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ORIGINAL ARTICLE

High Performance Liquid Chromatography–Size Exclusion Chromatography for Rapid Analysis of Total Polar Compounds in Used Frying Oils

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Abstract Analysis of used frying oil samples by high performance liquid chromatography–size exclusion chromatography (HPLC–SEC or HPSEC) was compared to AOCS Official Method Cd 20-91 (silica gel column chromatography) for the purpose of developing a rapid analysis of total polar compounds (TPC). In a direct comparison of the two analytical methods using four different sets of used frying oils (21 total oil samples) ranging from fresh to discard quality (4.3 to 35.4% TPC by column chromatography), the weight percent total polar compounds (%TPC) determined by HPLC–SEC averaged 0.71% higher than the values by silica gel column chromatography. Reproducibility of the HPLC–SEC method of $s_r = 0.30$ and $RSD_r\% = 1.22$ compares to the variability of $s_r = 0.29$ and $RSD_r = 1.3$ for samples of approximately the same %TPC, reported in AOCS Method Cd 20-91. Because the rapid method does not separate pure (non-polar) triacylglycerol (TAG) and polar, oxidized TAG (OX-TAG), a high concentration of OX-TAG will quantitatively affect the results. This places practical limits on the types of studies to which the method may be applied if a separate analysis for the OX-TAG is not performed. Advantages of the HPLC–SEC method include the following. It uses about 75% less solvent than standard column chromatography methods for determination of %TPC. This HPLC–SEC method is very similar to AOCS Official Method Cd 22-91, and thus, also separates and quantifies polymerized triacylglycerols. The HPLC–SEC method determines both TAG polymer concentration and %TPC of used frying oils in about 1 h.

Keywords Total polar compounds (TPC, TPM) · HPLC · HPSEC · Oxidized triacylglyerol · OX-TAG · Frying oil quality · Edible oil analysis

Introduction

The concentration of total polar compounds (%TPC) is rapidly becoming the most widely accepted parameter for determination of used frying oil quality. Several countries have established regulations for used frying oil quality in restaurants based on the %TPC. Maximum values ranging from 24 to 27% by weight TPC designate when oil is unfit for use [1]. The standard column chromatography methods for determination of total polar compounds, AOCS Official Method Cd 20-91 [2] and IUPAC Method 2.507 [3], require nearly 400 mL of organic solvents (if the polar fraction is also eluted) and take several hours to perform. Sebedio [4] and Dobarganes [5] demonstrated that column chromatography methods for determination of %TPC can be accurately performed on a much smaller scale, but the procedure is still time consuming. A faster method that also consumes significantly less solvent is desirable.

The development of the method of this paper benefitted from the previous work of a number of researchers including Perkins [6], White [7], Dobarganes [5, 8], and Abidi [9] who studied HPLC and gel permeation chromatography (GPC) for determination of total polar compounds and other lipids analyses. Size-exclusion chromatography (SEC), also known as gel permeation chromatography, is a chromatographic method in which molecules in solution are separated by their size which may not always correlate well with molecular weight. SEC has been used primarily for the analysis of large molecules such as proteins or polymers. SEC functions by trapping the smaller molecules

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in the pores of the particles. Larger molecules either do not penetrate the pores as deeply or not at all, and as a result, flow through the column quicker than smaller molecules. The smaller are retained longer, and the analytes elute in the order of largest first to smallest. (Although the stationary phases are slightly different, for simplicity SEC and GPC will be considered synonymous for the purposes of this paper.) Typical choices were polystyrene-divinyl benzene column (PSDVB) packings of 5–10 μm with 50–100 Å pore sizes, such as those recommended in the AOCS Official Method Cd 22-91, "Determination of polymerized triglycerides by gel-permeation HPLC." However, even a series of 4 or 5 small pore diameter columns resulted in poor separation of some oil components. Separation of the triacylglycerols (TAG) and diacylglycerols (DAG) was especially difficult because they are sterically similar in size. Maximum pressure restrictions limit the number of GPC columns in series to four columns or less.

Many earlier researchers used the calibration curves of styrene and similar synthetic polymers to determine the optimum columns for the separation of used frying oils. Evaluation of several styrene standards and used oil components (Table 1) show that the standard synthetic polymers are much more spatially compact than edible oil components with similar molecular weights. The fatty acids of TAG and DAG typically form an elongated, branched "pitch fork" configuration. Figure 1 shows that when the molecular weights of the polystyrene and lipid standards are graphed versus the retention times, the curves are nearly parallel, but distinctly separate. Comparing the retention times obtained from experiments with larger pore size columns demonstrated that a PSDVB packing of 5 μm particle size with 500 Å pores was optimal for this direct injection separation. When three 500 Å columns are used in series, baseline separation is achieved between the polymers and the TAG and with a resolution of greater than 1.0 between the TAG and DAG. These two separations are especially important because the DAG and polymers are typically the two largest polar components of used frying oils.

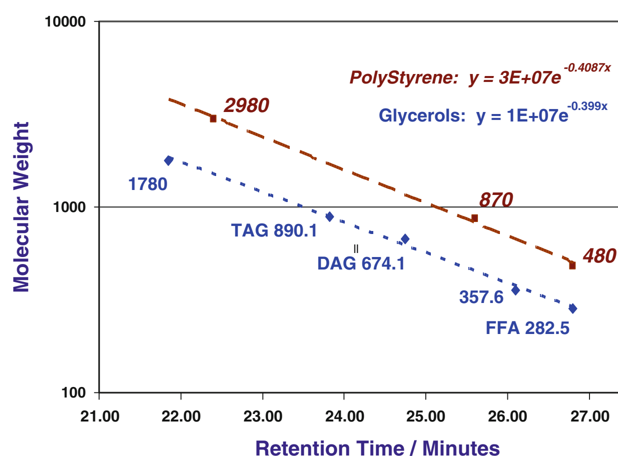

Fig. 1 Correlation of molecular weight and retention times for polystyrene and lipid standards

Materials and Methods

Silica Gel Column Chromatography

AOCS Official Method Cd 20-91 was followed using silica gel 60 (EMD # 7734-3, 70–230 mesh, ASTM) adjusted to 5 ± 0.2% H_2O. The nonpolar fraction was eluted with 150 mL of 87/13, V/V petroleum ether/diethyl ether, and the polar fraction with 150 mL of diethyl ether.

Lipid Standards

Purified dioleoylglycerol, mono-oleoylglycerol, and oleic acid were obtained from Sigma Chemical Company, St. Louis, Missouri. The triacylglycerol standard was a "zero *trans* fatty acid" blend of canola, corn, and partially hydrogenated soybean oil which was purified by elution through a silica gel chromatography column to remove any antioxidants and residual polar materials. Polymer standards for the calibration were prepared by heating a sample of the same fresh oil with mixing at 190 °C for about 16 h and purifying by elution through another silica gel column. The resulting polymerized sample was 73% mixed polymers and 17% TAG and DAG. Polystyrene standards were obtained from Phenomenex, Inc., Torrance, CA.

Table 1 Retention time of edible oil components and polystyrene standards

Oil component	Approximate MW	Retention time (min)	Polystyrene MW	Retention time, (min)
FFA	283	27.0	450–500	26.8
MAG	360	25.9	870	25.6
TAG Dimer	1880	21.9	2980	22.4

See Fig. 8.

HPLC–SEC System and Conditions

1. HPLC with degasser, auto injector, and column heater, Agilent Series 1100, Waldbronn, Germany. The column heater was set at 31 °C.
2. Phenogel 300 × 7.5 mm, PSDVB, 5 µm, 500-Å columns (three columns in series) obtained from Phenomenex, Inc., Torrance, CA
3. Evaporative light scattering detector (ELSD), Chromachem, ESA, Inc., Chelmsford, MA, with the nebulizer set at 30 °C and evaporator at 42 °C
4. Detector purge gas: Nitrogen at 26 psi
5. Mobile phase: unstabilized, chromatography grade tetrahydrofuran (Honeywell, Burdick and Jackson, B&J Brand, Muskegon, MI) at 1.0 mL/min
6. Typical injection size: 20–25 µg
7. Data acquisition and integration of the chromatograms was performed with ChromPerfect V 5.5 (Justice Innovations).

Note: Although an ELSD was used in this study, refractive index (RI) detectors have been used for this analysis. Because the sensitivity and stability of RI detectors has improved in recent years, some laboratories may wish to experiment with a late model RI detector. Early research comparing the two detectors was performed by W.H. Christie [10], and somewhat more recently by A.I. Hopia [11] and others.

Four sets of used, edible oil samples varying from nearly fresh to discard quality were obtained from two fast food restaurants. One restaurant fried French fries and chicken nuggets in canola oil, and the other fried the same two food products in palm oil. About 20–25 mg of oil sample were diluted with 5.0 mL THF and filtered through a 0.45 µ syringe filter before injection of 5.0 µL into the HPLC–SEC.

The TPM are calculated on the same basis as Cd 20-91, i.e., all of the components that are not included in the TAG peak (which may contain OX-TAG) are included in the TPM. By definition this includes all DAG, even though these compounds may be an acceptable component of some types of refined, edible oils. The major polar components include: polymerized TAG, DAG, polymerized DAG and FA, oxidized DAG and FA, FFA, and numerous smaller molecular weight hydrolytic and oxidation compounds that co-elute with the FFA. The effect of oxidized TAG is extensively discussed below.

Results and Discussion

Figures 2 and 3 show two calibration curves determined with the Evaporative Light Scattering Detector (ELSD).

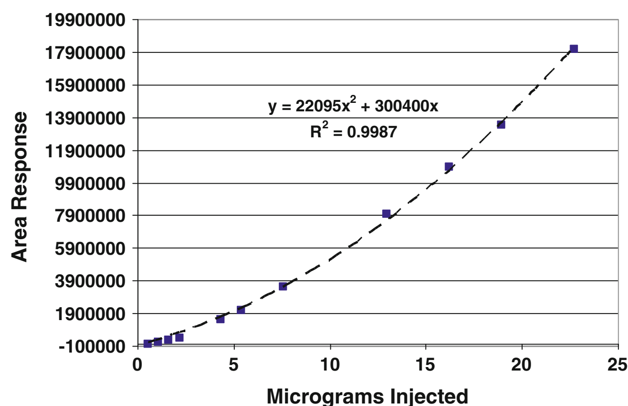

Fig. 2 Triacylglycerol calibration curve

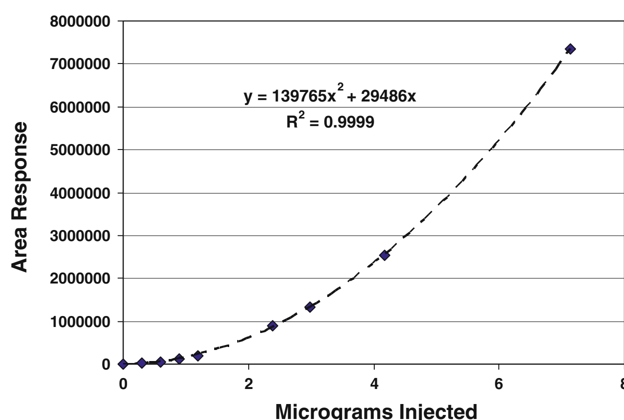

Fig. 3 FFA (oleic acid) calibration curve for HP–SEC

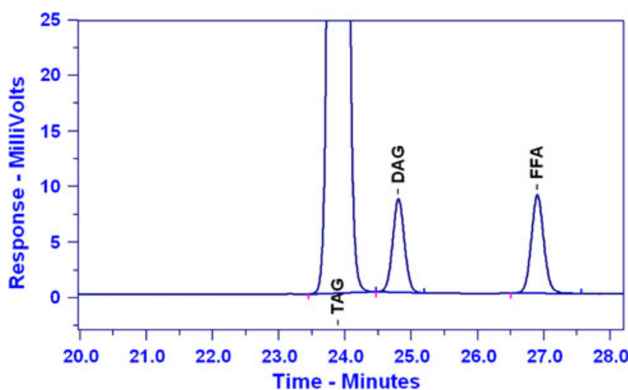

Fig. 4 Oleate glyceride standards ~15 µg total mass

Because the response factors are different for each edible oil component, separate calibration curves were determined. As is true of nearly all evaporative light scattering detectors, the response curves for this detector were nonlinear for all components [12]. Figure 2 shows the TAG calibration curve is nonlinear with a quadratic curve fit

over the given range up to about 24 µg. The detection limit for all components was about 0.25 µg and the upper limit was about 25 µg, or about 100 times the lower detection limit.

Figure 4 is the HPLC–SEC chromatogram from the injection of the TAG, DAG, and free fatty acid (FFA) standards roughly in proportion to their quantities in a discard quality used frying oil. The separations and symmetry support both reproducibility and accuracy. The FFA and other low molecular weight molecules elute in less than 28 min, so two replicate analyses can be run in less than an hour. Figure 5 is the HPLC–SEC chromatogram from the injection of a typical discard quality, used frying oil composed of about 25% TPC. The resolution (R) between the TAG trimer and TAG dimer was 1.6, the resolution between the TAG dimer and TAG peak was 2.7, and the resolution between the TAG and DAG was >1.0. The number of theoretical plates, *n*, for the TAG peak was 15,800. Figure 5 also shows the three column system even separated a small, unknown peak just before the main TAG peak. This could be either a DAG dimer or the addition reaction of a TAG and an FFA, but as of this writing it remains unidentified. Analyses of unused "virgin" or "fresh" oils by the HPLC–SEC method typically show they are more than 97.5% TAG, with 2–2.5% of low polarity, components that elute at the DAG retention time.

Table 2 shows the %TPC values for four sets of used frying oil as determined by both the standard column chromatography and new HPSEC methods. For HPSEC, TPC was determined by normalization of the sum of all detected components of the oil. The %TPC values of the oils from these four sets ranged from about 4% to a badly degraded 35%. The average difference between the two methods was 0.7%. Figure 8 shows a linear correlation between the HPSEC and Cd 20-91 values with a slope of 1.014 and R^2 of 0.995. Clearly, the HPSEC analysis replicates the column chromatography results for the various restaurant oils in this test.

The reproducibility for ten injections of the same oil sample, which was 24.6% TPC, was ±0.30, and the $RSD_r\% = 1.22$ (See Table 3). These values are consistent with the statistical precision of the column chromatography method, which is ±0.29 with an RSD_r of about 1.3% for an oil sample containing 22.28% TPC (AOCS Cd 20-91, page 4).

As previously stated, this HPLC–SEC procedure is similar to AOCS Official Method Cd 22-91 for the determination of polymerized triacylglycerols. The polymerized TAG concentrations of the oils in this study as determined by the HPLC–SEC method are included in Table 2. The polymer concentrations in all four sets increased rapidly, but then reached a plateau. The polymers in the palm oil used to fry French fries leveled out at about 13.5%, but the polymers in the other three oil sets all leveled off at about 8%. This polymerization pattern of a rapid increase and then stabilization has been observed by the authors in many controlled frying studies in which this method was employed. It was somewhat surprising that the type of oil (canola vs. palm) did not affect the results for this test. With the possible exception of the degree of polymerization, there was no clear indication that the food item (French fries or chicken nuggets) affected the results.

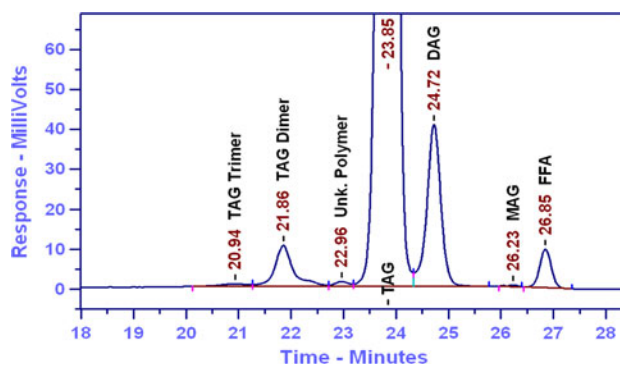

Fig. 5 Direct injection of used frying oil ≈25% TPC

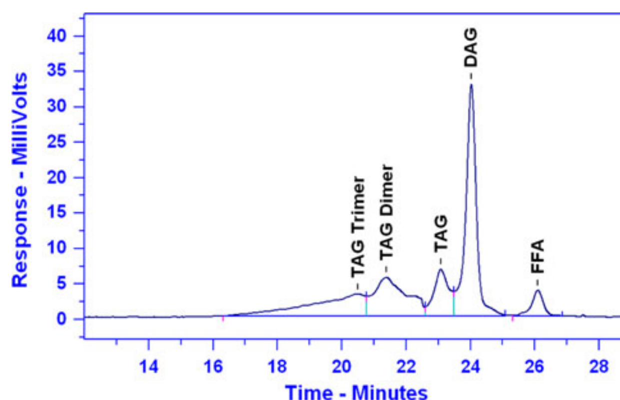

Fig. 6 Polar fraction from column chromatography

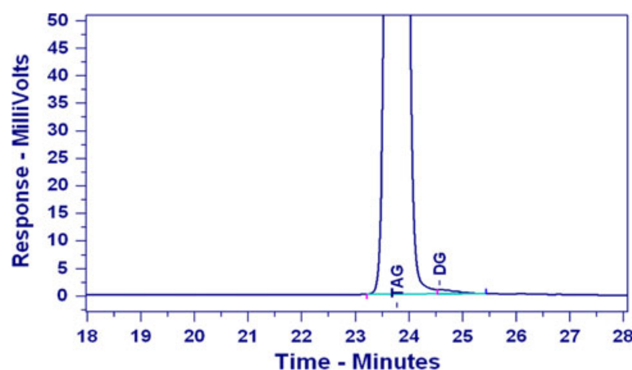

Fig. 7 Non-polar fraction from column chromatography

Table 2 Composition of used frying oils

Oil/food item	%TPC by column chromatography	%TPC by HPLC–SEC	Difference	%Polymers
Canola/FF	4.3	4.5	0.2	0.8
Canola/FF	12.8	14.0	1.2	5.1
Canola/FF	21.9	23.3	1.4	8.2
Canola/FF	29.9	31.3	1.4	8.0
Canola/FF	35.0	34.9	−0.1	7.9
Canola/CN	4.3	5.7	1.4	2.9
Canola/CN	13.9	14.1	0.2	7.9
Canola/CN	17.6	17.5	−0.1	7.8
Canola/CN	20.9	20.8	−0.1	7.2
Canola/CN	26.5	26.7	0.2	8.2
Canola/CN	29.7	30.4	0.7	8.4
Palm/FF	6.2	5.0	−1.2	0.2
Palm/FF	18.8	18.0	−0.8	8.5
Palm/FF	25.9	27.0	1.1	13.6
Palm/FF	28.7	29.3	0.6	13.3
Palm/FF	32.0	32.6	0.6	13.7
Palm/CN	6.3	6.4	0.1	1.4
Palm/CN	20.4	21.8	1.4	6.1
Palm/CN	23.7	24.6	0.9	6.8
Palm/CN	30.0	30.5	0.5	7.9
Palm/CN	35.0	35.4	0.4	8.2
Average			**0.7**	NA

Difference = %TPC by HPLC–SEC minus %TPC by column chromatography

%Polymers is composed of >98% polymerized TAG

FF French Fries, *CN* Chicken Nugget

The HPLC–SEC method was further applied to study the components of the polar fraction (ethyl ether elution) of an oil obtained after separation by silica gel, column chromatography. Figure 6 shows the polar fraction of this used oil contained high concentrations of polymers and DAG, indicative of both oxidation and hydrolysis of the oil. The polar fraction also contained a small TAG component indicating (OX-TAG) was present in the sample. The actual concentration of OX-TAG was 14.0% of the polar fraction. This oil was 1 day old and the TPM was 5.7%, thus the OX-TAG constituted ~0.8% of the total TAG. Because HPSEC does not separate non-polar TAG from OX-TAG, the polar OX-TAG will co-elute under the TAG peak. If there is a significant quantity of OX-TAG [13], this would quantitatively affect the results of the TPC value reported. A researcher could run an oil sample through AOCS Method Cd 20-91 to obtain the polar

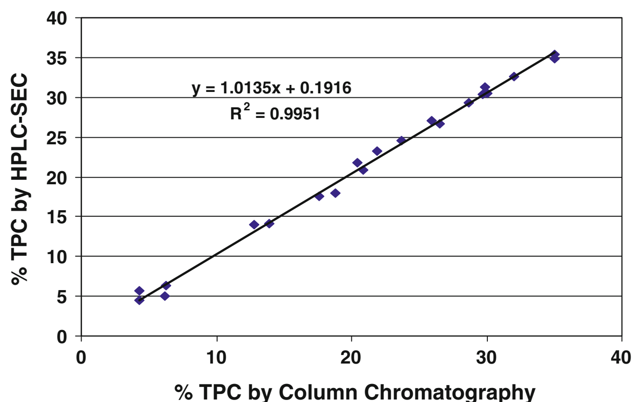

Fig. 8 Correlation of %TPC by HPLC–SEC versus column chromatography (AOCS Cd 20-91)

Table 3 Reproducibility of single FF oil sample injected 10 times

	%Polymer	%TAG	%TPC
	8.95	75.86	24.14
	9.11	75.69	24.31
	9.07	75.94	24.06
	8.71	75.24	24.76
	8.74	75.29	24.71
	8.69	75.46	24.54
	8.82	75.07	24.93
	8.77	75.37	24.63
	8.90	75.03	24.97
	8.83	75.29	24.71
Average	8.86	75.42	24.58
Std Dev	0.14	0.30	0.30
RSD_r%	1.60		1.22
2.8× s_r	0.39		0.84

fraction to quantify oxidized TAG [see Dobarganes, et. al., references 5, 14], but this would negate the time, labor and solvent savings of this direct injection method. This potential limitation of the method has been studied by Dobarganes et al., who reported oxidized FA as high as 12% in used frying oils [15], but merits further study. Because oxidized-DAG and oxidized-FFA co-elute with the pure DAG and FFA, unlike OX-TAG, they would be included in the TPM by this method.

Figure 7 is an example of HPSEC analysis of the non-polar fraction which typically contains a small quantity of material that elutes at the DAG retention time. These may consist of low polarity components that are not adsorbed by the silica gel during the column chromatography separation of the polar compounds and non-polar materials.

HPLC–SEC using three, 500-Å GPC columns effects nearly baseline separation for all the major components of used frying oil in less than 30 min. The oil sample is simply diluted, filtered, and injected onto the columns. While an ELSD is recommended, improvements in other types of detectors may allow substitution and addition of a fraction collector. Other detectors may allow recycling of the mobile phase for greater solvent reduction.

One of the eight recommendations of the 3rd International Symposium on Deep Fat Frying (March 2000, Hagen Halden, Germany) was "Analysis of suspect fats and oils should utilize two tests to confirm abuse. Recommended analyses should include total polar compounds (maximum of 24%) and polymeric compounds (maximum 12%) [13]." The HPLC–SEC method of this paper determines both oil quality parameters in a single analysis. Although not suitable for use in restaurants, for laboratories that need to analyze hundreds or even several thousand oils for TPC each year and can afford the HPLC system and maintenance expenses, this method correlates well with column chromatography results and is a faster, less laborious and less solvent intensive alternative. The method suggests further studies with other types of oils, frying conditions, and fried food items. The types of oils could include high oleic, genetically modified oils or recently developed "zero-trans" fatty acid blends formulated specifically for frying.

The OX-TAG question remains unresolved for the purpose of applying this method for research quality analyses. The question merits further study, and the analysis, quantification, and correlation of OX-TAG to other oil degradation parameters could constitute the basis of a separate research study in itself.

References

1. Firestone D (2007) Regulation of frying fat and oil. In: Erickson MD (ed) Deep frying: chemistry, nutrition, and practical applications, 2nd edn. AOCS Press, Champaign, pp 374–385
2. American Oil Chemists' Society (2001) Official methods and recommended practices of the American oil chemists' society, 5th edn. AOCS Press, Champaign
3. Paquot C, Hautfenne A (eds) (1987) IUPAC standard methods for the analysis of oils, fats and derivatives. Blackwell Scientific Publications, Oxford
4. Sebedio JL, Septier C, Grandgirard A (1986) Fractionation of commercial frying oil samples using sep-pak cartridges. J Am Oil Chem Soc 63:1541–1543
5. Dobarganes MC, Velasco J, Dieffenbacher A (2000) Determination of polar compounds, polymerized and oxidized triacylglycerols, and diacylglycerols in oils and fats. Pure Appl Chem 72:1563–1575
6. Perkins EG, Christopoulou CN (1986) High performance size exclusion chromatography of fatty acids, mono-, di-, and triglyceride mixtures. J Am Oil Chem Soc 63:679–684
7. White P, Wang W (1986) A high performance size-exclusion chromatographic method for evaluating heated oils. J Am Oil Chem Soc 63:914–920
8. Márquez-Ruiz G, Dobarganes MC (1996) Rapid, quantitative determination of polar compounds in fats and oils by solid-phase extraction and size-exclusion chromatography using Monostearin as internal standard. J Chrom A 749:55–60
9. Abidi SL, Kim IH, Rennick KA (1999) Determination of nonvolatile components of heated soybean oils separated with high-efficiency mixed-bed PSDVB columns. J Am Oil Chem Soc 76:939–944
10. Christie WH (1985) Rapid separation and quantification of lipid classes by high performance liquid chromatography and mass (light scattering) detection. J Lipid Res 26:507–512
11. Hopia AI, Ollilainen VM (1993) Comparison of the evaporative light scattering detector (ELSD) and refractive index detector (RID) in lipid analysis. J Liquid Chrom 16:2469–2482
12. Christie WW (1992) Detectors for high-performance liquid chromatography of lipids with special reference to ELSD, advances in lipid methodology-one. The Oily Press, Ayr, Scotland, pp. 239–271
13. Deutsche Gesellschaft für Fettswissenschaft (accessed November 2009) http://www.dgfett.de/material/recomm.htm
14. Dobarganes MC, Maruquez-Ruiz G, Berdeaux O, Velasco J (1999) Boskou D, Elmadfa I, (eds), Frying of foods. Technomic Publishing: Lancaster, PA, p. 143–161
15. Maruquez-Ruiz G, Dobarganes MC, Tasioula-Margari M (1995) Quantitation and distribution of altered fatty acids in frying oils. JAOCS 72:1171–1175