Fractionation of Commercial Frying Oil Samples using Sep-Pak Cartridges

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Commercial frying oil samples were fractionated by column chromatography on hydrated silicic acid according to the standardized DGF-IUPAC-AOAC method. The non-polar fraction was isolated using a mixture of petroleum ether:diethyl ether (87:13), while the polar fraction was eluted by diethyl ether. These used frying oil samples were also fractionated using Sep-Pak cartridges. The non-polar fraction was eluted with 20 ml of a mixture of petroleum ether:diethyl ether (92:8), while the polar fraction was eluted with methanol.

The purity of each fraction was studied by thin layer chromatography (TLC) and by the Iatroscan TLC/FID system using a mixture of hexane:tetrahydrofuran: acetic acid (97:3:1) as solvent system.

The Sep-Pak and the standardized methods gave similar results. This indicates that the state of degradation of a frying oil (detection of polar components) could be studied using Sep-Pak cartridges, which is less time- and solvent-consuming than column chromatography.

Following the work of Guhr and Waibel (1), the state of degradation of a frying oil is usually estimated by chromatography on a 5% hydrated silicic acid column (2,3). This standardized method allows fractionation of an oil into two fractions, a nonpolar fraction containing the unchanged triglycerides and a polar fraction including the polymerized and oxidized triglycerides. This method gives a good indication of the state of degradation of an oil even if the method does not separate all the altered components from the unchanged molecules. This is especially the case for the cyclic fatty acid monomers, which migrate in both the polar and non-polar fractions (4). However, this method is time- and solvent-consuming.

Sep-Pak cartridges already have been used to fractionate lipids and other molecules of all types (5-8) as an alternative to column chromatography. There is no need for cartridge preparation, and 80-90 mg of oil injected on a Sep-Pak (5) can be fractionated into a polar and non-polar fraction using 20 ml of a mixture of petroleum ether:diethyl ether (92:8) and 30 ml of MeOH.

MATERIALS AND METHODS

Separation of polar and non-polar fractions by column chromatography. The fractionation of used frying oils collected from restaurants was carried out by the standardized IUPAC-AOAC method (3). However, it was necessary to increase the volume of the mixture of petroleum ether:diethyl ether up to 300 ml in order to elute the non-polar fraction completely. The purity of each fraction was checked by TLC. The TLC plates (Merck 5721, 0.25 mm thickness) were developed with a mixture of hexane:diethyl ether:acetic acid (80:20:1), and the spots were detected by spraying with a solution of 2′7′ dichlorofluorescein in ethanol.

Separation of polar and non-polar fractions using Sep-Pak cartridges. An appropriate amount of sample (80-90 mg) was injected on a Sep-Pak cartridge (Waters, U.S. patent no. 4,211,658). The non-polar fraction was eluted with 20 or 30 ml of a mixture of petroleum ether:diethyl ether, while the polar fraction was eluted with 30 ml of methanol. Each fraction was weighted after evaporation of the solvent in order to determine the total recovery. The amount of the polar fraction (P) was calculated from the amount of non-polar fraction (NP) determined by weight using the equation $P = m - NP$ where m represents the amount of material injected on the column.

Analyses of polar and non-polar fractions by the Iatroscan TLC/FID system. Clean rods were spotted with about 10 μg of sample using a 1 μl micro-pipette as previously described (9,10). The rods were developed with a mixture of hexane:tetrahydrofuran:acetic acid (97:3:1) for 40 min, then dried. The rods were scanned (0.24 cm/sec) by the FID in an Iatroscan TH-10 apparatus (160 ml/min of hydrogen and 2000 ml/min of air).

RESULTS AND DISCUSSION

Two solvent systems were selected for the fractionation

TABLE 1

Influence of the Ratio of Petroleum Ether and Diethyl Ether on the Amount of Non-Polar Fraction Obtained after fractionation on Sep-Pak Cartridges

Sample	PE/Et_2O[a]	Non-polar fraction (Sep-Pak)(%)	Total recovery (%)	Non-polar fraction (column)[b]
A	90:10	90.5	94.0	91.8
	92:8	91.2	96.8	
	92:8[c]	91.5	96.8	
	95:5	67.4	94.5	
	98:2	70.5	98.4	
B	87:13	79.8	93.2	73.6
	90:10	79.7	91.7	
	92:8	76.8[d]	96.2	
	92:8[c]	76.4	96.2	
	95:5	59.7	96.0	
	98:2	56.7	91.1	
C	90:10	62.1	81.8	55.3
	92:8	63.0	85.8	
	92:8[c]	60.6	89.3	
	95:5	41.6	83.0	
	98:2	41.7	82.7	

[a] PE/Et_2O, petroleum ether:diethyl ether.
[b] Determined using a mixture of PE/Et_2O, 87:13.
[c] Determined using 20 ml of PE/Et_2O.
[d] Average of 6 analyses: 76.80 ± 0.15.

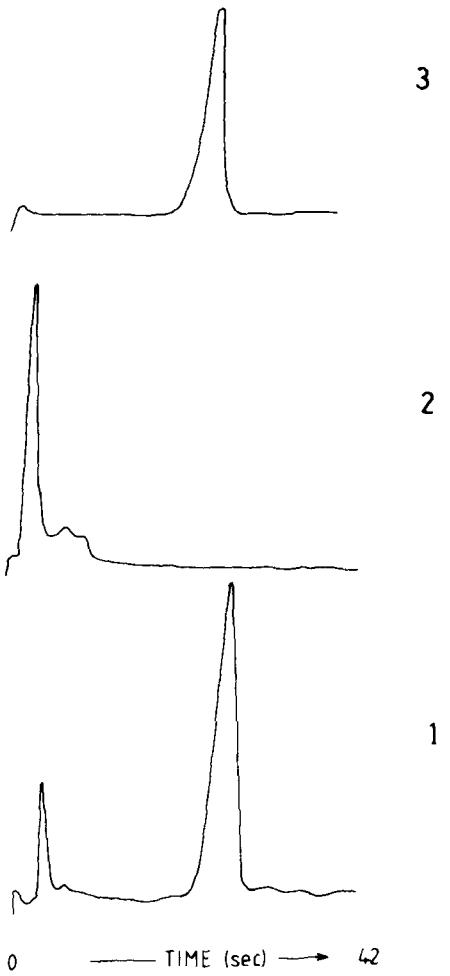

FIG. 1. Iatroscan analyses of (1) sample B and (2) the polar and (3) non-polar fractions obtained after fractionation on Sep-Pak.Solvent system:hexane:tetrahydrofuran:acetic acid (97:3:1).

TABLE 2

Determination of the Quantity of Polar Material in Commercial Frying Oils Using the Standardized Method and Sep-Pak Cartridges

	Column Chromatography[a]		Sep Pak[b]	
Sample	P[c] (%)	Total Recovery (%)	P[c] (%)	Total Recovery (%)
4 A	26.4	97.0	23.2	97.2
4 B	8.2	97.8	8.8	96.8
S	44.7	93.9	39.4	89.0
1 A	34.1	95.5	31.2	92.5
9 B	15.0	93.2	13.5	94.9
13 B	25.9	97.0	24.4	95.3
14 B	16.2	96.7	15.9	97.9
2 B	11.3	97.8	11.7	97.1
8 B	20.5	96.6	20.1	95.9
015211	10.5	97.8	8.6	99.9

[a]Solvent system, PE/Et_2O (87:13),.Et_2O.
[b]Solvent system, 20 ml PE/Et_2O (92:8), 30 ml MeOH.
[c]Polar fraction, P = m − NP where m is the amount of material injected on the column and NP the amount of non-polar fraction.

on Sep-Pak cartridges. The non-polar fraction was eluted with a mixture of petroleum ether:diethyl ether (PE/Et_2O), such as for the standardized method, while the polar fraction was eluted with methanol. Three commercial frying oils (Table 1) with different quantities of polar material (A,B and C) were selected to study the influence of the ratio of PE/Et_2O on the amount and purity of the non-polar fraction. One oil was rich in non-polar components (91.8%), one contained a fair amount of polar material (73.6% of non-polar fraction) and one was highly deteriorated (55.3% of non-polar fraction). The relative quantities of PE/Et_2O studied ranged from 98:2 to 87:13 (solvent system used in the standardized method). The fractionation was carried out on 80-90 mg of oil using 30 ml of the mixture of PE/Et_2O and 30 ml of MeOH to elute the polar fraction. The total recovery ranged from 82% for a highly deteriorated oil (Table 1) to 98% for a slightly deteriorated sample. The amount of sample left on the Sep-Pak seems to be greater if the oil is highly deteriorated. This was also observed for column chromatography (4,11,12).

A solvent system of PE/Et_2O from 98:2 to 95:5 was found not to be polar enough to elute all the non-polar fraction if compared with the results found with the standardized method. A solvent system of PE/Et_2O ranging from 92:8 to 87:13 gave results in the same range as those found by column chromatography.

The reproducibility of the method was studied using 80-90 mg of sample B, 30 ml of a mixture of PE/Et_2O (92:8), which seems to be a suitable solvent system to elute the non-polar fraction, and 30 ml of MeOH to elute the polar one. The analysis was carried out six times. The reproducibility was excellent, with an average of 76.80% ± 0.15 (Table 1). A good reproducibility also was obtained when using less sample (50 mg). However, 90 mg can be considered the upper limit because the utilization of more sample (∿110 mg) gave a non-polar fraction which was contaminated by traces of polar material.

It is also important to note that the elution of the non-polar fraction with 20 ml of PE/Et_2O (92:8) instead of 30 ml gave similar results for samples A and B and a slightly smaller amount of non-polar fraction for sample C. The purity of each fraction obtained from the three samples was studied by TLC and Iatroscan. No cross contamination was detected by TLC except for the non polar fraction of sample C collected with 30 ml of PE/Et_2O. Thirty ml is therefore too large a quantity of PE/Et_2O for the highly deteriorated samples. A good separation of the non-polar and polar fractions was obtained using a mixture of hexane:THF:acetic acid for the Iatroscan analyses (Fig. 1). A good separation is also obtained using a mixture of hexane:diethyl ether: acetic acid (97:3:1). No cross contamination could be detected under these analytical conditions for the fractions collected with 20 ml of PE/Et_2O. However, great care must be taken for the interpretation of the Iatroscan analyses. The analysis of the total sample B (26.4% polar fraction) seems to indicate a small response factor of the polar material compared to those of the non-polar fraction (Fig. 1). It is, therefore, obvious that minor contamination of polar material in the non-polar fraction would not be detected. However, it would be

possible to detect minor contamination of non-polar material in the polar fraction. The FID detection limit of polar material will be determined by studying the response factors as a function of the quantity of sample spotted on the rods.

Ten used frying oil samples were studied using the standardized column chromatographic method and Sep-Pak cartridges. The non-polar fraction was eluted from the Sep-Pak cartridges with 20 ml of a mixture of PE/Et_2O (92:8). These analytical conditions were selected considering the results reported in Table 1. The total recovery ranged from 93.2 to 97.8% for the column chromatographic method and 89.3 to 99.9% for the Sep-Pak cartridges (Table 2). The plot of the amount of the polar fraction obtained with Sep-Pak cartridges (y) versus the amount obtained by column chromatography (x) gave a linear regression, $y = 0.86\ x + 1.35$ with a correlation coefficient of 0.996. This indicates that the results obtained using the Sep-Pak cartridges are slightly lower than those from column chromatography.

There is a good correlation between the standardized DGF, IUPAC, AOAC method and fractionation using Sep-Pak cartridges. This method, which does not require either large quantities of solvent or time to prepare the column, could be a powerful tool for fast determination of the state of degradation of a commercial frying fat.

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Supplementary Consideration of the Triglyceride Matrix Model on Reverse Phase High Performance Liquid Chromatography

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The matrix model that has been expressed as linear relationship between the logarithm of the relative retention time of a molecular species of a triglyceride versus total acyl carbon number or total double bonds when only one acyl group differs in carbon number or number of double bonds was reviewed. A similar linear relationship was observed when the fatty acid residues were substituted in the triglyceride molecule. This relationship was demonstrated by introducing the theory of partition chromatography presented by A.J.P. Martin.

The empirically determined correlation graph (Fig. 1), the matrix model of triglyceride (TG) on high performance liquid chromatography (HPLC) presented previously (1,2), was reviewed because of the following reasons: Though (18:1, 18:1, 18:1)[1] has the same equivalent carbon number (ECN) as (16:0, 18:1, 18:1), the former elutes earlier than the latter, as can be observed in the chromatograms of Kuksis et al. (3) and of Pauls (4). Or, though (16:0, 18:1, 18:1) has the same ECN as (16:0, 16:0, 18:1), the former elutes earlier than the latter, as can be observed in the chromatograms of many others (5-12). This phenomenon may be attributed to the differences in chemical potential between 16:0 and 18:1 residues in the TG molecule. If the difference in chemical potential between these two fatty acid residues is expressed as $\Delta\mu_x$, $\Delta\mu_x$ is considered to be added every time the 16:0 residue substitutes for 18:1 in the TG molecule. Therefore, the linear relationship shown in Figure 2 should hold. $\Delta\mu_x$ is proportional to the logarithm of the relative retention time (RRT) because the ratio of the partition coefficients (α) of the two homologous series exactly denote RRT, and according to the theory of Martin (13), $\log \alpha = \Delta\mu_x/R \cdot T$ should hold where R is the gas constant; T is the absolute temperature ($1/R \cdot T$ can be considered constant in most chromatographic conditions). So, a linear relationship should also hold between the increase in log (RRT) and the number of substitutions of the 16:0 residue

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[1](18:1, 18:1, 18:1) means the same as $\begin{vmatrix}18:1\\18:1\\18:1\end{vmatrix}$, but the binding position of the acyl group is not discerned in this study.