Determination of Nonvolatile Components of Heated Soybean Oils Separated with High-Efficiency Mixed-Bed Polystyrene/Divinylbenzene Columns

S.L. Abidi[a,*], I.H. Kim[b], and K.A. Rennick[a]

[a]Food Quality and Safety Research, National Center for Agriculture Research, ARS, USDA, Peoria, Illinois 61604, and [b]Department of Food and Nutrition, College of Allied Health Sciences, Korea University, Seoul, Republic of Korea

ABSTRACT: Whole heated soybean oils and their polar fractions were analyzed for nonvolatile components by high-performance size-exclusion chromatography (HPSEC) with evaporative light scattering detection (ELSD). High molecular-weight (MW) polymer compounds with MW ≥ trimer were efficiently separated with new 3-μm mixed-bed styrene/divinylbenzene copolymer columns. Peaks of high MW polymer components in the new column system appeared to be sharper and more symmetrical than those obtained with other columns. In the model systems studied, continuous addition of water to partially simulate frying conditions resulted in a significant increase (up to 30%) in the polar lipid content of the heated oils evaluated. Due to relatively high concentrations of monomeric triglycerides (84.6–93.5%) present in the whole unfractionated oils, small but erratic variations in the compositional distribution of components were observed in oils containing different amounts of added water. On the other hand, HPSEC-ELSD analyses of the polar fractions (monomeric triglycerides, 25.4–62.6%) showed significant changes in the content and composition of nonvolatile components with the amount of water added. In general, prolonged heating with increasing amounts of water accelerated hydrolysis and polymerization of heated soybean oils. Discrepancies in total polymeric materials obtained from HPSEC composition data for whole oils and polar fractions are discussed in terms of nonuniformity in sample matrices, detection limitations for minor components, and a nonlinear ELSD response rationale.

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KEY WORDS: Frying stability, high-efficiency mixed-bed column, high-performance size exclusion chromatography, hydrolysis, polar fractions, soybean oil, thermal oxidation.

During deep-fat frying, oil is continuously or intermittently heated at elevated temperatures in the presence of air and moisture. A number of chemical reactions, including oxidation, hydrolysis, and polymerization, occur as do changes from thermal and oxidation products. As these reactions proceed, functional, sensory, and nutritional qualities of the oil change and the oil may eventually reach a point where it is no longer possible to prepare high-quality fried food, and the oil must be discarded. Under such conditions, oxidative and thermal decompositions take place with the formation of volatile and nonvolatile decomposition products, some of which in excess may be harmful to human health (1,2). Decomposition products also produce physical and chemical changes in frying oil including increased viscosity, polar components, polymer contents, free fatty acids, dark color, and foaming as well as decreased iodine value and surface tension (3,4). Factors affecting frying stability of oil include temperature, time of heating, frequency of frying food, exposure to oxygen, moisture of frying food, presence of antioxidants, and replenishment of oil during frying. Moisture is an important factor in deep-fat frying because water in food is heated quickly and vaporized. Resulting steam causes a boiling action in the oil which affects not only aeration but also triacylglycerol hydrolysis and increased oxidation of the oil with formation of nonvolatile and volatile components.

Nonvolatile components of heated oils are conventionally separated by high-performance size-exclusion chromatography (HPSEC) with a polymer column having particle size ≥ 5 μm and uniform porosity. In view of the inadequacy of existing methods for the separation of high molecular-weight (MW) polymer products of frying oils, we initiated exploratory experiments to examine the applicability of a newly developed mixed-bed 3-μm-polystyrene/divinylbenzene columns in frying oil separations. We found that separations of the hitherto unresolved bands in high-MW regions on HPSEC chromatograms were significantly improved. Thereby, the new columns were coupled to an HPSEC-evaporative light scattering detection (ELSD) system for the determination of the content and composition of degraded oils upon heating. The qualitative analytical data were used to evaluate effects of moisture on the distribution of nonvolatile components in the polar fractions of heated soybean oils. The results are reported in this paper.

EXPERIMENTAL PROCEDURES

Preparation of heated oil samples. Deodorized soybean oil (Procter & Gamble Co., Cincinnati, OH) was purchased at a local store. Oils (3 g) were placed in test tubes (i.d. 32.5 ×

*To whom correspondence should be addressed at Food Quality and Safety Research, National Center for Agriculture Research, USDA-ARS, 1815 N. University St., Peoria, IL 61604.
E-mail: abidis@mail.ncaur.usda.gov

L 125 mm) and heated at 190°C in an oil bath. The control had no water added and other samples had 1 and 2% deionized water (by oil weight) continuously added with a micro syringe for 1 h to partially simulate frying conditions. Oils were heated for 8 h daily for 4 d. Used oils were placed in sealed vials under nitrogen and stored at –20°C.

Determination and isolation of polar fraction. Total polar lipid content was determined by the AOCS column chromatographic method Cd 20–91 (5). The polar fraction isolated for HPSEC-ELSD analysis was obtained by elution with chloroform/methanol (1:1, vol/vol) instead of diethyl ether (6). Diethyl ether yielded lower recovery of polar materials. Separation of nonpolar and polar fraction was checked by thin-layer chromatography on 0.25 mm thick silica gel plates (20 × 20 cm glass). Plates were developed with hexane/diethyl ether/acetic acid (80:20:1, vol/vol/vol) and spots were detected by iodine vapor.

HPSEC-ELSD. MW standards of oleic acid, monolein, diolein, and triolein were obtained from Sigma (St. Louis, MO). HPSEC analyses were performed with a Thermo Separation Products (San Jose, CA, USA) Model P4000 liquid chromatograph equipped with an Alltech (Deerfield, IL) Model Varex IIA evaporative light-scattering detector. The detector was coupled with three Polymer Laboratory (Amherst, MA) PLGEL MIXED-E (3 μm, 300 × 7.5 mm i.d.) columns connected in tandem. Tetrahydrofuran (THF) containing 0.025% butylated hydroxytoluene (Fisher Chemicals, Fair Lawn, NJ) was used as the mobile phase and was pumped through the columns at a flow rate of 0.8 mL/min. Aliquots (10–20 μL) of samples (concentrations in the 10–20 mg/mL range) dissolved in THF were injected onto the columns *via* a Rheodyne (Cotati, CA) Model 7125 injector fitted with 50-μL loop. For identification of component MW, linear regression analysis of retention time vs. log standard MW was conducted. Statistical analysis of data for soybean oils heated with and without water was done by analysis of variance and chi-square test (7).

RESULTS AND DISCUSSION

Comparisons of published HPSEC separations of nonvolatile components of heated oils with our results indicated that the mixed-bed polystyrene divinylbenzene phase gave a better separation of the dimeric component from the trimeric product than conventional columns used by previous investigators. Improved separations of early-eluting components in the high polymer region (molecular weights ≥ trimer) were invariably observed (8) in another ongoing study on frying oils in food media. In the presence of foods, HPSEC of frying oils with new columns yielded sharper component peaks with more resolved components than those obtained by existing methods. Since no food was used in this study, the high MW peak profiles fortuitously resembled conventional separation patterns. Nevertheless, in relation to published column systems, overall component separations on the new columns were achieved with sharper peaks and improved peak symmetry. Evidently, the superior separation efficiency was benefited from the new column packings of variable porosity and small particle size (3 μm). In some cases where simple triglyceride (e.g., triolein) samples were used, baseline separations of monomer, dimer, trimer, and tetramer were achieved (Kim, I.H., and S.L. Abidi, unpublished results). We have in fact isolated the four nonvolatile components of heated triolein for molecular species studies (Abidi, S.L., I.H. Kim, W.E. Neff, and W.C. Byrdwell, unpublished data) by normal-phase and reversed-phase high-performance liquid chromatography .

Table 1 shows that the polar lipid content of oil heated with water was higher than for samples heated without water. For example, the polar lipid content of oil without water was 2.1% for fresh oil and 51.3% after 32 h of heating, whereas that with 2% added water increased from 2.1% for fresh oil to 67.0% after 32 h of heating. At the end of the heating duration, addition of 1 and 2% water to the oils resulted in, respectively, 12.3 and 30% increases in polar lipid contents. Obviously, the presence of added water in oil samples accelerated deterioration of oil during heating. Therefore, as expected, there was a significant difference between polar lipid contents in oils heated with and without added water ($P < 0.05$).

A HPSEC-ELSD technique was utilized to further examine unfractionated heated oil and its polar fractions because of its suitability to detect high MW components of low volatility (9). To determine MW of HPSEC peaks attributable to nonvolatile components of heated oil samples, triolein, diolein, monolein, and oleic acid standards were used (10). For each standard, retention time was measured under the same HPSEC conditions as used in our analysis of heated soybean oils. The logarithmic MW as a function of retention time was extrapolated on an extension of the regression line shown in Figure 1.

Figure 2 shows the HPSEC separation of whole oils heated for 8 and 32 h. Inspection of the HPSEC chromatograms in the figure revealed that the few weak polymeric peaks in the

TABLE 1
Polar Lipid Content (wt%) of Soybean Oils Heated at 190°C With or Without Water[a]

	Heating time (h)				
Water (%)	0	8	16	24	32
0	2.1 ± 0.02	14.9 ± 0.0	29.3 ± 0.0	43.5 ± 0.1	51.3 ± 0.1
1	2.1 ± 0.02	15.8 ± 0.1	32.5 ± 0.0	46.4 ± 0.1	57.6 ± 0.1
2	2.1 ± 0.02	17.3 ± 0.1	38.5 ± 0.1	49.5 ± 0.2	67.0 ± 0.1

[a]Mean values of three replicate determinations ± standard deviation.

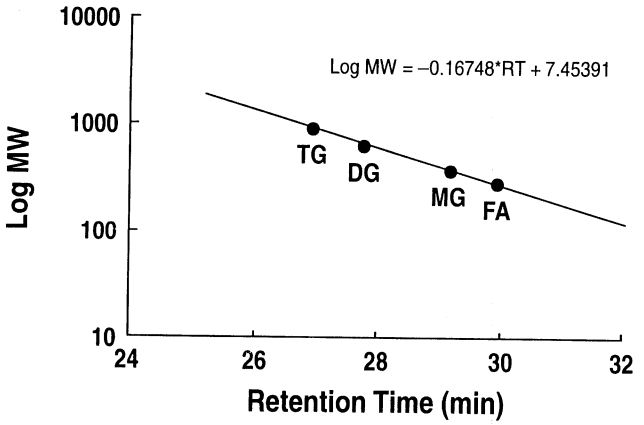

FIG. 1. Correlation of molecular weight (MW) and retention of standard compounds. TG = triglyceride, DG = diglyceride, MG = monoglyceride, FA = fatty acid.

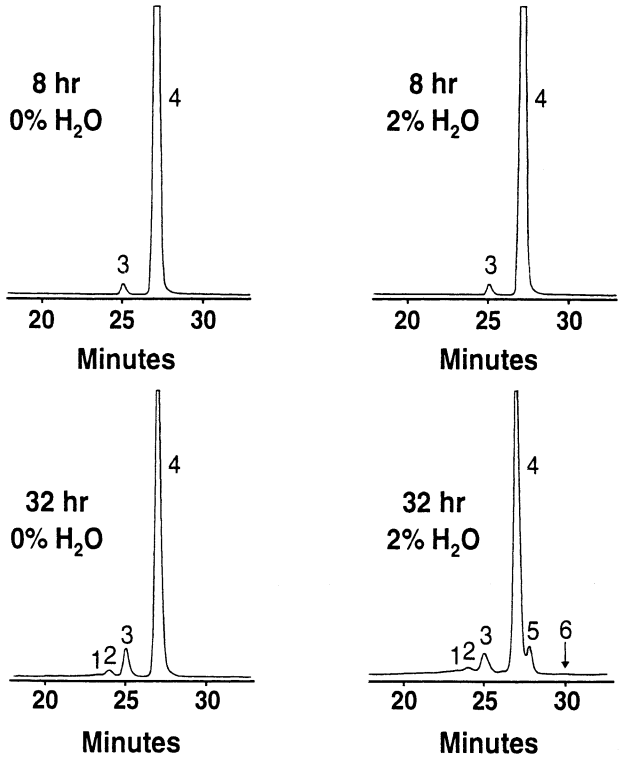

FIG. 2. High-performance size-exclusion chromatograms of nonvolatile components of whole heated soybean oils. Component designations: (1) polymer, (2) trimer, (3) dimer, (4) monomer, (5) diglyceride, (6) fatty acid.

high-MW region were flanked by a highly intense peak of monomer. The latter presumably consisted of nonpolar soybean triglycerides and polar oxidized triglycerides. As shown in all the separation profiles of the whole, unfractionated oil, the major polymerized lipid was the dimer (peak 3 in Fig. 2). A diglyceride peak of the oil heated with 2% water for 32 h was clearly detected (peak 5 in Fig. 2), but was not detected in oil heated without water. These observations indicated that added water increased hydrolysis during frying.

Table 2 shows the change in composition of nonvolatile components in response to the amount of water added to heated whole soybean oils. With the exception of the oil heated with 2% water for 32 h, HPSEC results of the unfractionated oil heated with or without water showed no significant differences ($P > 0.05$) between compositions of the oil heated with and without water. In other words, prolonged heating appeared to override the percentage of added water for causing apparent changes in compositional distribution of

TABLE 2
Composition (%) of Nonvolatile Components in Whole Soybean Oils Heated at 190°C With or Without Water

	Heating time					
	16 h			32 h		
	Water added (%)			Water added (%)		
Component	0	1	2	0	1	2
HPSEC of whole oils:						
Free fatty acid	ND	ND	ND	ND	ND	0.1 ± 0.13
Diglyceride	ND	ND	ND	ND	ND	6.2 ± 0.11
Monomer[b]	93.4 ± 0.0	93.2 ± 0.0	93.5 ± 0.1	89.3 ± 0.1	88.6 ± 0.1	84.6 ± 0.1
Dimer	6.0 ± 0.02	5.9 ± 0.02	5.9 ± 0.07	7.9 ± 0.09	7.9 ± 0.07	6.8 ± 0.02
Trimer	0.6 ± 0.02	0.6 ± 0.09	0.5 ± 0.14	1.9 ± 0.17	2.2 ± 0.05	1.8 ± 0.04
Polymer	0.1 ± 0.07	0.1 ± 0.14	0.1 ± 0.07	0.8 ± 0.10	1.3 ± 0.04	0.4 ± 0.04
Normalized values[c]						
Free fatty acid	ND	ND	ND	ND	ND	0.1 ± 0.13
Diglyceride	ND	ND	ND	ND	ND	9.3 ± 0.11
OX-monomer	77.2 ± 0.0	79.6 ± 0.0	83.1 ± 0.1	79.3 ± 0.1	80.2 ± 0.1	77.1 ± 0.1
Dimer	20.4 ± 0.0	18.2 ± 0.0	15.3 ± 0.1	15.4 ± 0.1	13.7 ± 0.1	10.2 ± 0.0
Trimer	2.0 ± 0.02	1.9 ± 0.09	1.3 ± 0.14	3.7 ± 0.17	3.8 ± 0.05	2.7 ± 0.04
Polymer	0.3 ± 0.07	0.3 ± 0.14	0.3 ± 0.07	1.6 ± 0.10	2.3 ± 0.04	0.6 ± 0.04

[a]Mean values of three replicate determinations ± standard deviation.
[b]Monomer contains triglycerides and oxidized triglycerides.
[c]Values were obtained after subtracting the percentage nonpolar triglycerides present in the whole oils based on the same components present in polar fractions. HPSEC, high-performance size exclusion chromatography; ND, not detected; OX-monomer, oxidized triglycerides.

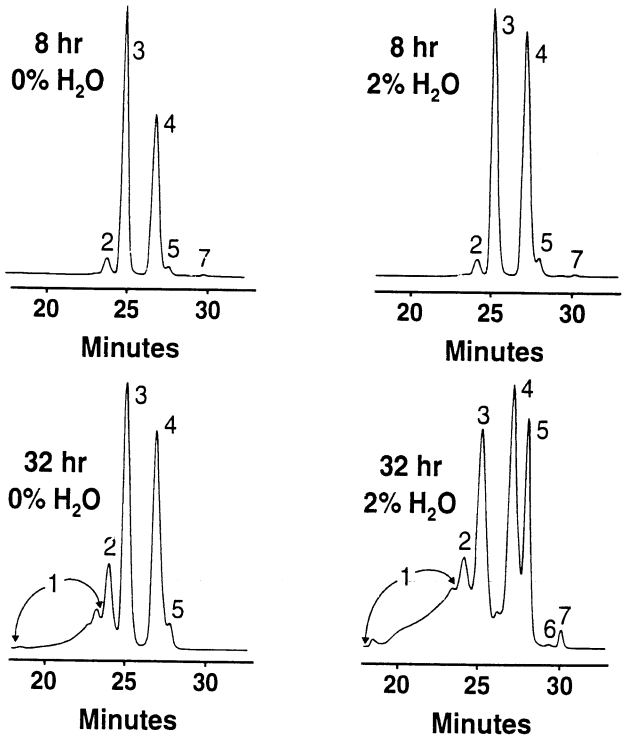

FIG. 3. High-performance size exclusion chromatograms of nonvolatile components in polar fractions isolated from heated soybean oils. Components: (6) monoglyceride, (7) fatty acid; for others, see Figure 2.

the degradation products of the whole heated oils. The observed variations in the compositional distribution of components with the amount of added water were small but erratic, because relatively high concentrations of monomeric triglycerides (84.6–93.5%) were present in the whole unfractionated oils. Removal of nonpolar triglycerides (see discussion in the following paragraph) should provide a higher degree of data accuracy to assess the impact of added water on the content and composition of degradation products of the heated oils. Therefore, it is highly recommended as general practice to use polar fractions instead of whole oils for analytical/oxidative stability studies of frying oils.

Compositions of polar lipid isolates of heated soybean oils were notably affected by both the amount of added water and heating times (Fig. 3). As clearly demonstrated in the figure, the HPSEC chromatograms of polar fractions isolated from the oil heated with or without water showed distinct separations of the nonvolatile components: free fatty acid, monoglyceride, diglyceride, monomer, dimer, trimer, and polymer. Since the primary objective of this study was to acquire compositional data for specific oil samples injected into the HPSEC-ELSD system, the chromatographic peak profiles shown in Figures 2 and 3 represent only those samples analyzed under conditions specified and do not reflect the actual distribution of nonvolatile components present in all the samples assayed. Owing to instrumental/graphic limitations as shown on the chromatograms in Figure 2, the minor component peaks tended to escape detection or to be buried under the very large monomeric peak of nonpolar soybean triglycerides and polar oxidized triglycerides. Often it required expansion of the particular minor-component region of a chromatogram by adjusting the monomeric peak with an off-scale setting. However, after removal of nonpolar triglycerides from a heated oil by silica gel chromatography to give polar fractions, there were more minor peaks discernible on the HPSEC chromatograms (Fig. 3) of the polar lipid fraction than those of the whole oil sample (Fig. 2 vs. Fig. 3). Therefore, as mentioned earlier, the separation of monomeric nonpolar triglycerides from monomeric polar triglycerides was mandatory in order to gain insight into the distribution patterns of nonvolatile components of heated oils under various frying conditions. In this regard, we are investigating an alternative approach to resolve monomeric components so that it would be possible in the future to do direct analysis of whole oils without fractionation.

The composition data presented in Table 3 were compiled based on amounts of polar fractions assayed. Accordingly, the percentage composition values of nonpolymeric components (e.g., free fatty acid, monoglyceride, diglyceride, and oxidized triglyceride monomer) of the polar fraction from the heated soybean oil at time zero were apparently higher than those from the oil heated for longer times, because amounts of polymerization products, if any, were relatively small. Examination of the HPSEC-ELSD data for nonvolatile components in polar fractions isolated from heated soybean oils showed that the diglyceride derived from oil heated for 32 h with 2% water accounted for 15.8% of the polar fraction, whereas that of oil heated for the same period of time but in the absence of water was only 2.2% (Table 3).

Previous frying oil studies have reported that the dimeric component was the major polymer formed during frying (11–13) consistent with our findings in this study. Analysis of the data in Table 3 showed that the dimer was the major product in all but one heated oil sample assayed. On the other hand, in experiments with oils heated for a long time in the presence of a high percentage of added water, both the content and composition of dimers of the polar fractions decreased with increasing amounts of water added to the heated oil. For example, the percentage/content of the dimer of polar fraction isolated from the oil heated for 32 h without water was 36.9%/18.9 mg per 100 mg oil, while the composition/content values of the dimeric component of polar fraction isolated from the oils heated for 32 h with 1 and 2% water were 31.7%/18.3 mg per 100 mg oil and 26.3%/17.6 mg per 100 mg oil, respectively. A similar trend of the effect of added water on the trimer composition was also observed in oils heated for 32 h, although an opposite trend was noted for the content values varying from 6.88 mg/100 mg oil to 7.57 mg/100 mg oil. When oils were heated for 16 h, addition of higher percentage of water tended to favor the formation of the trimer. As to the high polymer component with MW ≥ tetramer, an increase in the amount of water added to an oil heated for 16–32 h seemed to promote polymerization of heated soybean oils (Table 3).

TABLE 3
Composition and Content of Nonvolatile Components in Polar Fractions Isolated from Soybean Oils[a] Heated at 190°C With or Without Water

	Heating time						
		16 h			32 h		
	0 h	0%	1%	2%	0%	1%	2%
Composition (%)							
Free fatty acid	8.5 ± 0.07	0.1 ± 0.08	0.1 ± 0.10	0.1 ± 0.06	0.1 ± 0.07	0.2 ± 0.09	0.8 ± 0.19
Monoglyceride	1.5 ± 0.17	ND	ND	ND	ND	ND	0.1 ± 0.11
Diglyceride	23.7 ± 0.1	ND	1.2 ± 0.04	3.8 ± 0.05	2.2 ± 0.18	7.1 ± 0.16	15.8 ± 0.2
Monomer	62.6 ± 0.0	40.4 ± 0.1	42.1 ± 0.1	40.8 ± 0.1	33.9 ± 0.1	33.7 ± 0.1	25.4 ± 0.1
Dimer	3.7 ± 0.04	48.3 ± 0.1	46.8 ± 0.1	42.1 ± 0.1	36.9 ± 0.1	31.7 ± 0.1	26.3 ± 0.0
Trimer	0.1 ± 0.08	8.6 ± 0.06	7.7 ± 0.04	9.2 ± 0.10	13.4 ± 0.1	12.4 ± 0.0	11.3 ± 0.1
Polymer	ND	2.6 ± 0.10	2.1 ± 0.03	4.1 ± 0.03	13.5 ± 0.2	14.9 ± 0.2	19.8 ± 0.1
Content (mg/100 mg oil)							
Free fatty acid	0.2 ± 0.03	0.03 ± 0.0	0.03 ± 0.01	0.04 ± 0.00	0.05 ± 0.02	0.2 ± 0.04	0.5 ± 0.09
Monoglyceride	0.03 ± 0.0	ND	ND	ND	ND	ND	0.07 ± 0.11
Diglyceride	0.5 ± 0.02	ND	0.4 ± 0.02	1.4 ± 0.02	1.1 ± 0.10	4.1 ± 0.09	10.6 ± 0.2
Monomer	1.3 ± 0.02	11.8 ± 0.1	13.7 ± 0.1	15.7 ± 0.0	17.4 ± 0.1	19.4 ± 0.1	17.0 ± 0.0
Dimer	0.1 ± 0.01	14.1 ± 0.1	15.2 ± 0.0	16.2 ± 0.0	18.9 ± 0.0	18.3 ± 0.0	17.6 ± 0.0
Trimer	ND	2.5 ± 0.0	2.5 ± 0.0	3.5 ± 0.1	6.9 ± 0.1	7.2 ± 0.01	7.6 ± 0.1
Polymer	ND	0.8 ± 0.04	0.7 ± 0.0	1.6 ± 0.0	6.9 ± 0.1	8.6 ± 0.1	13.3 ± 0.0

[a]Mean values of three replicate determinations ± standard deviation. ND, not detected.

It must be stressed that the total number of nonvolatile components in whole oils was different from that in polar fractions, because the former contained an extra monomeric nonpolar triglyceride component co-eluting with the monomeric oxidized triglycerides. Comparisons of analytical data between the two sets of samples (whole oils vs. polar fractions) should be made on the basis of common species present in both oil matrices. This was done by subtracting the nonpolar triglyceride values from total monomer compositions followed by normalization of the composition data based on the same number of components (i.e., oxidized triglyceride monomer, dimer, trimer, and polymer) as in polar fractions. Thus, the normalized data for the whole oils are included in Table 2.

Table 4 compares the amount of total polymer products (i.e., dimer, trimer, and polymer) obtained by HPSEC-ELSD of whole oils and polar fractions. As discussed earlier, it is plausible to include normalized whole oil data in the same table. Obviously, the numerical values (mg/100 mg oil) of total polymer compounds found in whole oils are not in agreement with those derived from polar fractions. The discrepancy in the analytical data between the two different oil sample matrices (whole oil vs. polar fraction) may arise largely from nonlinear response of ELSD within the variable concentration range (14). When whole oils were directly subjected to HPSEC-ELSD analyses, polymer materials appeared on the chromatograms as very weak peaks flanked by a very large peak of unreacted triglycerides co-eluting with oxidized triglycerides. HPLC-ELSD analyses of the whole oil samples with such unbalanced peak distribution of minor polymer components (0.1–7.9%) invariably led to quantitation results with low precision, especially in the low concentration region of polymer compounds. The normalization of whole oil data (Table 4, column B) improved somewhat the observed HPLC-ELSD values (Table 4, column A), but failed to yield polymer content data in close agreement with those of polar fractions (Table 4, column C). Therefore, it is highly recommended to analyze polar fractions instead of whole oils for studying frying oil polymerization by HPSEC-ELSD.

TABLE 4
Total Polymer Compounds (mg/100 mg oil) in Soybean Oils Heated at 190°C With or Without Water[a]

	Dimer			Trimer			Polymer			Total		
Water (%)	A	B	C	A	B	C	A	B	C	A	B	C
16 h:												
0	6.0	20.4	14.1	0.6	2.0	2.0	0.1	0.3	0.3	6.7	22.7	16.4
1	5.9	18.2	15.2	0.6	1.9	1.8	0.1	0.3	0.2	6.6	20.4	17.2
2	5.9	15.3	16.2	0.5	1.3	1.4	0.1	0.3	0.2	6.5	16.9	17.8
32 h:												
0	7.9	15.4	18.9	1.9	3.7	6.9	0.8	1.6	6.9	10.6	20.7	32.7
1	7.9	13.7	18.3	2.2	3.8	7.2	1.3	2.3	8.6	11.4	19.8	34.1
2	6.8	10.2	17.6	1.8	2.7	7.6	0.4	0.6	13.3	9.0	13.5	38.5

[a]A, values obtained directly from HPSEC composition data for whole oils containing, among others, nonpolar triglycerides. B, normalized whole oil values based on the same number of components as in polar fractions containing no nonpolar triglycerides. C, values obtained from HPSEC composition data for polar fractions. Total, total amount of polymer compounds: dimer + trimer + polymer. For abbreviation see Table 2.

The use of a new mixed-bed polystyrene/divinylbenzene phase in the HPSEC-ELSD system allows simultaneous separations and quantification of nonvolatile components of heated oils with improved component resolution and analytical reproducibility. HPSEC of heated oils with the new columns yields much better defined peaks of the polymeric components than published methods. Application of the new column methodology to the present study facilitated the accurate analysis of heated soybean oils and enabled delineation of HPSEC characteristics of degradation products. The results of this study showed that water affected the deterioration and the content/composition of the nonvolatile components of heated oils. HPSEC-ELSD of polar fractions yielded much more reliable results than whole oils. The latter sample matrices proved unsuitable for quantitative analytical purposes.

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