Gel Permeation Chromatography of Heated Fats[1]

ABSTRACT

The high and low molecular weight components of heated fats have been separated with gel permeation chromatography. With Sephadex LH-20, as well as with Biobeads SX-1, the high molecular weight components of heated fats may be directly separated when chloroform, acetone, chloroform-methanol or tetrahydrofuran are employed as swelling agents and eluting solvents.

The determination of nonvolatile oxidation products in heated fats, in the form of intact triglycerides, presents a formidable problem. Little success has met efforts to separate oxidized or polymerized triglyceride from normal triglycerides. Various methods have been developed for the determination of higher molecular weight products in heated fats. These are urea nonadduct formation (1), solvent partition (2) and distillation (3). Urea fractionation will concentrate branched chain methyl esters and any others that deviate in structure from a molecule such as methyl oleate. However this method does not remove all linoleic acid present in the usual heated fat. Solvent partition methods, on the other hand, may be used to concentrate polar material, but may cause loss of nonpolar higher molecular weight products. Methods based on distillation residues are useful, although the heating required to effect distillation may cause increased values to be obtained for nondistillable material.

A column chromatographic method based on molecular exclusion chromatography has been found useful in separating higher molecular weight compounds from polymerized frying fats. Preliminary reports using Sephadex LH-20 in tetrahydrofuran and ethanol to separate the dimeric and polymeric materials from polymerized oleic acid, peanut oil and sunflower oil have been published (4-6). No systematic study of the effects of heating time on the formation of high molecular weight components has appeared. In the present communication we wish to report such results, as well as others, from our investigation of the separation of heated fat triglycerides with the use of gel permeation chromatography.

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MATERIALS AND METHODS

Oils employed for deep fat frying are normally heated at ca. 185 C. The corn oil used in the present experiment was heated at 200 C for an extended period of time for the express purpose of illustrating the relationship between heating time and temperature. Accordingly, samples of heated corn oil were obtained by allowing corn oil to heat in a small home type deep fat fryer (3.5 quart capacity) at 200 C ± 5 C for 314 hr and removing 5 ml aliquots each 12 hr. In order to emulate practical frying conditions, both corn oil and a commercial pourable hydrogenated vegetable oil were heated in the small fryer at 185 C. Fresh frozen french fries were fried at three times per hour in 100 g amounts. Samples of each oil were removed periodically and retained for analysis. The samples were stored at -20 C under an atmosphere of nitrogen until required. Methyl esters of each heated fat sample were prepared by transesterification with anhydrous methanol containing 3% H_2SO_4 and isolated in the usual manner (7). Thin layer chromatography was carried out according to Stahl (8) with 500 μ layers of Silica Gel G and developed with a solvent system consisting of hexane-diethyl ether 90:10.

Gel permeation chromatography was carried out with all-glass columns with Teflon and stainless steel adapter systems (Chromatronix, Inc.). Solvent flow was kept constant with a constant pressure reservoir. Samples (5-10 mg) were admitted to the gel surface via an all-Teflon sample injection valve (Chromatronix no. 803). A Waters model 401 differential refractometer was employed to monitor the flow of eluate from the columns.

Sephadex LH-20 was purchased from the Pharmacia Company and Biobeads SX-1 from the Bio-Rad Company. All gels were allowed to swell for 12 hr in the eluting solvents prior to slurry packing the columns. All solvents employed were either reagent grade or redistilled.

Samples of dimer and trimer fatty acids were obtained

TABLE I

Separation of Heated Corn Oil Triglycerides and Methyl Esters via Gel Filtration Chromatography[a]

	Sephadex LH-20		Sephadex LH-20			Biobeads SX-1				
	Sample state									
	Triglycerides, wt%		Methyl esters, wt%			Triglycerides, wt%				
	Component no. and mol wt range									
Hours heated/wt %	1 >1100	2 900-1100	1 >1100	2 600-850	3 ~300	1 >10,000	2 3500-4500	3 2300-3000	4 800-1000	5 300
0	1.7	98.2	—	—	100	—	—	0.7	95.5	3.6
24	2.3	97.6	—	—	—	—	—	—	—	—
48	13.7	86.2	3.1	2.3	94.4	—	—	2.5	94.0	3.3
96	14.4	85.5	9.9	9.2	80.8	—	—	6.6	89.5	3.8
144	23.4	76.5	7.7	7.7	84.4	2.2	3.4	13.8	77.1	3.3
192	33.1	66.8	13.0	15.9	71.0	6.9	9.0	16.3	60.5	7.1
240	47.1	52.8	13.9	18.1	67.9	15.9	10.0	15.50	50.5	7.9
354	59.0	40.9	—	—	—	—	—	—	—	—

[a]$CHCl_3$, swelling agent and solvent.

TABLE II

Comparison of K_{av} vs. Column Packing for Monomer, Dimer and Trimer

Column	Solvent	K_{av} Value x 100		
		Monomer	Dimer	Trimer
Sephadex LH-20	$CHCl_3$	22.93	7.45	6.94
	Acetone	23.3	9.7	9.7
	C/M[a]	30.25	18.67	12.68
Biobeads SX-2	THF	42.1	19.2	19.2
Biobeads SX-1	$CHCl_3$	79.6	36.17	33.89

[a]Chloroform-methanol 2:1.

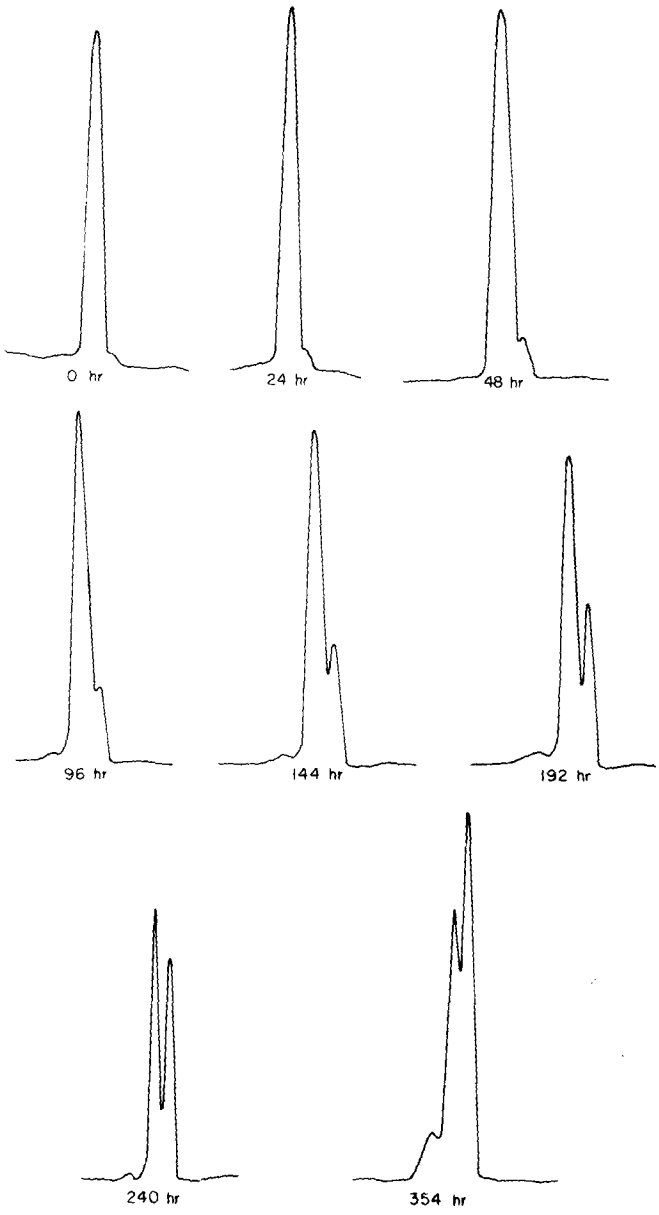

FIG. 1. Separation of heated triglycerides with LH-20 in chloroform. In all figures ordinate = detector response; abscissa = elution volume (mol wt).

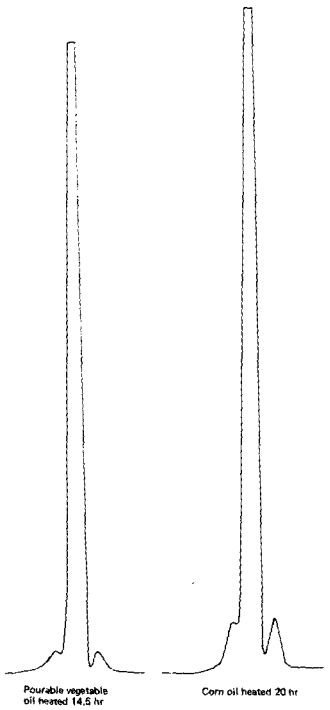

FIG. 2. Separation of used corn oil and pourable hydrogenated vegetable shortening with LH-20 in chloroform-methanol 2:1.

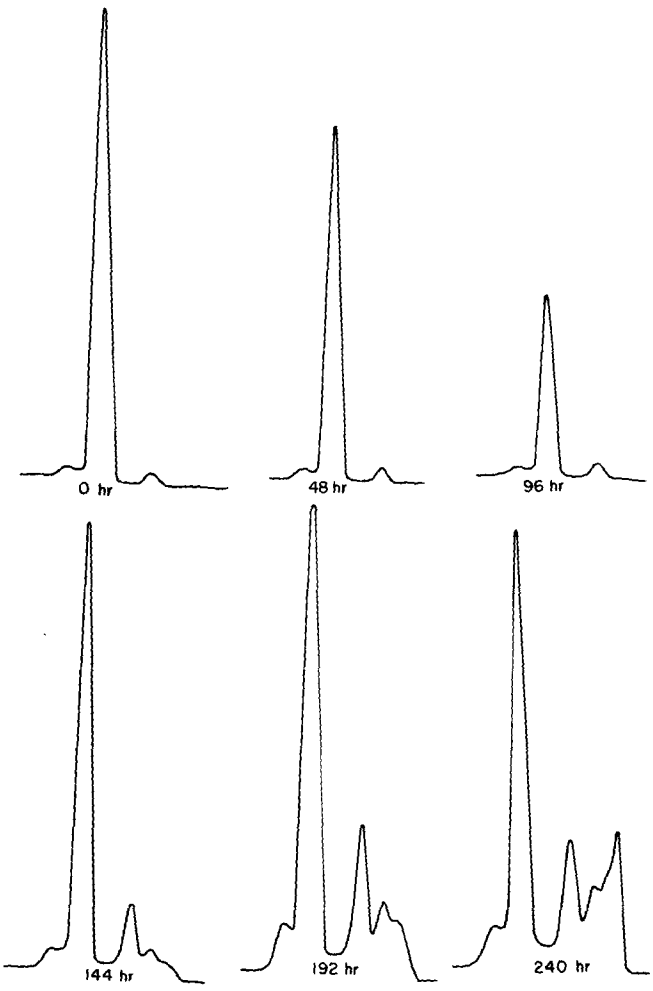

FIG. 3. Separation of heated triglycerides with Biobeads SX-1 in chloroform.

from Emery Industries and were converted to the corresponding methyl esters by reaction with anhydrous methanol containing 3% H_2SO_4 prior to use (7).

Molecular weight determinations of the different fractions separated by the column were based on K_{av} vs. logarithmic molecular weight plots of known standards. The standards consisted of a series of triglycerides and methyl esters of different fatty acids.

RESULTS AND DISCUSSION

Inoue et al. have reported a separation of thermal polymeric methyl esters with a Sephadex LH-20 column and DMF as solvent (9). While this may be a useful separation, the use of DMF as a solvent is not desirable since it has a rather high boiling point and is difficult to remove from collected fractions. Also, a direct separation of the triglycerides by molecular weight is more desirable. Such a separation has been previously demonstrated by Aitzetmüller (5) and in the present work is illustrated on a continuously heated corn oil sample, as shown in Figure 1. Since Sephadex LH-20 has an exclusion limit in chloroform for triglyceride of ca. 1100, one peak is obtained for all components with molecular weight greater than the triglyceride mixture made up of normal fatty acids. Therefore relative amounts of total high molecular weight may be obtained in a straightforward manner. A tabulated list of percentages obtained is given in Table I.

Examination of the fractions collected from this column by thin layer chromatography indicated that the high molecular weight fraction consisted of dimer and other higher polymeric and oxidized components of triglycerides; while the low molecular weight fraction consisted primarily of normal triglycerides, the leading edge of the peak consisted primarily of free fatty acids.

The application of exclusion chromatography to fats that have been used for deep fat frying is shown in Figure 2. The results were obtained with a gel of LH-20 swelled with a mixture of chloroform-methanol 2:1. The peaks for high molecular weight material represented 4.9 and 3.0%, respectively, for the used corn oil and pourable hydrogenated shortening. The percentage of fatty acids and other low molecular weight products formed were 6.6 and 4.6%, respectively.

The use of a gel with a higher exclusion limit, in the present case Biobeads SX-1, with a limit of ca. 6700, would allow further fractionation of heated fat components eluted at the exclusion limit of Sephadex LH-20. Accordingly, such a separation is illustrated by the data listed in Table I and shown graphically for heated corn oil in Figure 3. The use of the corresponding methyl esters of heated fats would allow separation of the esters into more discreet fractions. For example, the methyl esters of heated corn oil used in the separation illustrated in Figure 1, when separated on a Sephadex LH-20 column with chloroform as solvent, separated into two discreet fractions of higher molecular weight than the normal methyl ester of corn oil (Fig. 4, Table I). The separation observed represents concentrates of dimeric and trimeric fatty acids, as determined by comparison of their calculated K_{av} values (and elution volumes) with those from known K_{av} values for monomeric, dimeric and trimeric fatty acids as their methyl esters (Table II). A separation of all three of these components was achieved by the use of chloroform with Sephadex LH-20 and Biobeads SX-1 and with LH-20 with chloroform-methanol 2:1 as a solvent.

The preliminary results obtained above indicate that gel permeation chromatography with either Sephadex LH-20 swelled in chloroform or Biobeads SX-1 allow a separation and determination of the high molecular weight components of either the methyl esters or triglycerides of a heated or polymerized fat.

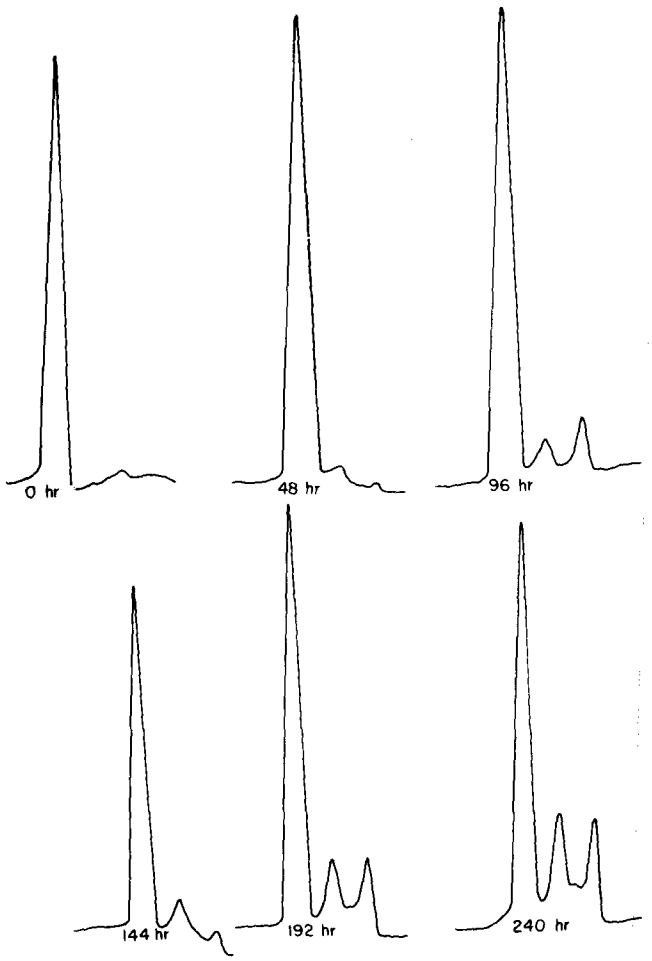

FIG. 4. Separation of heated fat methyl esters with Sephadex LH-20 in chloroform.

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