Chemical Analysis of Polymerization Products in Abused Fats and Oils[1]

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ABSTRACT

For more than a decade, numerous analytical methods have been proposed for the detection and measurement of polymers in vegetable fats and oils. Many of the methods have been little more than laboratory curiosities, either because they were concerned with only very specific compounds or were too cumbersome and time consuming to become very popular. More recently, a number of methods in common use for analysis of fats and oils has been shown to be useful for indirectly measuring polymeric materials in heat abused oils. The present report shows, by the use of gel filtration chromatography, the validity of two of the indirect methods of estimating polymeric products of abused fats and oils. These methods are: the estimation of polymers through changes in the iodine value and the measurement of retention materials on a gas liquid chromatographic column. A new simplified internal standard gas liquid chromatographic procedure utilizing triglyceride standards also is presented. This latter method permits estimating the degree of degradation of vegetable fats and oils by any laboratory capable of determining the fatty acid composition of a sample by gas liquid chromatography.

INTRODUCTION

As early as 1953 in the publications of Crampton, et al. (1,2), chemical compounds were found in heated fats which were shown to be toxic to animals when ingested. Since that time a controversy has waged between two groups of researchers. The one group has isolated compounds from heated oils and speculated on their effects upon humans or have obtained measurable responses after introducing these compounds to animals (1-17). Many of these researchers dramatically have cautioned about the potential toxicity of heated fats (4,6-8,14-16). On the other hand are other researchers who generally have taken commercially used frying oils and either attempted to measure the amount of the questioned components or fed animals the whole used fat as part of a balanced diet (18-37). These latter researchers have contended that the suspect components are not present in commercially used oils in a significant quantity. The conclusions of the latter researchers have been supported by the lack of detectable deleterious effects in their animal studies.

Included in the studies of both groups were attempts to develop methods which would be meaningful for answering the questions of what compounds and how much are produced during use of frying oils. The principal emphasis on new techniques has been in the field of analytical chemistry, in part because of extended periods of time required by biological procedures (23,34) and in part because, unless the experimental diets are nutritionally adequate, results of feeding studies may be misleading and liable to misinterpretation (26). Analyses of the various degradation or oxidation products have resulted in reports of the presence in heated oils of epoxides (17,38,39), lactones (39,40), polymers (12,13,39,41), cyclic monomers (1,2,33), phthalates (9,32,36), etc. However, Melnick (18) has suggested that the major commercial concern should be directed at polymers, and he demonstrated that oils containing a relatively small quantity of oxidative polymers would possess an objectionable flavor, whereas a measurable concentration of thermal polymers may be present without any organoleptic indication. A measure of polymer development as indicated by a drop of ca. 5 in the iodine value is the level of change shown by Lassen, et al., (42) and subsequently confirmed by Alfin-Slater, et al., (26) to be necessary before there is a significant biological response. Various other degradation products of heated oils can be shown to be of little consequence. Crampton, et al., and later Andrews and his associated (1,43) showed that simple oxidation products do not interfere significantly with the nutritional well being of test animals, and it also may be concluded from the work of Crampton (1,2) that formation of measurable quantities of toxic cyclized monomeric acids is not possible at commercial frying temperatures in oils not containing linolenic acid. It has, therefore, been concluded (19) that the problem of concern in frying oils (if any) is related to the presence of thermal polymers of the nonoxidative type, particularly since these polymers even enhance the flavor stability of an oil. A report by Witting, et al., (44) supported this position by also indicating greater concern about thermal than oxidative polymers. More recent researchers have shown that thermal polymers, per se, are nontoxic, and only the nonadductible monomer and oxidative dimers possess toxic properties (33,36).

Of all the procedures that have been proposed for the determination of fatty acid polymers, it is uncertain that any of them yield absolute values. Comparisons have involved the use of viscosity (45); iodine value (18,43); hydroxyl value (46); distillation of the methyl esters (1,2); thin layer (47,48), paper (49), column (50,51), liquid-liquid (52), and reversed phase (53,54) chromatographies; urea adduction (55-57); counter current distribution (58); sublimation (41); gas liquid chromatography (GLC) (14,59,60); and many others.

All have shown definite relationships to the deterioration of the quality of the fat. Although viscosity, iodine value, and hydroxyl values are relatively simple tests, they require a base value from the identical oil sample before abuse to make any reasonable estimate of the extent of degradation. The most popular among these is the iodine value, since it is a routine quality control tool in the fat and oil industry.

Data collated from the literature (61) showed that, whether the loss of unsaturated acids was the result of what could be termed thermal polymers, oxidative polymers, or probably just oxidation products, the drop in iodine value was ca. equal to the decrease in content of the dienoic acids. However, even though the method is an accurate measure of the loss of polyunsaturates from which polymers are formed, it can be affected by such obvious interferences as alteration of sample density and commingling of oils expelled from the food products.

Certain of the other more complex methods also has drawbacks, many of which have been reviewed by Artman (62). In our own experience with the chromatographic procedures of Frankel, et al. (52), Seibert and Sliwiok (48),

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TABLE I

Polymer Content of Oil Samples

Sample	Hr heated @ 185 C	Original IV (Wijs)	Updated[a] IV (Wijs)	Change in IV (Wijs)
Corn oil	24	121.5	121.1	− 0.4
	40	115.2	115.9	+ 0.6
	48	108.1	108.1	0.0
Soybean shortening	40	75.8	74.1	− 1.7
Cottonseed oil	40	82.8	79.5	− 3.3
Soybean oil	40	102.3	95.2	− 7.1
Lightly hydrogenated and winterized soybean oil	40	90.4	84.6	− 5.8

[a]Iodine value (IV) after storage at −4 C for 3 years.

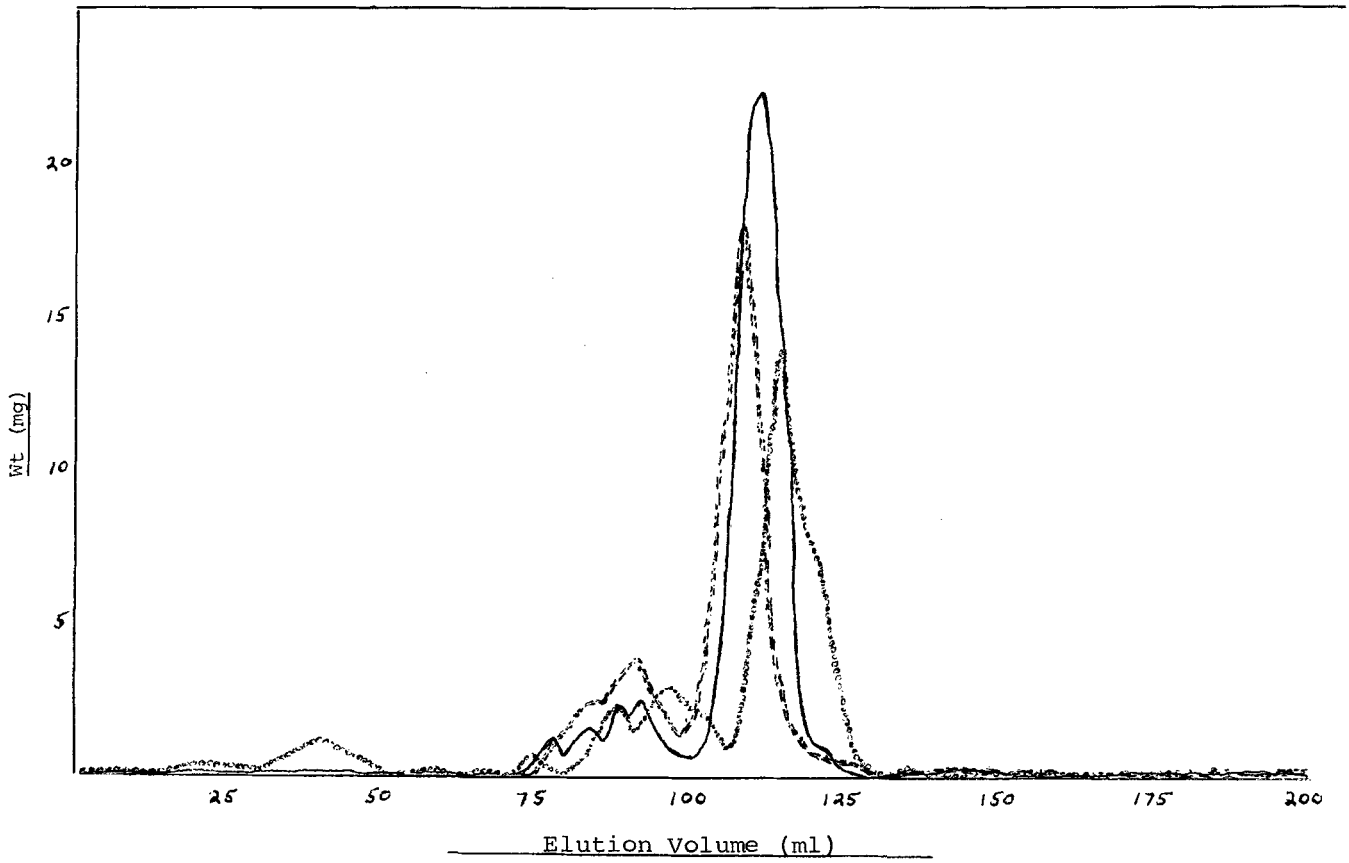

FIG. 1. Chromatograms of oil esters. —— = Corn oil, ∘∘∘∘ = soybean oil, and == = cottonseed oil.

and Sahasrabudhe and Farn (51), they are tedious, complex, and sometimes of questionable value with samples of fresh or only slightly abused oils. Since our principal aim had been to ferret out a simple and rapid screening technique, we soon became intrigued with the GLC procedures of Zielinski (60) or Thompson, et al., (14) in which the value for the material which did not elute from the column under standard operating conditions appeared to correlate with polymer content.

In 1970, we reviewed these methods (61) and reported on the comparative studies we had made. Recently, an additional attempt has been made to correlate these results with a more direct measure of polymers using gel filtration chromatography. This procedure involves the separation of the methyl esters of the components of a heat abused oil on a column of a hydroxylpropylated dextran gel (Sephadex LH-20) with chloroform. This is a procedure developed by Downey, et al., (63) as utilized by Ferren and Seery (64). Some prefer to call this technique "gel permeation chromatography" and admittedly have good reason on the basis of the chemical properties involved, but we have utilized the designation "gel filtration chromatography" used by the gel manufacturer and of investigators who have used Sephadex gels in the past (63,64). Bly (65) extensively discussed the pros and cons of the terminology and suggested "size exclusion chromatography" as better defining the mechanism which he and Determann (66) have explained as the passing of molecules through the gel bed in the order of their decreasing size. Thus, in contrast to the other chromatographic procedures mentioned earlier, the sample fractions of interest (the polymers, trimers, and dimers) are eluted first from this column before the monomer esters.

Sufficient proof of the specificity of the technique of gel chromatography has been obtained by previous researchers (63,64,67-71) and clearly are demonstrated by Perkins et al. (72). However, the application to heat abused oils has been limited (67,68,72) and involved equipment of such

TABLE II

Comparison of Methods for Polymer Content of Oil Samples[a]

Sample	Hr heated @ 185 C	Change in IV (Wijs)	Percent polymer by gel chromatography	Percent noneluted material from GLC
Blended salad oil			0.9	1.0
Blended salad oil	+ 18.2% Dimer		18.5	20.0
Corn oil	24	5.6	7.0	7.4
	40	10.8	14.3	15.0
	48	18.6	18.7	20.5
Soybean shortening	40	22.7	17.1	26.6
Cottonseed oil	40	37.7	25.5	47.0
Soybean oil	40	41.0	26.0	40.7
Lightly hydrogenated and winterized soybean oil	40	32.5	24.9	36.8

[a]IV = iodine value and GLC = gas liquid chromatography.

relative sophistication that it would not be generally available in the average food processor's laboratory. In contrast, the apparatus used, the same as reported by Ferren and Seery (64), is relatively simple.

EXPERIMENTAL PROCEDURES

Some of the oil samples prepared in 1969 were removed from cold storage and reanalyzed. After 3 years storage, almost entirely at -4 C, a reevaluation of the iodine values of these samples was made as shown in Table I to determine if further deterioration had occurred. Some samples, specifically the corn oil and the hydrogenated shortening, still possessed iodine values which agreed with the original values, but the samples of soybean oils, as well as the cottonseed oil, showed further deterioration during storage. Just the same, all samples were analyzed by the following procedures.

Gel Chromatography Procedure

The gel chromatography column consisted of a glass chromatographic column (1.5 x 120 cm) with a sintered glass disc and a flow regulating Teflon stopcock at its base and a 250 ml reservoir above. The eluate from the column was obtained using a Buchler fraction collector but could have been done manually. After esterification using the AOCS procedure (73), the esters from a 1 g oil sample were dried by washing through a funnel containing anhydrous sodium sulfate and concentrated by evaporation of the solvent on a steam bath under an atmosphere of nitrogen. They were applied to the column in the manner described by Ferren and Seery (64). Fractions were obtained every 2 min for this group to compare with the chromatograms of standard polymer and monomer esters. Routinely, it was not found necessary to obtain separate fractions, except during the time of the polymers passage from the column. The column composition and dimensions define the elution volume for the components, and the rate of flow determines the separation. In Figure 1 are indicated superimposed curves of the corn, cottonseed and soybean oil samples which had been abused for 40 hr.

GLC Procedure

The GLC procedure uses carefully weighed portions of an internal standard (trimargarin or triheptadecanoin) which are esterified together with the oil sample. The details of this esterification can be found in the earlier publication (61). Since that time, the procedure has been simplified by the relating of the internal standard directly to the total area only and thereby directly to the noneluted components of the sample. From a solution of known concentration of the internal standard in petroleum ether, an aliquot is added with an aliquot of a similar solution of the oil sample to an esterification flask, such that the internal standard is close to 20% of the wt of the total sample. The esters were prepared according to the AOCS method (73) and injected into an F&M 700 gas chromatograph with a flame ionization detector under the conditions and column parameters suggested by the AOCS method (74). The individual peak areas of the chromatograms are obtained by planimetry or an electronic integrator. If the analysis being made is of a marine oil or an animal fat, a different internal standard is used; or a second ester is prepared for analysis without the internal standard, and a correction is made for the area of the minor component at the same retention time found in the second chromatogram. The noneluted materials retained by the column are calculated by the following formula:

$$\frac{\text{Internal standard area}\left[\frac{\text{internal standard + sample wt}}{\text{internal standard wt}}\right] - \text{Total area of chromatogram}}{\text{Internal standard area}\left[\frac{\text{internal standard + sample wt}}{\text{internal standard wt}} - 1\right]} \times 100$$

Calculation is simplified if the internal standard is added at a fixed percentage (20%) of the sample wt. The calculation corrects the total area of the sample to the level it would have if all components would be eluted from the column and the procedure by-passes the extensive use of reference curves by assuming equal response of each component when using a GLC flame ionization detector.

RESULTS AND DISCUSSION

The results obtained by the GLC technique are in agreement with the estimates of polymer content by gel chromatography and the change in the iodine value of the samples of abused corn oil, as well as of a sample of blended salad oil spiked with a standard dimer as shown in Table II. The agreement would indicate that the three methods are measuring the same thing. However, with the other oils significant differences are noted.

Since it was hard to believe that the gel chromatographic values could be in error because of its proven ability to sort molecules on the basis of size, we theorized that the higher

TABLE III

Evaluation of Heated Oils

Sample	Change in iodine value (Wijs)	Percent polymer by gel chromatography	Percent noneluted material from GLC[a]
Laboratory abused			
Oil heated at 600 F under N_2 (15 min)	6.1	4.6	3.2
Oil heated at 600 F under air (2 min)	5.8	3.9	6.9
Commercially abused			
Hydrogenated cottonseed oil used for roasting peanuts	1.3	2.2	2.1
Hydrogenated soybean oil shortening used for miscellaneous fryings	4.9	2.2	3.8

[a]GLC = gas liquid chromatography.

values by the GLC and for the iodine value difference were the result of other oxidation products of linoleic acid whose added polarity interfered with their elution from the GLC column and not polymers.

In an attempt to confirm our suspicions, we decided to prepare oils similar to the two oils prepared by Melnick (19) which were considered to contain only polymers of either thermal or oxidative origin. These oils, shown in Table III, were abused until an iodine value change of 5 or more had occurred. The results did not show a significant difference for the oils thermally abused in the absence of oxygen, but the thermally and oxidatively abused oil contained significantly less polymer by gel chromatography than GLC nonelution materials; a phenomenon repeatedly seen before with all but the corn oils. It is questioned whether an oxidative polymer, as such, really exists, and work is continuing to test the premise that all of the polymers indicated here were of thermal origin.

We suspect that the corn oil probably was protected, to a degree, from oxidation by traces of silicone (61) and, therefore, contains principally thermal polymers. Remembering the agreement shown for the 40 hr samples between different methods, including the percent urea nonadductables (61), and the elucidation by recent researchers of the many compounds which were not polymers found in the urea nonadducting fractions of heated oils (32,33), it is quite logical to expect the gel chromatographic values to be lower if they are indicating only polymers.

Thus, the difference between the percent noneluted materials and the polymers is apparently polar oxidation products amounting to 10-20% for all of the oils heated for 40 hr with the exception of the corn oil.

The GLC and gel chromatographic methods likewise were applied to two samples discarded from different commercial operations compared initially in our earlier paper (61) and also shown in Table III. These oils, like the corn oils, did not exhibit any significant further deterioration during storage. On the basis of what we have just seen, it would appear that the degradation products involved were probably ca. 2% thermal polymers in both samples and an additional 1-2% of oxidation products in the oil used for miscellaneous fryings.

It is obvious that, for estimating the degree of degradation of vegetable fats and oils, the simplest method is the determination of the change in iodine value (providing the proper unheated reference sample is available). If a reference sample is not available, the internal standard GLC method proposed can be used as a rapid screening technique which will quite simply provide a good estimate of the maximum level of degradation. For samples which are found to contain a significant response by the GLC technique, additional analysis by gel chromatography then can provide a means of estimating the thermal polymer and, by difference from the GLC values, the oxidation products in an abused oil after taking into account the contribution of the natural unsaponifiables and nonlipid food additives present.

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