (UV) wavelength detector. In this manner, only those compounds that have undergone change with heating will be measured. The purpose of the present study was to use high performance size-exclusion chromatography (HPSEC) with a UV wavelength detector as a means for examining the high-MW compounds that form in heated oils.

EXPERIMENTAL PROCEDURES

Oil samples. For laboratory tests, refined, bleached and deodorized soybean oils (A and B) were obtained from Anderson Clayton and Company, Richardson, Texas, and from A.E. Staley Manufacturing Company, Decatur, Illinois, respectively. Oil A also had citric acid added. Three hundred g of each oil was heated in 500-ml Pyrex beakers at 182 ± 2 C for seven hr each day for eight days. The oils were cooled to room temperature the remainder of the time. Samples were removed daily and stored under nitrogen at −18 C before analysis.

Oils C and D were ones that had been used in commercial frying operations and were partially hydrogenated soybean oils with added methyl polysiloxane and citric acid. Oil C also contained BHA and BHT and had been used for frying chicken, french fries, doughnuts and breaded vegetables at 180 ± 3 C in a stainless steel pressure fryer for eight 9-hr days. After each day's use, the oil was filtered and allowed to cool to room temperature. The oil was replenished as needed to maintain the original level. Oil D had been used for frying breaded chicken at 176.5 C for seven 9.5-hr days. It also was filtered and allowed to cool to room temperature after each day's use, but was not replenished. Samples from oils C and D contained food debris, so they were filtered after being mixed with HPLC-grade methylene chloride before flushing with nitrogen and storage at −18 C.

High Performance Size-Exclusion Chromatography (HPSEC). The HPSEC system was composed of a Beckman 110A pump, 20-μl injector loop, Beckman 210 sample injector, Hitachi 100-10 variable wavelength UV/VIS detector and a Beckman 10-inch strip-chart recorder. Two μ-Spherogel columns (Beckman), 500 Å (0.8 × 30 cm) and 1000 Å (0.77 × 30 cm), were used. A column inlet filter between the injector and the column prevented blockage of the column inlet frit. HPLC-grade methylene chloride (Fisher Scientific) served as the mobile phase, with a flow rate of one ml/min.

Sample preparation for HPSEC analysis of the heated oils is as follows: dissolve 0.500 g ± .005 g of a heated oil sample in 4.5 ml of HPLC-grade methylene chloride. Centrifuge for two min at 1600 rpm and retain supernatant. Inject desired concentration onto the HPLC.

Amounts of each sample were injected according to the sizes of the polymer peaks obtained. In this study, the final concentrations injected onto the column were 6.6 μg/20 μl, 10 μg/20 μl and 5 μg/20 μl for soybean oils A, B and the commercial oils, respectively. Analyses for each oil were run in duplicate.

Polystyrene standards (Supelco, Inc., Bellefonte, Pennsylvania) of various molecular weights (800, 2,000, 4,000, 9,000, 17,500, 35,000 and 50,000 g/mole) were used as external standards to determine the approximate MW separations on the columns.

The peak areas of the compounds were measured by either height × width at half height or by use of a planimeter. Peak areas that were irregular or that were smaller than 0.3 cm^2 were measured by planimeter tracings. If peaks overlapped, their areas were measured by extrapolation to the baseline. All HPSEC runs were duplicated and the results averaged.

Three UV wavelengths (234, 254 and 270 nm) were tested for detecting functional groups expected to form in the heated oils. These expected functional groups and their absorbance wavelengths follow: conjugated dienes are detected at 234 nm (33,34), conjugated linolenic acid and other polar compounds at 254 nm (34) and ketone groups and conjugated linolenic acid and other trienes at 270 nm (33,34). Each wavelength detected the same peaks from the abused oils in approximately the same ratios, although the sensitivities varied. A wavelength of 234 nm produced the largest peaks, followed by 254 nm and then 270 nm. No advantage of one wavelength over another due to functional group selectivity was evident, so a wavelength of 234 nm was selected on the basis of sensitivity.

Column chromatography (CC). Work done by Billek et al. (25) suggested that a measure of the total polar components by separation of polar and nonpolar components in a used frying fat by CC on silica gel is one of the best methods for assessing the overall deterioration of heat-abused oils. In our study, selected oil samples were analyzed in duplicate by the CC method (35) to better evaluate the HPSEC procedure.

Column Chromatography/High Performance Size-Exclusion Chromatography. Further evaluation of the HPSEC method was accomplished by combining CC and HPSEC. Selected oil samples from CC of the polar and nonpolar fractions were analyzed in duplicate by HPSEC.

Peroxide Value (PV). Peroxide values of oil samples

were determined in duplicate by official method Cd 8-53 of the American Oil Chemists' Society (36).

RESULTS AND DISCUSSION

Method development. The polymers formed in heated oils range from 700 to 6000 Daltons, depending upon the oil source, heating conditions and time (37,38). In accordance with these results, 500-Å and 1000-Å size-exclusion columns were combined in that order and used to separate compounds having MW of 5×10^2 to 5×10^4. Each column also was tested separately, but, as expected, yielded poorer resolution than with both in series.

Data on retention time versus MW separations on the columns was obtained by using polystyrene standards. In general, retention volumes increased by about one ml for every doubling of MW for the standards ranging in MW from 800 to 50,000 g/mole. A comparison of the retention volumes of the oil polymers with those of the standards gave an estimate of MW for the polymers and suggested the following MW distribution: peak 1, 1,000 g/mole (triglycerides and fatty acid trimers); peak 2, 2,000 g/mole (dimeric triglycerides); peak 3, 4,000 g/mole (tetrameric TG); and peak 4, 4,000-6,000 g/mole (larger than tetramers).

Reproducibility of the HPSEC method was tested by injecting triplicate samples of a standard known compound (polystyrene) and of the unknown sample. The coefficient of variation for a single peak ranged from 2 to 9%, depending upon the size of the peak being measured. Smaller peaks gave variations closer to 9%, while larger peaks had smaller variations of around 2%. The polystyrene standards all gave similar peak areas for the weight of compound injected, amounting to 1 cm^2 for 0.63 μg of pure polystyrene compound (recorder range = 0.02, chart speed 1 cm/min). The minimum detectable level was estimated as follows. We assumed that a TG molecule has a MW of 1,000 and that each TG contains one conjugated diene group with a molar absorbance (molar extinction coefficient) of 29,000 $mole^{-1}cm^{-1}$ per point of conjugation. Considering the characteristics of the instrument used, we calculated a minimum detectable level of around 38 ng. If one assumes only 10% conjugation of the TG molecules, the method gives a sensitivity of 380 ng.

Because we do not know the extinction coefficient of the compounds detected, measures of the peak area cannot be translated into concentrations. Nonetheless, a measurement of peak areas gives an estimate of heat abuse.

Heating tests. To test the suitability of HPSEC as a measure of heat abuse in frying oils, two oils were heated under laboratory conditions (oils A and B), and oils from two commercial frying operations (oils C and D) were sampled as described previously. Soybean oil A was heated several times in preliminary studies, and the results were always similar.

Two chromatograms of soybean oil A at seven and 56 hr of heating are shown in Figure 1. These give pictures of the peak separations and comparative sizes. Changes in average peak areas over the 8-day (56-hr) heating period are shown in Figure 2 and Table 1. Only peaks 1 and 2 were present in the unheated oil. Peak 1, which probably is conjugated triglycerides, increased rapidly, reached a maximum at two days (14 hr) and then declined gradually. The decrease in peak 1 was likely caused by polymerization of triglycerides into dimers or higher-MW compounds. Peak 2 (probably dimeric

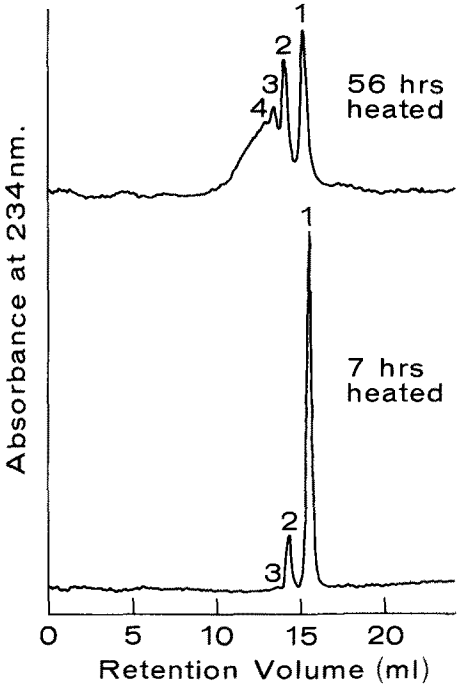

FIG. 1. Typical HPSEC chromatograms for soybean oil A at 7 and 56 hr of heating. Recorder range, 0.02; chart speed, 1 cm/min; UV detector, 234 nm.

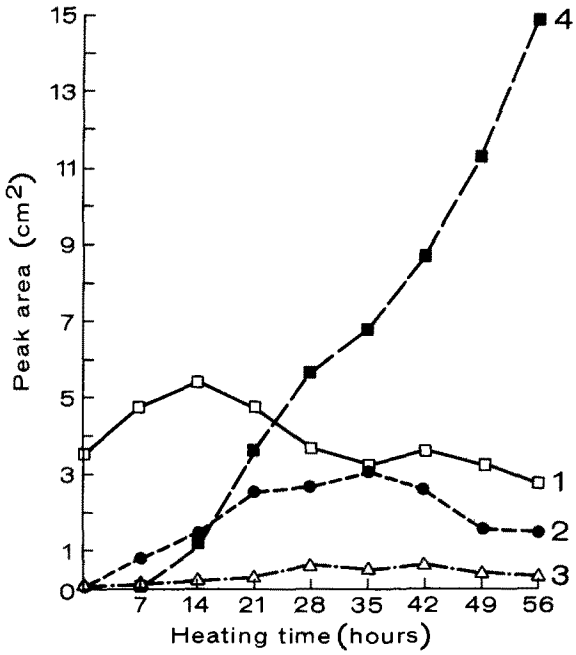

FIG. 2. Changes in peak areas from HPSEC analysis of heated soybean oil A. Peak areas are the average of two measurements and are calculated for 10 μg of injected oil. Recorder range, 0.02; chart speed, 1 cm/min; UV detector, 234 nm.

triglycerides) increased for five days (35 hr) of heating and then declined. This pattern likely represents an almost steady state between dimer formation and then further polymerization into higher-MW compounds. Peak 3 (probably tetrameric triglycerides) appeared at day 1 (7 hr) of heating, increased for four days (28 hr) and then declined gradually as the tetrameric TG were further polymerized to form the compounds represented by peak 4. Peak 4 (highest-MW compounds) appeared at day 2 (14 hr) and continued to increase dramatically for the remainder of the heating period. It reached an area of 14.85 cm^2 by day 8 (56 hr), which was accompanied by the eventual decrease of peaks 1, 2 and 3.

These chromatograms can be compared with those of Perrin et al. (32), who determined triglyceride polymers from fried sunflower oil on 50-Å and 100-Å size-exclusion columns in series. They obtained chromatograms similar to ours. They believed that their peak 1 represented triglycerides and oxidized triglycerides and that peak 2 was dimers. This is similar to the HPSEC results reported here, except that unchanged TG were not detected in our method and, thus, peak 1 was much smaller. Trimers and polymers in their study eluted together in peak 3. In our experiment, higher-MW compounds are partly separated into peaks 3 and 4.

Fritsch et al. (19) reported that peroxides are very unstable during the heat abuse of oils. The peroxide values of our samples increased very little with heating. After eight days (56 hr) of heat abuse, oil A had risen to only 3.48 from a value on day 0 of 0.21. These values were typical of all the samples analyzed.

The second oil, soybean oil B, was tested and the results similar to those previously described for oil A (Table 1). However, the peaks were slightly larger overall, possibly because it did not contain citric acid. Peroxide values for soybean oil B were comparable to those of soybean oil A.

The HPSEC results from soybean oils A and B indicate that a successful measure of heat abuse is being determined. To get a better comparison, soybean oil A was also analyzed by column chromatography (CC) of the polar components. Results are shown in Table 2. There was a large increase in the percentage of polar components seen in soybean oil A as heating progressed. By eight days (56 hr), the percentage of polar materials was almost 66%. In this experiment, recovery of the oil from the column by addition of the polar and nonpolar fractions was between 95.5% and 98.7%. Waltking and Wessels (17) reported that, for samples containing substantial amounts of polar materials, recovery might be incomplete because small amounts of highly polar compounds, generally 1-2%, are not eluted from the column.

Paradis and Nawar (20,21) recommended that 27% polar components from CC analyses be the maximum level for use of a frying oil. A possible method for comparing this standard with the HPSEC method presented here is to measure the area of peak 4, which represents the most polymerized compounds and which increases steadily throughout heating. The CC method also measures polar low-MW compounds such as free fatty acids and mono- and diglycerides. In our experiment, a measure of 27% polar compounds from CC corresponded to a peak 4 size of about 5 cm^2 (recorder

TABLE 1

Changes in Peak Areas of Heated Soybean Oils A, B, C, and D by HPSEC Analyses

Sample[a]	Peak no.	Peak areas (cm^2) per days of heating								
		0	1	2	3	4	5	6	7	8
Oil A (7 hr)	1	3.5	4.3	5.5	4.3	3.7	3.4	3.6	3.3	2.9
(citric acid)	2	0.2	0.9	1.6	2.6	2.7	3.2	2.7	1.6	1.5
	3	0.0	0.2	0.2	0.3	0.6	0.5	0.7	0.4	0.3
	4	0.0	0.0	1.2	3.7	5.7	6.8	8.7	11.2	14.9
Oil B (7 hr)	1	2.4	3.3	5.6	6.6	6.9	5.9	4.7	5.0	4.3
(no additives)	2	0.6	4.1	6.8	7.5	6.8	7.3	6.1	5.6	5.9
	3	0.0	0.4	1.1	1.4	1.5	1.4	1.4	1.3	1.1
	4	0.0	1.6	7.1	13.2	13.9	19.3	26.8	29.3	32.7
Oil C (9 hr)	1	8.1	7.7	6.0	7.0	7.9	7.6	7.7	7.7	6.7
(BHA, BHT, Si[b], citric	2	0.4	1.7	1.5	1.9	2.6	2.3	2.4	2.0	1.8
acid, mixed menu)	3	0.0	0.4	0.2	0.4	0.4	0.6	0.3	0.3	0.2
	4	0.0	0.2	2.0	1.0	1.9	1.6	2.3	2.2	2.7
Oil D (9.5 hr)	1	5.1	6.6	5.5	5.3	5.0	4.7	4.7	4.8	NA[c]
(Si, citric acid, breaded	2	0.2	0.4	0.4	0.4	0.4	0.3	0.5	0.5	NA
chicken)	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA
	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA

[a]Sample source (heating time/d). Peak areas are the average of two measurements and are calculated for 10 μg of injected oil. Range 0.02; chart speed, 1 cm/min; UV detector, 234 nm.
[b]Methyl polysiloxane.
[c]Not analyzed.

TABLE 2

Percentages of Polar Fractions (F-2) from Column Chromatography Analysis of Heated Oils A, C and D

Sample[a]	Days of heating								
	0	1	2	3	4	5	6	7	8
Oil A (7 hr) (citric acid)	3.1	6.0	12.4	23.2	33.6	44.3	54.1	59.9	65.8
Oil C (9 hr) (BHA, BHT, Si[c], citric acid, mixed menu)	2.9	8.1	8.7	12.9	14.9	16.0	18.6	15.1	17.2
Oil D (9.5 hr) (Si, citric acid, breaded chicken)	6.0	5.1	6.0	5.0	7.4	7.9	6.6	7.0	NA[b]

[a]Sample source (heating time/d).
[b]Not analyzed.
[c]Methyl polysiloxane.

range = .02) when 10 μg oil was injected. This occurred between three and four days (21 and 28 hr) of heating of soybean oil A. This information could be used as a guide to determine when to discard used frying oil.

Direct HPSEC analyses of a heated oil can provide a visual picture of the deterioration and information about the MW distribution as well as giving a numerical value for its abuse. This provides more detailed information than does CC about the deterioration pattern.

To further compare the HPSEC and CC methods, the nonpolar (F-1) and polar (F-2) fractions from the CC method were injected onto the HPLC by using the HPSEC method previously described. Theoretically, the total size of the peaks from F-1 and F-2 should equal the size of these peaks from an HPSEC analysis alone. The results of CC/HPSEC analyses of both fractions are summarized in Figure 3 and Table 3. The nonpolar fractions contained only peak 1, which decreased gradually throughout heating. This pattern indicates that most conjugated triglycerides (TG) in the fresh oil are not very oxidized and are thus nonpolar. The eventual decrease of unoxidized TG from F-1 was accompanied by an increase of oxidized TG that appeared as peak 1 in the polar fraction. Although F-2 of the fresh oil had no peaks, peaks 1, 2, 3 and 4 in this fraction increased with heating and, with the exception of days 7 and 8 (49 and 56 hr) for peak 4, duplicated the results from direct HPSEC analyses. After day 7 (42 hr), there was incomplete recovery of the very polar polymers represented by peak 4 by the CC method. It is for this reason that the official IUPAC-AOAC method (35) suggests that the percentage polar components be calculated indirectly from the percentage nonpolar fraction.

In the final part of this study, oil samples from the fryers of two commercial frying operations in Ames, Iowa, were tested. The HPSEC results are shown in Table 1. In both samples, fresh oil revealed only peaks 1 and 2. The size of peak 1 changed little throughout the heating period in both oils, most likely because of continual change in its composition from nonpolar to more polar TG. In addition, lipids from the supply of fresh foods to the fryer likely contributed additional TG which then underwent conjugation and contributed to

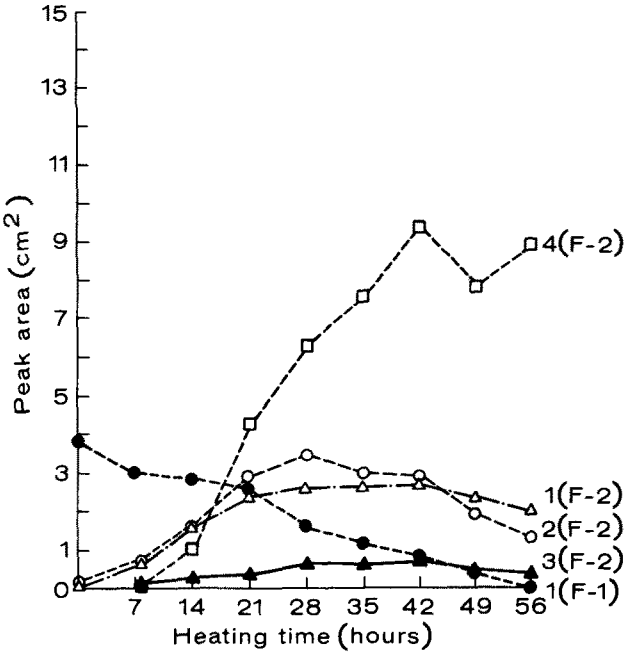

FIG. 3. Changes in peak areas of heated soybean oil A by column chromatography followed by HPSEC analyses of the nonpolar (F-1) and polar (F-2) fractions. Peak areas are the average of two measurements and are calculated for 10 μg of injected oil. Recorder range, 0.02; chart speed, 1 cm/min; UV detector, 234 nm.

the size of peak 1. With additional heating, peak 1 likely would decrease as the TG polymerized into dimers and higher-MW compounds. In oil C, peaks 2 and 3 rose with heating but showed a slight drop at the end, probably as these compounds polymerized into higher-MW compounds. The area of peak 4 in this sample was still relatively small, even after eight days (72 hr) of heat abuse, indicating that oil C was not very deteriorated. Peak 2 from oil D increased only slightly during heating and peaks 3 and 4 were not detected, again indicating minimal abuse.

The CC results of commercial oils C and D are shown in Table 2. For oil C, percentages of the polar fraction increased steadily and reached a maximum at day 6 (54 hr) of heating. By day 7 (63 hr), the percentage dropped and even by day 8 (72 hr) had not regained the level

TABLE 3

Changes in Peak Areas of Heated Soybean Oils A, C and D by Column Chromatography Followed by HPLC Analyses of the Nonpolar (F-1) and Polar (F-2) Fractions

Sample[a]	Peak (fract)	Peak areas (cm^2) per days of heating								
		0	1	2	3	4	5	6	7	8
Oil A (7 hr)	1 (F-1)	3.7	3.0	2.9	2.2	1.7	1.2	0.9	0.4	0.0
(citric acid)	1 (F-2)	0.0	0.9	1.7	2.4	2.6	2.7	2.7	2.3	2.0
	2 (F-2)	0.0	0.7	1.6	2.9	3.1	3.0	2.8	1.9	1.2
	3 (F-2)	0.0	0.0	0.3	0.3	0.4	0.6	0.6	0.5	0.2
	4 (F-2)	0.0	0.0	1.0	4.2	6.2	7.5	9.3	7.8	8.9
Oil C (9 hr)	1 (F-1)	8.4	NA[c]	NA	6.2	NA	NA	6.9	NA	6.0
(BHA, BHT, Si[b], citric	1 (F-2)	0.0	NA	NA	1.0	NA	NA	0.8	NA	0.9
acid, mixed menu)	2 (F-2)	0.0	NA	NA	1.8	NA	NA	2.5	NA	2.1
	3 (F-2)	0.0	NA	NA	0.2	NA	NA	0.3	NA	0.4
	4 (F-2)	0.0	NA	NA	0.8	NA	NA	2.4	NA	2.6
Oil D (9.5 hr)	1 (F-1)	4.2	6.8	NA	NA	4.7	NA	NA	4.8	NA
(Si, citric acid, breaded	1 (F-2)	0.0	0.3	NA	NA	0.3	NA	NA	0.5	NA
chicken)	2 (F-2)	0.0	0.0	NA	NA	0.4	NA	NA	0.4	NA
	3 (F-2)	0.0	0.0	NA	NA	0.0	NA	NA	0.0	NA
	4 (F-2)	0.0	0.0	NA	NA	0.0	NA	NA	0.0	NA

[a]Sample source (heating time/d). Peak areas are the average of two measurements and are calculated for 10 μg of injected oil. Sensitivity, 0.02; chart speed, 1 cm/min; UV detector, 234 nm.
[b]Methyl polysiloxane.
[c]Not analyzed.

reached at day 6. Recoveries, when totaling both fractions for commercial oil C, ranged from 96.5% to 100.4%. The drop in percentage of the polar fraction at days 7 and 8 may have been because the oil was replenished with fresh oil, thus diluting the polar components. The oils from days 6, 7 and 8 were tested again to see if an error had been made in the procedure, but these values were similar to the ones reported. The high-MW compounds (peak 4) by direct HPSEC did not drop at days 7 and 8 (Table 1). Percentages of the polar fraction of oil D were even smaller than for oil C , and again, were erratic within a small range. Both oils were discarded by the restaurants before reaching 27% polar components, the level recommended by Paradis and Nawar (20,21). In addition, the size of peak 4 did not reach 5 cm^2 (10 μg oil injected, r = .02), the size that earlier was suggested to correspond to 27% polar components.

Because deterioration of commercial oils C and D was fairly slow, only a few oil samples were selected for analysis of the CC fractions by HPSEC (Table 3). As with oil A, fresh oil from C and D contained only peak 1 in the nonpolar fraction and no peaks in the polar fraction. In addition, the areas of peak 1 from the fresh oil samples were fairly close to those from direct HPSEC analyses (see Table 1). For peaks 2, 3 and 4, values from CC/HPSEC were very similar to those of direct HPSEC analyses, which corresponds to the CC/HPSEC and direct HPSEC comparisons of oil A. Peroxide values for oils C and D rose very little with heating.

The deterioration of oil D was much slower than that of oil C. Oil C had been used to fry a variety of foods, including chicken, french fries, doughnuts and breaded vegetables, while oil D had been used to fry only chicken. According to McGill (39), fatty foods, wet foods and foods that easily break apart will accelerate the chemical changes of a frying oil. The breaded vegetables likely enhanced the deterioration of oil C. Thompson and Aust (40) also reported that the quantity of food fried contributes to the decreased quality of a frying oil. More total food was reported to have been fried in commercial oil C than in D. The frying temperature was also reported to be higher in oil C than in D.

In all, oils A and B, heated to frying temperature under laboratory conditions, deteriorated much more quickly than did oils C and D, which were used to fry foods in a commercial setting. This was confirmed by both CC and HPSEC in oils A, C and D and by HPSEC in oil B. Commercial oils C and D were partially hydrogenated and contained dimethyl polysiloxanes as an additive. Both treatments are very effective in slowing down oxidation of frying oils (41). Oil C also had BHA and BHT added and was replenished, as needed, to its original level. Oils A and B were untreated, were not replenished and had no additives, except for citric acid in oil A. In addition, oils A and B were heated in small beakers and had higher surface/volume ratios than did oils C and D. Waltking and Zmachinski (13) claimed that cooking a food in frying oil produces a steam layer which can decrease the deterioration of a frying oil. All these factors would account for more rapid deterioration in the laboratory-heated oils. In addition, foods fried in the commercial oils may have carried off some of the polymers that are measured during heat abuse, thus resulting in lower values for these parameters. Some researchers have found that the oil removed from a fried food contains greater amounts of polymers than does

the oil in which it was fried (15,42). This does, however, contradict the work of McGill (39) and Thompson and Aust (40) in which oil use was reported to increase its rate of deterioration. However, this may help to account for the small amount of polar components and/or polymers formed in oils C and D when compared with oils A and B.

These results suggest that the HPSEC method with μ-Spherogel columns can give qualitative measures to heat abuse in frying oils by partially separating the high-MW compounds found in frying oils. In addition, some estimate of the relative quantity of high-MW compounds formed can be given. The method is very quick, involving a 2-min centrifugation followed by a 20-min HPSEC run. In contrast, the time necessary to run one sample by CC is at least 3.5 hr. Practically, this HPSEC method could be used in the laboratory, as in the current study, to observe high-MW compounds formed in heated oils. It might also be used by the oil industry as a quick test to compare heat stabilities of various frying oils. The major expenses of this procedure involve the initial purchase of the HPSEC equipment and columns, but, because many large quality control laboratories already own HPLC or HPSEC systems, the costs would be minimal.

ACKNOWLEDGMENTS

This work was supported in part by research grants from the Iowa Soybean Association and the National Institutes of Health.

REFERENCES

1. Gere, A., *Fette, Seifen, Anstrichm. 85*:111 (1983).
2. Perkins, E.G., *Food Technol. 21*:611 (1967).
3. Howard, R., and P.R. Stanley, *Baker's Dig. 46*:38 (1972).
4. Tangel, F.P., J.G. Leeder and S.S. Chang, *J. Food Sci. 42*:1110 (1977).
5. Crampton, E.W., R.H. Common, F.A. Farmer, A.F. Wells and D. Crawford, *J. Nutr. 49*:333 (1953).
6. Crampton, E.W., R.H. Common, E.T. Pritchard and F.A. Farmer, *Ibid. 60*:13 (1956).
7. Nolen, G.A., J.C. Alexander and N.R. Artman, *Ibid. 93*:337 (1967).
8. Iwaoka, W.T., and E.G. Perkins, *Lipids 11*:349 (1976).
9. Iwaoka, W.T., and E.G. Perkins, *J. Amer. Oil Chem. Soc. 55*:734 (1978).
10. Meltzer, J.B., E.N. Frankel, T.R. Bessler and E.G. Perkins, *Ibid. 58*:779 (1981).
11. Johnson, O.C., and F.A. Kummerow, *Ibid. 34*:407 (1957).
12. Stevenson, S.G., L. Jeffrey, M. Vaisey-Genser, B. Fyfe, F.W. Hougen and N.A.M. Eskin, *Can. Inst. Food Sci. Technol. J. 17*:187 (1984).
13. Waltking, A.E., and H. Zmachinski, *J. Amer. Oil Chem. Soc. 47*:530 (1970).
14. Sims, R.P.A., *Ibid. 34*:466 (1957).
15. Alim, H., and I.D. Morton, *Proc. IV Int. Congr. Food Sci.Technol. 1*:345 (1974).
16. Firestone, D., W. Horwitz, L. Friedman and G.M. Shue, *J. Amer. Oil Chem. Soc. 38*:253 (1961).
17. Waltking, A.E., and H. Wessels, *J. Assoc. Off. Anal. Chem. 64*:1329 (1981).
18. Graziano, V.J., *Food Technol. 33*:50 (1979).
19. Fritsch, C.W., D.C. Egberg and J.S. Magnuson, *J. Amer. Oil Chem. Soc. 56*:746 (1979).
20. Paradis, A.J., and W.W. Nawar, *Ibid. 58*:635 (1981).
21. Paradis, A.J., and W.W. Nawar, *J. Food Sci. 46*:449 (1981).
22. Aitzetmüller, K., *Fette, Seifen, Anstrichm. 75*:256 (1973).
23. Aitzetmüller, K., *J. Chromatogr. 79*:329 (1973).
24. Aitzetmüller, K., *Ibid. 83*:461 (1973).
25. Billek, G., G. Guhr and J. Waibel, *J. Amer. Oil Chem. Soc. 55*:728 (1978).
26. Aitzetmüller, K., *Fette, Seifen, Anstrichm. 74*:598 (1972).
27. Perkins, E.G., R. Taubold and A. Hsieh, *J. Amer. Oil Chem. Soc. 50*:223 (1973).
28. Harris, W.C., E.P. Crowell and B.B. Burnett, *Ibid. 50*:537 (1973).
29. Firestone, D., *Ibid. 40*:247 (1963).
30. Guillaumin, G., *Rev. Fr. Corps Gras. 24*:211 (1977).
31. Waltking, A.E., W.E. Seery and G.W. Bleffert, *J. Amer. Oil Chem. Soc. 52*:96 (1975).
32. Perrin, J.L., F. Redero and A. Prevot, *Rev. Fr. Corps Gras 31*:131 (1983).
33. Gray, J.I., *J. Amer. Oil Chem. Soc. 55*:539 (1978).
34. Kates, M., in *Lab Techniques in Biochemistry and Molecular Biology*, edited by T.S. Work and E. Work, Elsevier Publishing Co., New York, NY, 1975, pp. 382-383.
35. Association of Official Analytical Chemists (AOAC), Polar components in frying fats chromatographic method, in *AOAC Official Methods of Analysis*, 14th edition, AOAC, Inc., Arlington, VA, 1984, pp. 516-517.
36. *Official and Tentative Methods of the American Oil Chemists' Society* (AOCS), Vol. 1, 3rd edition, AOCS, Champaign, IL, 1971.
37. Aitzetmüller, K., *J. Chromatogr. 83*:461 (1973).
38. Perkins, E.G., and F.A. Kummerow, *J. Amer. Oil Chem. Soc. 36*:371 (1959).
39. McGill, E.A., *Baker's Dig. 54*:38 (1980).
40. Thompson, L.U., and R. Aust, *Can. Inst. Food Sci. Technol. J. 16*:246 (1983).
41. Freeman, I.P., and F.B. Padley, *J. Amer. Oil Chem. Soc. 50*:101 (1973).
42. Sahasrabudhe, M.R., and V.R. Bhalerao, *Ibid. 40*:711 (1963).

[Received July 29, 1985]