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HIGH-PERFORMANCE SIZE-EXCLUSION CHROMATOGRAPHY OF OILS AND FATS*

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SUMMARY

A simple high-performance size-exclusion chromatographic (HPSEC) method for the determination of the molecular weights of various oils and fats and their binary mixtures is described. The weight-average molecular weights, ($\bar{M}_w$), of refined oil samples thus obtained are in close agreement with those predicted from conventional saponification data. The results obtained for pure butter fats and binary mixtures of coconut oil and castor oil by HPSEC are discussed. The method may be suitable for the determination of adulteration in oils and fats.

INTRODUCTION

Oils and fats are essentially triglyceride mixtures of long chain fatty acids of carbon number ranging from C_4 to C_{24}. Generally, they differ in their fatty acid composition depending on their origin and nature. In addition to the above components, a number of foreign substances such as diglycerides, monoglycerides, free fatty acids and unsaponifiable matter are also present in oils and fats.

Several analytical methods[1,2] and specifications[3,4] based on physical, chemical and physicochemical aspects are available to characterize oils and fats. Among these, the saponification value (SV) occupies the most important position as it correlates the average molecular weight (SE) of fatty acids of glycerides. The relationship between SV and SE is :

$$SE = 56\,100/SV \quad (1)$$

The average molecular weight of oils and fats can be predicted by[5,6]

$$\text{Average molecular weight} = \frac{3 \cdot 56\,100}{SV} + 38 \quad (2)$$

but is not truly representative of all the components present in the sample as it does not

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include the contributions of low molecular weight and unsaponifiable fractions. Therefore, the accuracy of this prediction is considered to be very low, particularly for crude samples. Further it does not convey any information about the molecular weight distribution (MWD). The MWD is a key property which is needed to understand the compositional nature of oils and fats. It may also be helpful in studying adulteration in oils and fats.

Many techniques such as cryoscopy, ebullioscopy, osmometry, viscometry, light scattering, sedimentation and ultracentrifugation based on colligative and bulk properties are used for determining molecular weights[7]. However, all these techniques yield approximate data.

High-performance size-exclusion chromatography (HPSEC) is a valuable technique in providing information about sample complexity, accurate molecular weights and their distribution pattern. In a SEC column, the analytes are eluted in accordance with their molecular sizes, *i.e.*, hydrodynamic volume in a mobile phase solvent.

Though the technique was originally developed for characterizing macromolecules, it has also been successfully used for studying low-molecular-weight mixtures by introducing more efficient column packings and making innovations in instrumentation[8–10]. A thorough literature survey has revealed that only limited studies have been carried out with on the SEC retention behaviour of oils and fats. White and Wang[11] employed HPSEC for evaluating heated soya bean oil.

The present investigation was undertaken with a view to collecting information about the HPSEC retention behaviour of various oils and fats and to extend the utility of this technique to the prediction of saponification values of unknown samples and also the relative contributions of impurities and their detection. This technique may also find application in the detection of adulteration in oils and fats.

EXPERIMENTAL

Apparatus

HPSEC was performed on an LC-6A high-performance liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) with a loop injector (capacity 12 μl) having an high-pressure six-way valve. A refractive index detector Model RID-6A was connected after the column for eluate monitoring. The work was carried out on a high speed gel permeation chromatographic column (HSG-15H, Shimadzu) 300 mm × 7.9 mm I.D. As per the specifications from the supplier, the number of theoretical plates of the column ranges between 20 000 to 30 000 per meter. The column was packed with spherical beads (size 10 μm) of styrene–divinylbenzene copolymer. The chromatograms and the integrated data were recorded by a Chromatopac C-R3A processor (Shimadzu).

Reagents and samples

HPLC-grade tetrahydrofuran (THF) was obtained from Spectrochem Pvt. (India) and other chemicals such as potassium hydroxide, hydrochloric acid, etc., were of reagent grade. Ethanol was freshly distilled before use. Polyethylene glycols of different molecular weights (800, 580, 400 and 200) were obtained from S.D. Fine Chemicals (India).

Samples of vegetable oils and fats were obtained from local industries. Butter fats were extracted from different samples of processed buffalo milk collected from two different regions. Animal body fats such as mutton tallow, broiler chicken and beef fats were obtained by solvent extraction of the respective crude animal tissues.

Chromatographic conditions

The mobile phase used was the HPLC grade THF with a flow-rate of 0.3 ml/min. The samples were dissolved in the mobile phase used. A 12-μl loop with an high-pressure six-way valve was used for injecting the sample. Chromatograms were recorded on $16 \cdot 10^{-6}$ refractive index unit scale (RIUS) ranges and the sample concentration was about 15 mg/ml. Analyses were performed at ambient temperature. The samples were filtered using 0.45-μm pore size filters supplied by Waters Assoc. (Milford, MA, U.S.A.) before injection.

Reference materials

Polyethylene glycol standards of various molecular weights (800, 580, 400 and 200) were employed for calibrating the retention data of oils and fats. Stearic acid and the unsaponifiable fraction extracted from palm oil were used as reference materials to study the retention behaviour of free fatty acids and the unsaponifiable matter of oils and fats. To estimate the theoretical efficiency of the method, the $\overline{M}_w$ obtained (in case of refined samples) was compared with that predicted from the experimental saponification value. By use of the AOCS method[1], the SV data for refined samples were determined.

Calibration of the retention data

HPSEC retention times of standard polyethylene glycols on a single HSG-15H (Shimadzu) column were determined by injecting individual samples with THF as the eluent at a flow-rate of 0.3 ml/min. The retention data and a linear calibration graph of retention *vs.* log $\overline{M}_w$ are shown in Table I and Fig. 1 respectively. The data for the calibration graph were stored on a floppy disk FDD-1A (Shimadzu) coupled with the

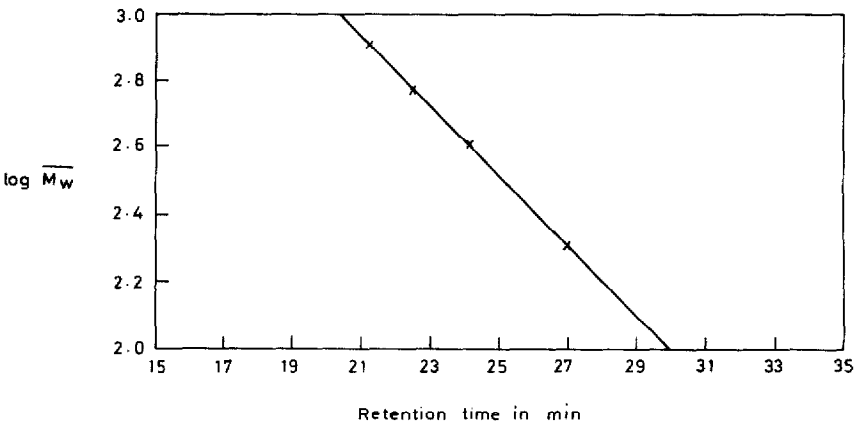

Fig. 1. Calibration graph using polyethylene glycols as standards.

TABLE I

HPSEC RETENTION DATA FOR STANDARD POLYETHYLENE GLYCOLS (PEG)

Sample No.	*Average molecular weight of PEG, $\bar{M}_w$*	*Retention time (min)*
1	800	21.3
2	580	22.5
3	400	24.2
4	200	27.0

Chromatopac C-R3A processor for molecular weight determination of the analytes. The reproducibility of this method was tested by injecting triplicate samples of standards and analytes. The coefficient of variation for molecular weights ranged from 1 to 2% and the $\bar{M}_w$ obtained for refined samples is in close agreement with that predicted from the average experimental saponification value.

RESULTS AND DISCUSSION

Determination of molecular weights

$\bar{M}_w$, the number-average molecular weight, $\bar{M}_n$, the viscosity average molecular weight, $\bar{M}_v$, and the Z-average molecular weight, $\bar{M}_z$, of some oils and fats are given in Table II and HPSEC chromatograms of refined and crude oil samples are shown in Fig. 2A and B respectively. The $\bar{M}_w$ and M_v values for all samples are found to be almost identical. The difference between $\bar{M}_w$ and M_n is much less for refined samples. In case of the crude samples it is about 100 units. This large difference between M_w and $\bar{M}_n$ values in crude samples is indicative of a significant contribution of low-molecular-weight fractions like free fatty acids and unsaponifiable matter. A standard

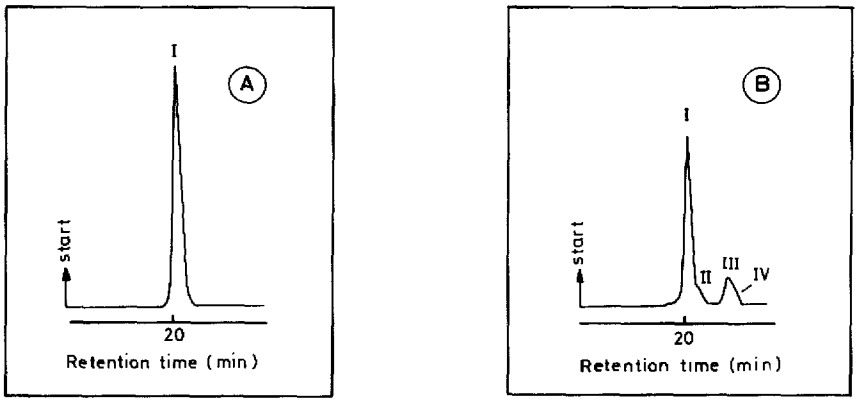

Fig. 2. (A) HPSEC chromatogram of refined safflower oil showing the molecular weight distribution of glycerides (I). (B) HPSEC chromatogram of crude neem oil showing the molecular weight distribution of triglycerides (I), mono- and diglycerides (II), free fatty acids (III) and the unsaponifiable fraction (IV).

TABLE II

MOLECULAR WEIGHTS OF VARIOUS OILS AND FATS DETERMINED BY HPSEC

Calculated from the total constituents present in the analyte.

Sample No.	*Name*	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_z$	$\bar{M}_v$	$\bar{M}_w/\bar{M}_n$
1	Coconut oil	712	701	723	720	1.0157
2	Butter fats					
	a $(BF)_W$	814	799	829	812	1.0188
	b $(BF)_H$	831	808	850	828	1.0285
3	Palm oil	886	876	896	884	1.0114
4	Cotton seed oil	889	869	915	886	1.0230
5	Soya bean oil	845	837	854	844	1.0096
6	Linseed oil	916	902	927	914	1.0155
7	Safflower oil	898	878	915	885	1.0228
8	Sesame oil	875	866	884	874	1.0104
9	Groundnut oil	903	894	911	901	1.0101
10	Ricebran oil					
	a Neutral oil	910	900	919	909	1.0201
	b Oil with 14.8% acid value	856	798	890	849	1.1152
11	Castor oil	965	953	979	964	1.0126
12	Mustard oil	1019	1014	1023	1018	1.0049
13	Corn oil	874	847	890	871	1.0319
14	Neem oil	811	703	876	799	1.1536
15	Vanaspathi					
	a S	896	887	906	894	1.0101
	b D	898	885	911	896	1.0147
	c T	906	892	919	904	1.0157
	d A	928	912	944	926	1.0175
16	Mahua oil	903	870	922	900	1.0379
17	Ram fat	941	917	951	939	1.0262
18	Broiler chicken fat	928	925	932	928	1.0032
19	Beef fat	918	877	932	915	1.0468

sample of stearic acid and an unsaponifiable fraction extracted from palm oil were injected for comparison of retention times. Stearic acid was eluted after 24.1 min which corresponds to the molecular weight, $\bar{M}_w$, of 380 according to the standard calibration graph (Fig. 1). However, the true molecular weight of stearic acid is lower than the value obtained by HPSEC. This may be due to strong hydrogen bonding interactions between fatty acids and the mobile phase solvent.

From Table III and Fig. 3, in the case of refined samples the $\bar{M}_w$ obtained by HPSEC and that predicted from their SV data are seen to be in good agreement. The slope and intercept of the correlation graph are in accord with eqn. 2.

Molecular weight distributions of oils and fats

Fig. 2A shows that the molecular weight distribution for mixtures of glycerides present in all vegetable oils and fats and animal body fats is represented by a symmetric gaussian peak at their respective retention times. However, two partially resolved peaks are observed in the case of butter fats. The first peak which corresponds to $\bar{M}_w$ of *ca.* 900 is due to the presence of high-molecular-weight glycerides containing $C_{18:1}$

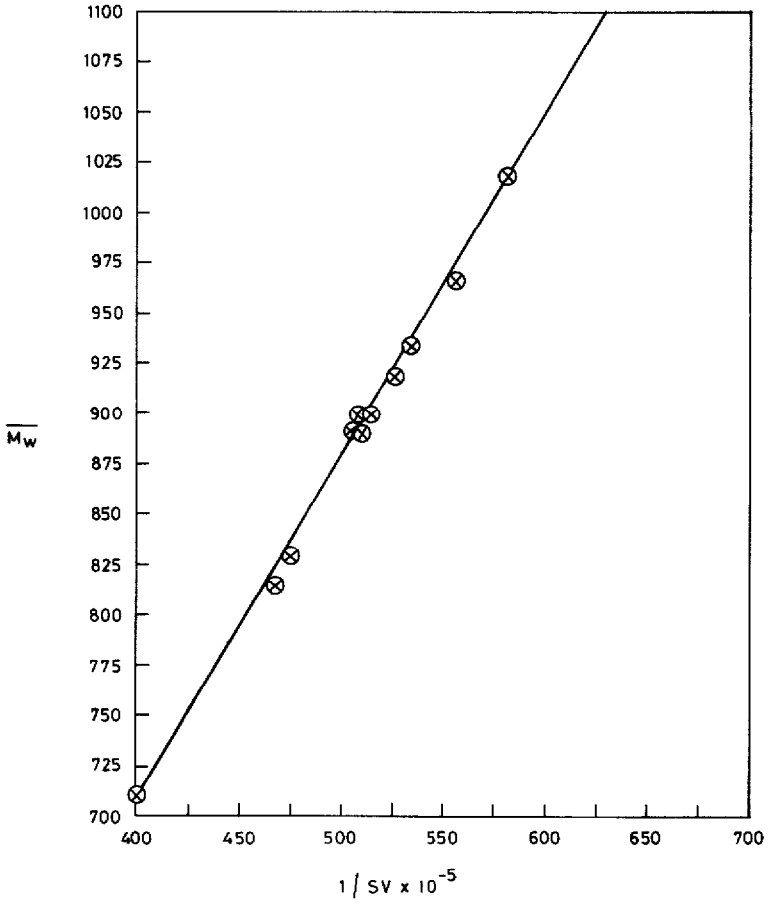

Fig. 3. Correlation of weight-average molecular weight, $\overline{M}_w$, and reciprocal of average experimental saponification value, 1/SV.

unsaturated fatty acid, whereas the second peak corresponds to $\overline{M}_w$ of *ca.* 700 due to low-molecular-weight glycerides containing saturated fatty acids of carbon numbers less than sixteen.

Two butter fats from different regions were studied in respect of their MWD and fatty acid composition using HPSEC and conventional gas–liquid chromatographic[12] techniques respectively. Fig. 4A and B show the MWD of two butter fats labelled as $(BF)_H$ and $(BF)_W$ respectively. It is seen that the high-molecular-weight glycerides are relatively more abundant in sample $(BF)_H$ than in $(BF)_W$. The fatty acid compositions of these samples are given in Table IV, and are in close agreement with the MWD.

MWD of binary mixtures of oils and fats

The MWD of glycerides for a pure oil is symmetric, whereas binary mixtures of oils having molecular weight differences of more than 100 units have unsymmetric molecular weight distributions. A number of binary systems, *viz.*, coconut oil (CCO)–castor oil (CO), butter fat (BF)–Vanaspathi (V), BF–CCO and CCO–mineral oil

TABLE III

COMPARISON BETWEEN $\overline{M}_w$ OBTAINED BY HPSEC AND THE MOLECULAR WEIGHT PREDICTED FROM SV DATA USING EQN. 2 FOR REFINED SAMPLES

Sample No.	*Name*	M_w	*Mol.wt. predicted*	*SV*	*% Error*
1	Coconut oil	712	708	251	0.5
2	Palm oil	886	892	197	0.7
3	Cotton seed oil	889	892	197	0.4
4	Safflower oil	898	903	194.5	0.5
5	Groundnut oil	903	915	192	1.3
6	Mahua oil	903	910	193	0.8
7	Linseed oil	916	920	191	0.5
8	Castor oil	965	973	180	0.9
9	Mustard oil	1019	1013	172.5	0.6
10	Butter fats				
	a $(BF)_w$	814	817	216	0.4
	b $(BF)_{fl}$	831	836	211	0.6
11	Vanaspathi	928	933	188	0.6
12	Broiler chicken fat	928	905	194	2.5

(liquid paraffin) were studied to understand the MWD of binary mixtures of oils and fats.

Typical HPSEC chromatograms of these mixtures are presented in Fig. 5A, B, C and D respectively. Two partially resolved peaks for their respective triglyceride groups are seen in a CCO–CO mixture containing more than 5% CO. The resolution of the two peaks increases with increasing percentage of CO. A similar pattern was obtained for mixtures of CCO with other oils and fats, except BF.

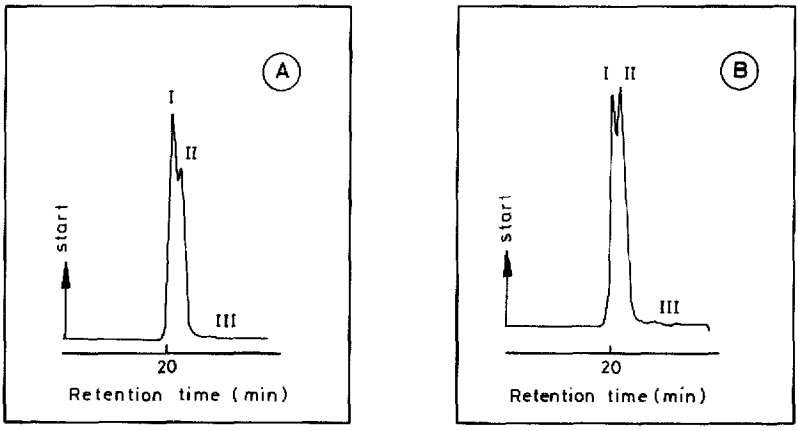

Fig. 4. HPSEC chromatograms of butter fat $(BF)_{fl}$ (A) and butter fat $(BF)_w$ (B) showing the molecular weight distribution of relatively high-molecular-weight glycerides (I), relatively low-molecular-weight glycerides (II) and free fatty acids and the unsaponifiable fraction (III).

TABLE IV

FATTY ACID COMPOSITION (%) OF BUTTER FATS

	Fatty acid	*Sample code*	
		$(BF)_H$	$(BF)_W$
(A)	*Saturates*		
1	Butyric acid ($C_{4:0}$)	1.8	1.6
2	Caproic acid ($C_{6:0}$)	0.4	0.8
3	Caprylic acid ($C_{8:0}$)	0.3	0.7
4	Capric acid ($C_{10:0}$)	0.6	1.0
5	Lauric acid ($C_{12:0}$)	1.0	1.7
6	Myristic acid ($C_{14:0}$)	5.8	7.7
7	Palmitic acid ($C_{16:0}$)	23.2	27.6
8	Stearic acid ($C_{18:0}$)	16.4	14.8
9	$C_{20:0}$, $C_{22:0}$, etc.	6.6	6.7
(B)	*Unsaturated*		
1	Myristoleic acid ($C_{14:1}$)	1.6	2.1
2	Palmitoleic acid ($C_{16:1}$)	4.6	4.7
3	Palmitolinoleic acid ($C_{16:2}$)	3.1	2.9
4	Oleic acid ($C_{18:1}$)	23.8	18.0
5	Linoleic acid ($C_{18:2}$)	5.0	4.7
(C)	*Unusual fatty acids*	5.8	5.0

It has already been mentioned that BF gives two peaks for its glycerides. The addition of any other oil or fat to it changes the intensities of these two peaks. It is seen from Fig. 5C that when CCO is added to BF the intensity of the low-molecular-weight peak is increased whereas Fig. 5B reveals that when vanaspathi is added to BF the intensity of the high-molecular-weight peak is increased. This observation may find application in detecting the adulteration of some oils and fats.

It is also observed that the ratio $\bar{M}_w/\bar{M}_n$ is almost unity for pure samples. Any change in the value of $\bar{M}_w/\bar{M}_n$ is indicative of adulteration. A typical example for admixtures of CCO–CO is shown in Fig. 5A(a) to (e) and Table V. It is also possible to detect the adulteration of mineral oil in edible oil by applying this method, as seen from Fig. 5D.

TABLE V

MOLECULAR WEIGHTS OF CCO–CO BINARY MIXTURES

Calculated from MWD of glycerides only.

Sample No.	*CCO (%)*	*CO (%)*	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
1	100.0	0.0	718	716	1.0028
2	97.5	2.5	734	721	1.0180
3	95.0	5.0	748	732	1.0218
4	90.0	10.0	768	747	1.0281
5	85.0	15.0	788	765	1.0301
6	80.0	20.0	801	775	1.0335

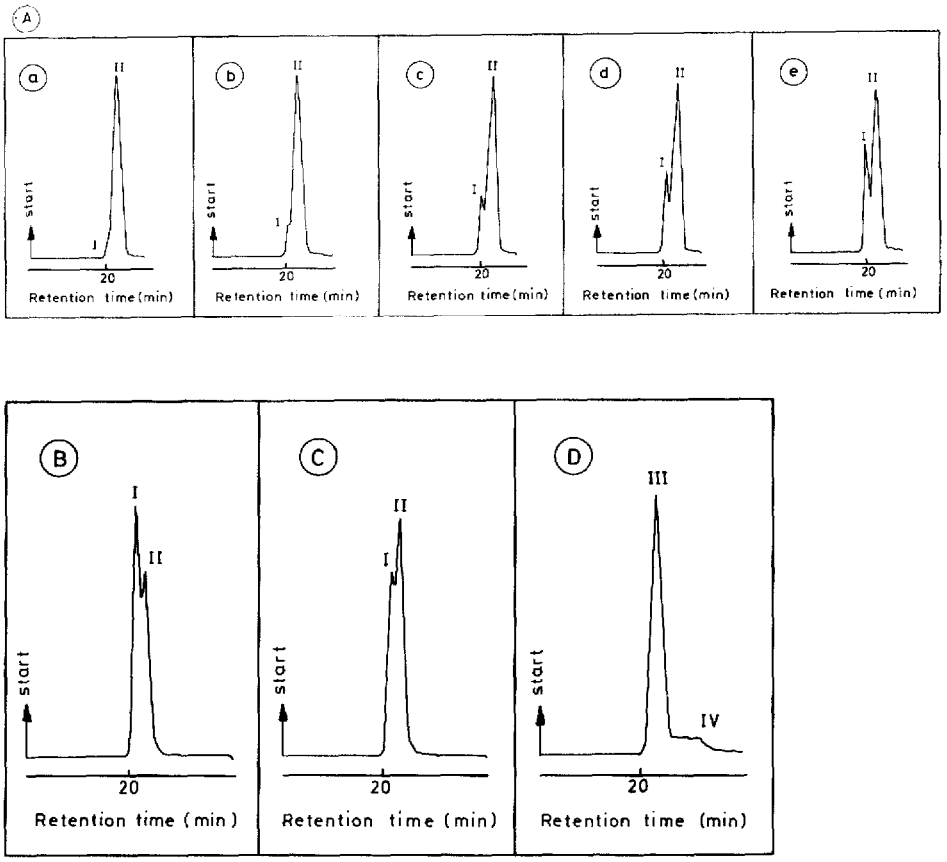

Fig. 5. (A) HPSEC chromatograms of coconut oil (CCO)–castor oil (CO) binary mixtures: (a) 97.5:2.5, (b) 95.0:5.0, (c) 90.0:10.0, (d) 85.0:15.0 and (e) 80.0:20.0. Molecular weight distributions: I, CO glycerides; II, CCO glycerides. (B) HPSEC chromatogram of $(BF)_w$ containing vanaspati, (C) containing CCO and (D) CCO containing mineral oil. Molecular weight distributions: III, CCO glycerides; IV, mineral oil fraction.

CONCLUSION

The determination of molecular weights and their distribution in oils and fats by HPSEC is reported for the first time. The data can be used for the characterization of various oils and fats. This method can be extended to the determination of adulteration in oils and fats.

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