Molecular Weight Averages as Criteria for Quality Assessment of Heated Oils and Fats[1,2]

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A simple method for quality assessment of heated oils and fats is described. The proposed method involves precise determination of molecular weight averages (MWA) *viz.*, the weight average molecular weight ($\overline{M}$w), the number average molecular weight ($\overline{M}$n) and the Z-average molecular weight ($\overline{M}$z) by high performance size exclusion chromatography (HPSEC) and their quantitative correlation to percent polar material obtained by column chromatography (CC). Change in MWA on heat treatment of fourteen different edible oils and fats at 180° ± 2°C for eight 8-hr days is studied. Relative standard deviations and regression coefficients of correlation between MWA and their ratios ($\overline{M}$w, $\overline{M}$n, $\overline{M}$z, $\overline{M}$z/$\overline{M}$n, $\overline{M}$w/$\overline{M}$n and $\overline{M}$z/$\overline{M}$w) *vs* percent polar material have been reported. Probable discard time was predicted for all the oils based on the above-mentioned correlations and also from the percent of high molecular weight (H. Mwt) species formed. It was observed that the oils which are generally recommended for coronary patients deteriorated faster. A possibility of extending this methodology to the frying oils is suggested.

KEY WORDS: Column chromatography, discarding time, heated oils, high molecular weight species, high performance size exclusion chromatography, molecular weight averages, quality assessment.

Deep-fat frying is one of the most commonly used procedures for the preparation of food (1). On repeated frying, oil is prone to undergo several oxidative and thermal reactions which ultimately change the physical, chemical, physico-chemical, physiological, nutritional and sensory properties of the oil which therefore is considered as heat abused (1,2). The measurement of heat abuse in an oil and its discard time is important.

Different analytical methodologies and criteria (3) for quantitative evaluation of heated oils are available. The quality assessment of heat abused oils is generally done based on the level of petroleum ether insoluble oxidized fatty acids (4) and percent polar compounds (5). The level of percent polar material is determined by silica gel column chromatography (CC) proposed by Guhr and Waibel (3,4) and is a standard official procedure since 1984 (6). Subsequently, high performance liquid chromatographic (HPLC) methods using silica gel (3,7–9) and size exclusion column packings (10–13) for determination of total polar artifacts in deep-fried oils are reported and the results are also correlated with those of CC method and petroleum ether insoluble oxidized fatty acids for studying quality deterioration in heated oils.

The composition of heated oils is complex in nature. In this connection we are of the opinion that their molecular weight averages (MWA) *viz.*, $\overline{M}$w, $\overline{M}$n and $\overline{M}$z and distribution patterns *viz.*, $\overline{M}$z/$\overline{M}$n, $\overline{M}$z/$\overline{M}$w and $\overline{M}$w/$\overline{M}$n may be more useful in understanding the nature of composition particularly in terms of quality deterioration. High performance size exclusion chromatography (HPSEC) is a powerful tool providing precise MWAs and their distribution patterns. Recently, we have reported the use of HPSEC technique for accurate determination of MWA for many oils, fats and their binary mixtures, and correlated them with conventional chemical data (14,15). Now, we report the utility of HPSEC technique for quantitative assessment of a wide variety of heated oils.

EXPERIMENTAL PROCEDURES

Apparatus: high performance size exclusion chromatography. HPSEC was performed on a LC-6A High Performance Liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) with a loop injector of capacity 12 μL having a high pressure six-way valve. A refractive index detector model RID-6A was connected after the column(s) for eluate monitoring. The work was carried out on high speed gel permeation columns of different exclusion limits (HSG-15H and HSG-20H, Shimadzu Corporation) each having 300 mm L × 7.9 mm i.d. and packed with 10 μm size spherical beads of styrene-divinyl benzene co-polymer connected in series. The chromatograms and the integrated data were recorded by a Chromatopac C-R3A processor (Shimadzu).

Column chromatography. A glass column (Borosil) 45 cm long × 2.1 cm i.d. with a Teflon stopcock and ground glass joint was fabricated in the laboratory.

Solvents, standards and samples. HPLC grade tetrahydrofuran was obtained from Spectrochem Pvt. Ltd, New Delhi, India. Solvents like n-hexane and diethyl ether were obtained from Apex Enterprises, New Delhi, India. Polypropylene glycols of different average molecular weights ($\overline{M}$w) 4000; 2000; 1000; 800 and 400) were obtained from S.D. Fine Chemicals, New Delhi, India. Silica gel adsorbent of particle size 70–230 mesh ASTM Merck No. 7734 (Darmstadt, Germany) was used for CC. Vegetable oils and fats, *viz.*, coconut oil (Cco), peanut oil (Pno), sesame oil (Seo), ricebran oil (Rbo), sunflower oil (Sun. o), safflower oil (Saff. o), soyabean oil (Soy. o), corn oil (Co), cottonseed oil (Cso), palm oil (Po), olive oil (Oo), rapeseed oil (Ro) and vanaspathi (hydrogenated fat) of edible grade were purchased locally.

Sampling procedure. Fourteen different edible oils were used for the present studies. 200 mL of each oil sample was taken in a glass beaker of 250-mL capacity, the surface area to volume ratio of which is 1:7.25. All the oils were heated under laboratory conditions in an air oven at 180° ± 2°C for eight 8-hr days. Samples were collected periodically (8 hr) and subjected to HPSEC and CC.

Column chromatography (CC). CC work was carried out

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according to the standard procedure recommended for determination of polar and nonpolar materials (6).

HPSEC and method development. The average molecular weights of the heated oils (which contain both polymers as well as degraded products) range from 700–7000 amu depending on the nature of the oils and heating conditions. Accordingly, two size exclusion columns *viz.,* HSG-15H and HSG-20H having exclusion limits 1000 and 10,000 respectively weré connected in series to separate the various molecular species present in heated oils. The mobile phase used was HPLC grade tetrahydrofuran (THF) with an optimized flow rate of 0.5 mL/min. Although a 0.5 mL/min flow rate is considered as low, that had provided a well-defined molecular weight distribution (MWD) pattern most probably due to low mass transfer. Samples were dissolved in the mobile phase used. Chromatograms were recorded on 32×10^{-6} refractive index units scale and the sample concentration was about 30 mg/mL. The samples were filtered using 0.45 μm pore size filters, supplied by M/s Waters Associates, Milford, MA, before injection and the analysis was performed at ambient temperature.

Calibration of the HPSEC retention data and determination of MWA. Based on the functionality and polar nature associated with the eluting solvent and samples, polypropylene glycols of different average molecular weights ($\overline{M}w$) *viz.,* 4000, 2000, 1000, 800 and 400 were chosen assuming that the status of their effective molecular size and hydrodynamic volume in THF was similar to that of heated oils for calibrating the HPSEC retention data (14). Retention times of standard polypropylene glycols were determined by injecting individual standards employing the same conditions established earlier. The retention data and a linear calibration graph (within the exclusion limits of column system) are shown in Table 1 and Fig. 1, respectively). Data for the calibration are stored in a floppy disk FDD-1A (Shimadzu) coupled with the chromatopac C-R3A processor as per the procedure given in the instruction manual of chromatopac C-R3A, GPC program supplied by M/s Shimadzu corporation for the determination of MWA. The reproducibility of the method was tested by multiple injection of samples. The coefficient of variation for MWA ranged from 0.5–1.0% (n = 5).

TABLE 1

SEC Retention Behavior of Reference Materials (standards: polypropylene glycols)

Sample no.	Average M.wt	Retention time in min
1	4000	24.29 ± 0.03
2	2000	26.88 ± 0.03
3	1000	28.55 ± 0.04
4	800	30.24 ± 0.06
5	400	33.35 ± 0.06

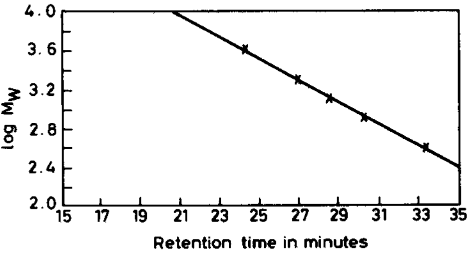

FIG. 1. Calibration graph.

RESULTS AND DISCUSSION

One-hundred-and-ten heated samples of fourteen different oils were subjected to HPSEC. Chromatograms of Saff. o at 0,8,16,32,48 and 64 hr of heating are shown in Figure 2 as a typical example which gives information regarding MWD, peak separation, peak identifications and their relative concentrations. Relative changes in monomer, dimer, trimer and tetramer triglyceride concentrations with respect to heating time from zero to 64 hr are shown in Table 2. The unheated oils contain only three peaks whereas a steady increase in the number of peaks of high molecular sizes is seen in heated oils. It is further observed

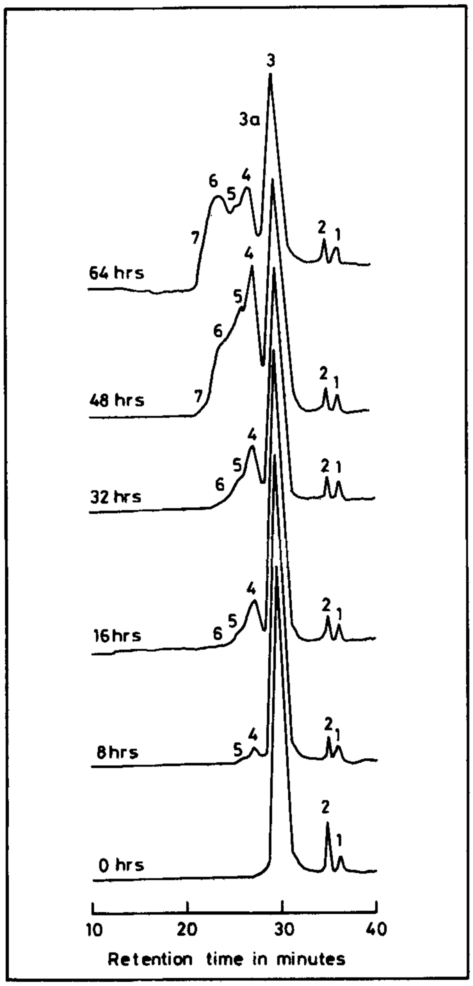

FIG. 2. Typical HPSEC chromatograms for safflower oil at 0, 8, 16, 32, 48 and 64 hr of heating. Peak identifications based on molecular weight distribution are (1) monoglycerides and free fatty acids (2) diglycerides (3) triglycerides (TG) (3.a) oxygenated TG (4) dimeric TG (5) trimeric (6) tetrameric TG (7) oligomeric TG.

TABLE 2

Changes in Relative Peak Percent of Heated Safflower Oil by HPSEC Analyses

Hours of heating	Peak no.						
	1	2	3	4	5	6	(6 + 7)
0	2.1	4.1	93.8	0.0	0.0	0.0	0.0
8	2.0	3.8	90.0	3.3	0.0	0.0	0.0
16	2.0	3.7	86.4	6.0	1.9	0.0	0.0
24	2.0	3.8	75.3	11.8	7.1	0.0	0.0
32	2.0	3.7	69.5	14.0	8.9	2.1	2.1
40	1.8	3.7	65.1	16.2	9.1	4.4	4.4
48	1.8	3.7	54.0	18.5	10.6	—	11.3
56	1.8	3.7	42.5	16.9	10.8	—	24.8
64	1.8	3.6	39.2	12.0	11.4	—	32.0

that peak 3 decreased progressively on heating. These chromatograms have been compared with those reported by Perrin *et al.* (16) and White and Wang (13) and on comparison of the retention times with the standards of similar molecular sizes, it is inferred that peak 3 in heated oils represents triglycerides and oxidized triglycerides and that peaks 4, 5 and 6 depict dimers, trimers and tetramers respectively. These results substantiate the deterioration of oils on heating as a result of formation of high molecular weight species (H.Mwt species). However, the information is qualitative and the approach is not adequate when H.Mwt species starts forming particularly below 48 hr of heating due to peak overlapping. Calculation of individual SEC peak areas using a planimeter may also give erroneous results. Therefore, slicing the entire SEC elution pattern helps in determining the various species formed at different time intervals quantitatively in terms of MWA.

The recommended criteria for assessing the quality of heated oils is either based on the value of petroleum ether insoluble fatty acids (0.7 to 1.0%) or percent polar material (27%) obtained by CC. Although these methods provide good indication of the substances which are responsible for oil deterioration, they are not specific. It has also been pointed out by White and Wang (13) that the H.Mwt compounds may serve as a more reliable indicator for heat abuse because of their low volatility and resultant increase of stability. Hence, it has been considered that a correlation may be drawn between percent polar material and MWA which may serve as a better criterion for quality assessment of heat-abused oils.

The different kinds of MWA dealt with in this paper for several heated oils are $\overline{M}$w, $\overline{M}$n and $\overline{M}$z. These are found to increase with heating time and the extent of increase also depends on the nature of the oil. Among the oils studied, it is observed that the change in MWA is maximum for Ro and minimum for Cco. On examination of the MWA of different samples, it is observed that a striking difference between the pairs, *viz.*, $\overline{M}$z-$\overline{M}$w and $\overline{M}$z-$\overline{M}$n exists. This difference goes on increasing with the increase in heating time and is believed to be due to formation of polar species of H.Mwt, which are responsible for oil deterioration.

A graph was drawn between percent polar material and MWA for 110 heated samples belonging to fourteen different oils as illustrated in Figure 3. By employing regression analysis, a linear relation is seen. Table 3 enlists the values of regression coefficients and also includes the MWA data at 27% of polar material. It is seen from the table that the best linear fit of the data is obtained for $\overline{M}$z followed by $\overline{M}$w and $\overline{M}$n. This is perhaps due to the contribution of progressively increased formation of H.Mwt species in the heated oil. On the other hand the presence of low molecular weight component (monomeric glycerides, mono and diglycerides, free fatty acids etc.) is believed to influence $\overline{M}$n slightly. It could be seen from Table 3 that the results obtained for $\overline{M}$n is in accordance with this assumption.

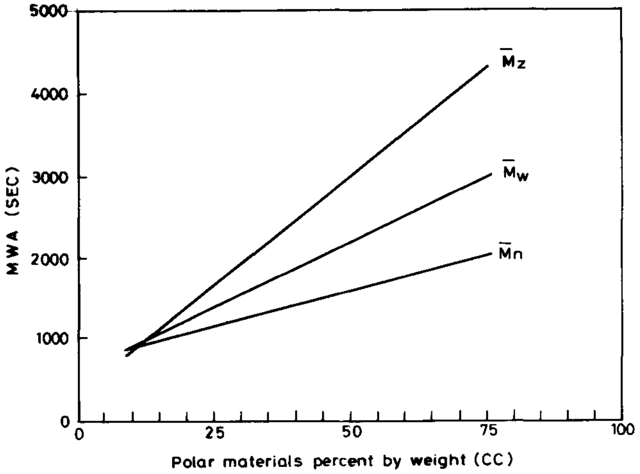

FIG. 3. Correlation graph between per cent polar material (CC) and MWA (SEC).

The detailed knowledge about dispersivity is considered very important for characterization of H.Mwt species. The different ratios (*viz.*, $\overline{M}$z/$\overline{M}$n, $\overline{M}$w/$\overline{M}$n and $\overline{M}$z/$\overline{M}$w) of MWA demonstrate the complete nature of MWD for the product which in turn has been utilized for the quality assessment of heated oils. Figure 4 gives a graph between percent polar material and ratios of MWA for all the samples under investigation. It is seen from it that all pure oils have a value of almost unity. Any change in the value of MWA ratios is indicative of deterioration of oils. Further, it is seen from the graph that these ratios are increased with the progressive heating. Table 4 records the regression analysis data for the above correlations and also includes the values at 27% of polar material. Irrespective of the nature of the oil, the different ratios of MWA at 27% of polar material are absolute indicators for its discard level. The regression data for the correlation of

TABLE 3

Regression Data for the Correlations of MWA (SEC) *versus* Percent Polar Material (CC) (regression equation: Y = A + BX n = 110)

Sample no.	Type of MWA	A	B	Multiple correlation coefficient (R^2)	F ratio	Value of MWA at 27 (%) of polar material
1	$\overline{M}$z	274	52.6	0.948	1997	1695
2	$\overline{M}$w	526	31.4	0.934	1538	1375
3	$\overline{M}$n	703	17.1	0.875	773	1165

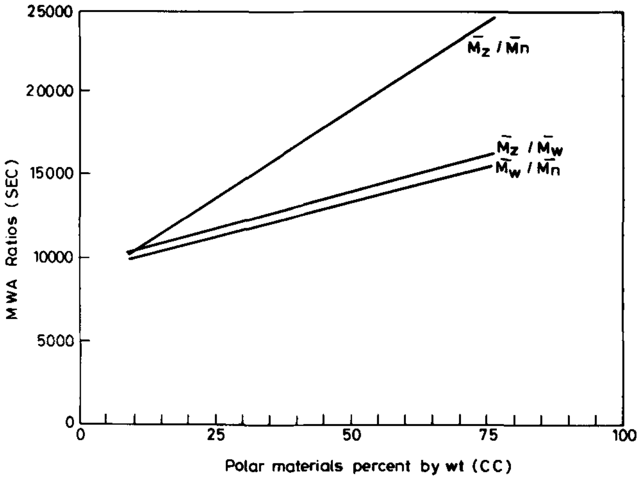

FIG. 4. Correlation graph between per cent polar material (CC) and MWA ratios (SEC).

TABLE 4

Regression Data for the Correlations of MWA Ratio *versus* Percent Polar Material (regression equation: Y = A + BX n = 110)

Sample no.	Type of MWA ratio	A	B	Multiple correlation coefficient (R^2)	F ratio	MWA ratio value at 27(%) of polar material
1	$\bar{M}z/\bar{M}n$	0.8151	0.0211	0.924	1337	1.38
2	$\bar{M}w/\bar{M}n$	0.9248	0.0084	0.917	1222	1.15
3	$\bar{M}z/\bar{M}w$	0.9469	0.0089	0.833	549	1.19

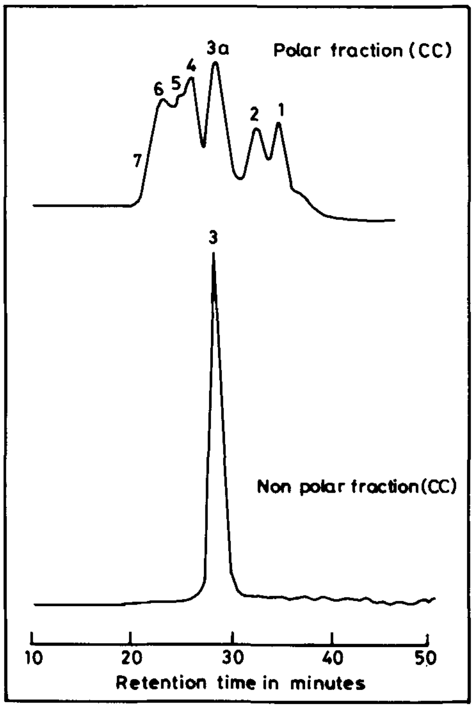

FIG. 5. Typical HPSEC chromatograms of nonpolar and polar fractions obtained by CC for safflower oil at 64 hr of heating. (Peak identifications are same as in Fig. 2.)

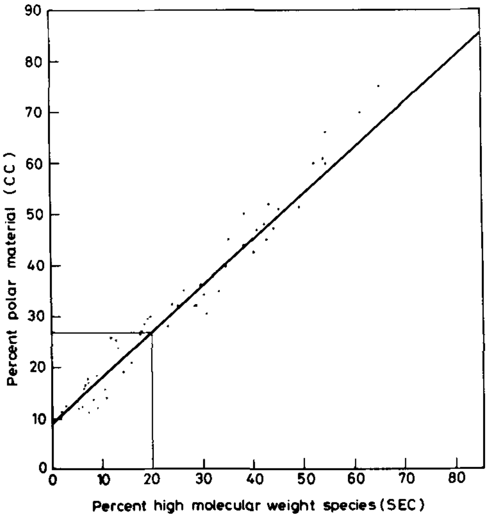

FIG. 6. Correlation between per cent H.Mwt species (SEC) and per cent polar material (CC).

percent polar material *vs* ratios of MWA reveal that the $\bar{M}z/\bar{M}n$ gives the best linear fit followed by $\bar{M}w/\bar{M}n$ and $\bar{M}z/\bar{M}w$.

The HPSEC also gives a clear idea about total polar artifacts by taking the combined relative percentage of all peaks except for pure triglycerides. In this connection, polar and nonpolar fractions collected in column chromatographic analysis are monitored by SEC. The nonpolar fraction gives only one peak corresponding to pure triglycerides whereas the polar fraction gives several peaks as depicted in Figure 5.

A graph also was drawn between the relative percent of H.Mwt species obtained by HPSEC and percent polar material for all the samples (Fig. 6). The following facts are observed: (i) 27% polar material corresponds to 20% of H.Mwt species in heated oils; (ii) Intercept on the Y-axis (percent PM) corresponds to 7% which is attributed to mono and diglycerides, free fatty acids and unsaponifiable matter originally present in the oil.

Based on the above correlations, we have extended this methodology for predicting the most probable discard time for a specific oil by comparing the MWA values and H.Mwt species with corresponding percent polar material at different time intervals. Table 5 records the MWA and their ratios and percent polar artifacts for pure and maximum heated oils obtained by HPSEC. These data are in conformity with the changes in fatty acid composition determined and are also in accordance with the observation made by Dobarganes and Perez-Camino (17). The formation of relatively H.Mwt species content observed for rapeseed oil is also due to the presence of the long-chain monoenic fatty acid, erucic acid (C-22:1).

The oils under study can be classified into four groups containing highly saturated, monoene-rich, diene-rich and triene-rich fatty acids. Their discard times are approximately 56 hr, 32 hr, 24 hr and 16 hr, respectively. Table 6

TABLE 5

Molecular Weight Averages (MWA) and Their Ratios and Percent Polar Artifacts for Pure and Maximum Heated Oils Determined by SEC

Sample no.	Oil	$\bar{M}$w		$\bar{M}$n		$\bar{M}$z		$\bar{M}$w/$\bar{M}$n		$\bar{M}$z/$\bar{M}$w		$\bar{M}$z/$\bar{M}$n		Percent polar artifacts[a]	
		Time in hrs													
		0	64	0	64	0	64	0	64	0	64	0	64	0	64
1	Cco	724	968	710	810	730	1281	1.0197	1.1951	1.0083	1.3233	1.0282	1.5815	0	18.10
2	Po	886	1486	876	1254	896	1895	1.0114	1.1845	1.0113	1.2752	1.0288	1.5109	0	23.77
3	Pno	903	1828	894	1395	911	2479	1.0101	1.3106	1.0090	1.3561	1.0190	1.7771	0	44.33
4	V	906	1878	892	1450	919	2670	1.0157	1.2646	1.0143	1.4217	1.0303	1.8408	0	35.34
5	Seo(1)	875	1820	866	1408	884	2488	1.0104	1.2925	1.0103	1.3670	1.0208	1.7673	0	38.14
6	Seo(2)	870	1760	862	1365	876	2305	1.0093	1.2894	1.0069	1.3097	1.0046	1.9671	0	37.40
7	Cso	889	2128	869	1547	915	3003	1.0230	1.3756	1.0292	1.4112	1.0529	1.9412	0	41.20
8	Rbo	910	2144	900	1450	919	3183	1.0110	1.4786	1.0099	1.4846	1.0211	2.1507	0	42.11
9	Sun. oil	880	2231	866	1576	919	3194	1.0162	1.4155	1.0443	1.4316	1.0612	2.0259	0	49.49
10	Co	874	2306	847	1557	890	3480	1.0319	1.4811	1.0183	1.5091	1.0508	2.2351	0	50.60
11	Saff. oil	898	2408	878	1517	915	3570	1.0288	1.5865	1.0893	1.4825	1.0421	2.3526	0	60.82
12	Co	895	2501	867	1692	910	3630	1.0323	1.4773	1.0168	1.4514	1.0496	2.1444	0	52.71
13	Soy. oil	845	2532	837	1688	854	3696	1.0096	1.5000	1.0107	1.4595	1.0203	2.1890	0	54.10
14	RO	1019	3363	1014	2211	1023	4658	1.0049	1.5209	1.0039	1.3850	1.0089	2.1052	0	64.71

[a] Refers to relative percent of H.Mwt species.

TABLE 6

Discard Time in Hours Predicted for Heated Oils of Specific Nature

Sample no.	Oil	Discard time in hr From the correlation of H.Mwt species *vs* polar material (Fig. 6)	From the correlation of MWA *vs* polar material (Fig. 3)
1	Cco	more than 64	more than 64
2	Po	52	54
3	Pno	35	35
4	V	32	30
5	Seo (1)	33	32
6	Seo (2)	35	35
7	Cso	30	30
8	Rbo[a]	24	25
9	Sun. oil[a]	20	20
10	CO[a]	22	20
11	Saff. oil[a]	30	32
12	Oo	20	20
13	Soy. oil[a]	20	20
14	RO[a]	14	12

[a] Oils containing polyunsaturated fatty acids and generally recommended for coronary patients.

gives the probable discard time predicted for heated oils of specific nature. Although the data are restricted to the air-heated oils, the same methodology may be extended to the quality assessment of deep-frying oils. It is presumed that in case of frying oils, their discard time may be shorter when compared to the respective air-heated oil due to the differences in dynamics of hydrolysis and food oil interactions particularly in case of coconut oil which contains short-chain fatty acids and would hydrolyze quickly and produce volatile products leading to bad smoking problems.

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