formed in the oil. This spectrophotometer also can be used for indirect monitoring of hydrogenation. Figure 10 shows the application of this instrument for the previously mentioned pilot plant hydrogenation. Apparently, the progression of *trans* isomer formation is inversely related to the decrease of IV. Because the rate of *trans* isomer formation is greatly dependent on e.g., the catalyst and temperature used during hydrogenation, each set of hydrogenation conditions requires a special set of monitoring parameters.

Monitoring the clarity of a filtrate after hydrogenation or bleaching is also of interest to the oil processing industry. Another optically based process control instrument is on the market which can measure suspended particles in the process stream by means of nephelometry. Nephelometry measures the scattered light intensity at a right angle to the path of illumination caused by suspended particles. This instrument may be useful in monitoring filtration efficacy.

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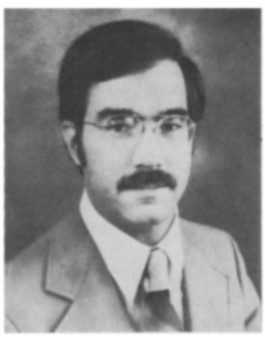

Analysis of Processed Soy Oil by Gas Chromatography

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ABSTRACT

Two basic gas chromatographic approaches are currently used for the analysis and characterization of processed soy oil and other lipid materials. One approach separates the intact glycerides after silylation whereas the complementary approach determines the fatty acid composition of the glycerides by the formation and subsequent separation of the corresponding fatty acid methyl esters. A brief history of the development of these gas chromatographic procedures and a more detailed discussion of current packed and capillary column technology used for the separation of both intact glycerides and fatty acid methyl esters is presented. Emphasis is placed on the advantages of the newly emerging capillary techniques over the more conventional packed column separations. High resolution chromatograms are shown for the separation of mixed glyceride isomers and also for the separation of unsaturated methyl esters with respect to the number, geometry and position of the double bonds. The separation of the methyl esters can provide information equivalent to classical *cis,cis*-lipoxygenase, iodine monochloride titration and infrared *trans* analyses. Finally, the use of short capillary columns to achieve rapid low cost separations (with resolution equivalent to packed column separations), ideal for quality control, also are discussed.

INTRODUCTION

Gas chromatography (GC) has become well established as a powerful tool in lipid research. The high resolving power, speed of analyses, sensitivity and adaptability to automation make GC a highly desirable technique where it is applicable. Two basic gas chromatographic approaches are currently used for the analysis and characterization of processed soy oil. One approach separates the intact glycerides after silylation to provide what will be defined as a carbon number profile (CNP) whereas the complementary approach determines the fatty acid composition (FAC) of the glycerides by the formation and subsequent separation of the corresponding methyl esters.

The fatty acid composition method, the older and more commonly used of these two methods, involves the transesterification of the triglycerides with sodium methoxide. The resulting methyl esters are suitable for chromatography. The quantitative data sometimes can be used to identify the source oil, provided that it has not been altered by hydrogenation or other processing beyond refining, bleaching and deodorization. The method is now well established and accepted in the lipid industry.

The separation of intact triglycerides by GC has been viewed as a valuable complement to GC-FAC. This has been recognized for quite some time, as investigations were started in 1959 (1). Since that time, Litchfield (2) and Kuksis (3) have worked independently to establish the quantitative aspects of triglyceride analysis by GC. More recently, Monseigny et al. (4) have used capillary column GC for the quantitation of triglycerides; however, the columns used in their work are not commercially available. The analysis of triglycerides is more difficult than the analysis of the fatty acid methyl esters, generally because of their higher boiling points. Because of this difficulty, there has been a significant time lag in the development of the triglyceride method. The advances in equipment and the state-of-the-art now make this method routine. The separation of intact lipid samples provides a characteristic profile or distribution of glycerides which often can be used to identify a specific fat or mixture of fats. The method aids in interpreting data where ambiguities exist in FAC. It is especially valuable in that the results are not affected

when an oil has been hydrogenated.

This paper presents a detailed discussion of the current packed and capillary column technology used for the separation of both intact glycerides and fatty acid methyl esters. Emphasis is on the advantages of the newly emerging capillary technique over the more conventional, packed column separations. High resolution chromatograms are shown for the separation of the methyl esters with respect to the number, geometry and position of the double bonds. The separation of the methyl esters can provide information equivalent to classical *cis,cis*-lipoxygenase, iodine monochloride titration and infrared *trans* analyses. Finally, the use of short capillary columns to achieve rapid, low-cost separations (with resolution equal to or better than packed column separations) for quality control are discussed.

SEPARATION OF INTACT GLYCERIDES

General Principles and Terminology

The mechanism for separation is based on differences in boiling points. Because all of the components are similar in structure and chemical nature, the boiling point is largely determined by the molecular weight (MW). Thus, the retention time is primarily determined by the MW of the component.

Temperature programming allows the wide range of MW components to be eluted efficiently. The temperature is low initially and the higher MW components condense at the column head. As the temperature is increased, the components are vaporized at their effective boiling points and carried down the column. The efficiency of the separation is such that glyceride components differing by two carbon atoms can be totally resolved.

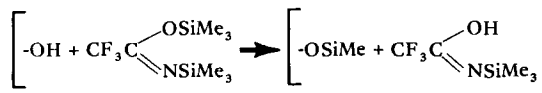
For convenience, an unofficial convention has been employed to describe the resolved components by referring to their carbon number. The term carbon number is defined as the total number of carbon atoms in all the acyl chains of the glyceride. The carbon atoms in the glycerol moiety are not counted, so the carbon number of the triglyceride would simply be equal to the sum of the carbon numbers of the three component fatty acids. An example would be tristearin, which is a triglyceride composed of three stearic acid groups and glycerol. Each stearic acid has 18 carbon atoms and thus the tristearin is referred to as a C54 triglyceride. Because the separation is by total carbon number, certain components are not resolved. For instance, C50 could be a triglyceride composed of two palmitic groups (C16) and one C18 fatty acid, regardless of unsaturation. Also, it would be possible to have C14, C18, C18 or any other combination which results in a total of 50 acyl carbon atoms. A second convention will be employed in this report to prevent possible ambiguities. Because a diglyceride and a triglyceride could both have the same carbon number, e.g., C18, C18 diglyceride and C12, C12, C12 triglyceride, a letter is used to designate the class of glyceride: A = acid; M = monoglyceride; D = diglyceride; T = triglyceride. Thus, tristearin can be referred to as C54T and distearin as C36D, for example. This method is called the GC-carbon number profile, or GC-CNP method, because it gives a fingerprint based on the resolution by carbon number. The chromatogram of a standard mixture shown in Figure 1 illustrates the separation. In practice, a C36T would have the same retention time as a C34D, but most oils analyzed have predominantly C16 and C18 acids.

When this is the case, the classes are cleanly separated and there is no difficulty interpreting the chromatogram. The classes of compounds are shown to elute in order of decreasing volatility, i.e., fatty acids first, then monoglycerides, diglycerides and triglycerides. The internal standard shown is tricaprin (C30T). The propylene glycol monoesters (PGME) are common emulsifiers; they usually are not found in processed soy oil. If present in a specific sample, they elute between the fatty acids and monoglycerides. The triglycerides, especially, illustrate the baseline separation by the two-carbon-number increment.

Derivatization

Derivatization is necessary for good chromatography of the fatty acids, PGME, mono- and diglycerides. The hydroxyl groups are prone to adsorption and generally cause tailing problems. A further bad side effect was noted with injections of underivatized mono- and diglycerides. The monoglycerides would give rise to additional peaks corresponding to the respective diglyceride and triglycerides. The diglycerides would produce additional peaks corresponding to triglycerides. After derivatization, the additional peaks disappeared. The conclusion is that the mono- and diglycerides decomposed and rearranged on the column or in the injection port to form the higher glycerides.

Derivatization of the hydroxyl groups on the mono- and diglycerides to form the corresponding trimethyl silyl (TMS) ethers prevents both tailing and the rearrangement reactions. The best derivatizing reagent investigated was BSTFA [(N,O)-bis (trimethyl silyl)] trifluoroacetamide which was found to react quantitatively within 15 min at 100 C. The reaction involved is:



The reaction products are the derivatized glyceride and N-(trimethylsilyl)trifluoroacetamide. N-Brimethylsilyl)trifluoroacetamide is very volatile and coelutes with glycerine and the solvent causing no interferences in the chromatogram. The derivatized glycerides were analyzed over a period of time and were found to be stable at room temperature for three days. They are thermally and chromatographically stable and do not give any evidence of decomposition on the column.

The fatty acids react with the BSTFA to form their respective TMS esters. Again, the tailing is reduced in the chromatography of the derivatives.

Packed Column Separation

Packed column chromatographic conditions. The chromatographic conditions for this method were established primarily for vegetable oils, such as palm, cottonseed and soybean oils. These conditions are listed in Table I but some of the conditions could be altered in order to analyze less common fats and oils.

While Table I gives a self-explanatory description of the chromatographic conditions, a brief explanation of the rationale behind the choice of these conditions is important if any alteration of conditions is to be considered.

All of the conditions were chosen to provide a good separation in a reasonable time period. Because of the high MW of the triglycerides, and the high temperatures required to elute them, it is desired to minimize the residence time of any component on the column in order to prevent decomposition. This has been done by resorting to low loadings of liquid phase, short column lengths, high flow rates and temperature programming.

The basic chromatograph is a Hewlett-Packard 5700A equipped with a flame ionization detector and on-column injection. The on-column feature is essential to minimize dead volume and insure that the sample is placed on the column in a small slug. Without the on-column injection,

the mono- and diglycerides were adequately separated but the component triglycerides showed poor resolution.

The choice of liquid phases was restricted by the chromatographic requirements. The phase had to be stable to 350 C with minimal column bleed during temperature programming. This limited the liquid phase selection to organo silicone polymers and carborane polymers, OV-1, OV-17, OV-101, SE-30, JXR and Dexsil 300. The phases tried were OV-17, SE-30, JXR and Dexsil 300. JXR is essentially the same as SE-30, in terms of McReynold's constants and performance, so SE-30 and JXR could be interchanged. The JXR in these studies gave consistently good separations and the best quantitative reproducibility. Problems with reproducibility were often encountered with the more polar OV-17 and Dexsil 300 liquid phases.

The liquid phase was a 3% loading which was conditioned by programming from 150 C to 350 C at 1 C/min at 50 ml/min flow and allowing it to remain at 350 C overnight. The low loading level still gives a good separation and allows the triglycerides to elute at an earlier retention time than with higher liquid phase levels.

The packing material was Gas Chrom Q (100-120 mesh) which is a diatomaceous earth, acid-washed to remove metal impurities, and silanized to reduce peak tailing. The dimensions of the stainless steel column are 1/8" × 18". The short column was again intended to minimize residence time of the glycerides and yet provide the required resolution.

The injection port and detector temperatures must be kept in excess of any column temperature in order to prevent condensation of the sample in either location. Both were set at 350 C. The initial column temperature is 140 C, which allows myristic acid to be separated from the solvent peak. This is the lower MW limitation of the method (∿200). Due to the wide distribution of MW, temperature programming is necessary to get a good separation and elute all the components as sharp, well defined, peaks. Generally, the C56 triglycerides are eluted when the temperature program reaches the final temperature of 340 C.

Quantitative analysis by packed column. Tricaprin was chosen as an internal standard because (a) it is similar to the components being analyzed since it is a triglyceride, (b) it is not present in most samples analyzed, (c) it does not interfere with other typical glyceride components, and (d) it has a retention time approximately midway in the analysis. The tricaprin peak is then assigned a relative retention time (RRT) of 1.00. Standards of other components are analyzed with the tricaprin internal standard and each component can be identified by its characteristic RRT.

TABLE I

Packed Column Chromatographic Conditions for Separation of Intact Glycerides

Gas chromatograph:	Hewlett-Packard 5700A with with FID and on-column injection	
Recorder:	Sargent Model SRL, 1 mV, 0.5 in./min	
Column:	1/8 in. × 18 in. stainless steel 3% JXR on 100/120 Gas Chrom Q	
Temperatures:	Injection port	350 C
	Detector	350 C
	Column	140-340 C @ 8 C/min 340 C for 8 min
Flow rates:	Carrier	50 ml/min He
	Hydrogen	∿50 ml/min
	Air	∿350 ml/min
Sample size:	∿20-40 mg sample in 1 ml BSTFA 2 μl injected	

A primary standard was prepared by weighing pure individual components. Each component was checked for purity by thin layer chromatography (TLC) and the GC method. Corrections were made when necessary for purity and contamination. This primary standard was used to establish response factors (to be discussed later) and to adjust any chromatographic conditions.

A secondary standard was prepared for the purpose of having an available, inexpensive, large volume of sample to work with. The standard was prepared by melting equal portions of a soybean shortening and Palm Duex in a 2-ℓ beaker. The sample was stirred well and then distributed into l-dram vials while still melted. Thus, small portions of this standard are available without causing any contamination to the bulk of the standard. The composition was established by using the response factors from the primary standard. The secondary standard is then available to be run daily to check the quantitation and separation. Resolution factors were calculated for all separations involved and they were all greater than 0.95, which is excellent for quantitation. If the resolution factor is 1.0 for two equal area peaks, it means that the resolution is 98% complete.

Areas arising from compounds are not directly proportional to the percent composition because different compounds have different detector responses. Therefore, it is necessary to determine response factors for each compound to be analyzed. The flame ionization detector (FID) response is dependent on the weight of a compound injected. The results of an analysis are then generally calculated as weight percents.

For analogous series of compounds, general trends are known to exist and thus response factors can be predicted. In the hydrogen flame detector, the plot of the ratio of weight percent area vs the carbon number for long chain fatty acid methyl esters approximates a horizontal line. Litchfield et al. (2) have pointed out that the molecule of tristearin has the same atomic composition and the same number of C-C, C-H and C-O bonds as one molecule of methyl stearate plus two molecules of methyl oleate. Thus, a plot for triglyceride response similar to that for the methyl esters should yield a corresponding trend. The assumptions needed are that all the injected material lands on the column and eventually reaches the detector intact.

The response factors were calculated according to the internal standard method.

$$RF_A = (wt_A)\,(area_{IS})/(wt_{IS})(area_A),$$

Where RF_A = response factor of component$_A$; wt_A = weight of component$_A$; wt_{IS} = weight of internal standard (tricaprin).

The response factors determined are the average values from several determinations of several individual standards and these results are listed in Table II. The relative retention time was shown to be related to the MW.

The relationship was not truly linear, but it was predictable enough in order to assign a response factor to a given retention time. Thus, material can be quantitated even though no standard has been run.

In theory, the FID response is proportional to the hydrocarbon content of each glyceride. The response factors of unsaturated triglycerides should approximately equal those of saturated molecules of the same carbon number. However, some workers have reported difficulties and have observed losses of high MW unsaturated glycerides due to thermal degradation. These losses are strongly influenced by the column, operation conditions and chromatograph configuration employed. We determined response factors for triolein and tristearin and found them to be within 2% of each other. Also, palm oil was quanti-

TABLE II

Relationships among Molecular Weight, RRT and RF

Component	MW	TMS-MW[a]	RRT	RF
C16A	256	328	0.114	.687
C16M	331	475	0.456	.740
C18M	359	503	0.585	.755
Int. std.	–	–	1.000	1.000
C32D	569	641	1,215	.795
C34D	597	669	1.306	.806
C36D	625	697	1,394	.818
C48T	807	807	1.769	.872
C50T	835	835	1.844	.888
C52T	864	864	1.905	.901
C54T	892	892	1.980	.912

[a]TMS-MW = molecular weight of the TMS derivative; RRT = relative retention time; RF = response factor.

TABLE III

Comparison of Response Factors for Triglycerides

Component	Number of double bonds	Litchfield (2)	Kuksis (3)	This lab
Tristearin	0	.912	.912	.912
Triolein	3	.862	.912	.923
Trilinolein	6	.921	.958	–
Trilinolenin	9	.937	–	–

TABLE IV

Statistical Evaluation of the Known Glyceride Mixture Analysis (N=8)

Component	Theor.	Mean (%)	Std. dev. (±)	Coeff. var. (±%)
C16A	5.5	5.3	0.1	1.9
C16M	5.5	5.6	0.2	3.6
C18M	5.5	5.9	0.3	5.0
C32D	5.6	5.6	0.1	1.8
C34D	5.6	5.7	0.2	1.8
C36D	5.6	5.5	0.2	1.8
C46T	–	0.2	–	–
C48T	17.1	16.2	0.2	1.2
C50T	17.0	17.3	0.3	1.7
C52T	16.1	15.3	0.4	2.6
C54T	16.4	15.9	0.2	1.3
C56T	–	0.3	–	–

TABLE V

Statistical Evaluation of the Working Glyceride Mixture Analysis (N=8)

Component	Mean (%)	Std. dev. (±)	Coeff. var. (±%)
C16A	0.2	–	–
C18A	0.3	–	–
C16M	8.2	0.1	1.2
C18M	10.7	0.1	0.9
C32D	4.4	0.1	2.3
C34D	10.6	0.2	1.9
C36D	8.5	0.2	2.4
C46T	0.1	–	–
C48T	1.2	0.02	1.6
C50T	5.5	0.2	3.6
C52T	16.8	0.2	1.2
C54T	30.3	0.5	1.7
C56T	1.4	–	–
C58T	0.3	–	–
Other	1.3	0.1	7.7

tated before and after hydrogenation and the results agreed well within the standard deviation. Some values are available in the literature and these have been normalized to our value of 0.912 as the response factor for tristearin for comparison purposes (Table III). The average response factor for the nine determinations was 0.912 ± .027.

The overall coefficient of variation for quantitating the individual components is ∿3%. The response factors for the corresponding saturated and unsaturated triglycerides vary within these limits, and for all practical purposes are the same. Thus, quantitation is unaffected by degree of unsaturation.

A 10-component mixture of glycerides was prepared by weighing pure standard materials and the internal standard into a volumetric flask and diluting to volume with CH_2Cl_2. A fresh aliquot was taken daily, the CH_2Cl_2 was evaporated off, and the sample was analyzed according to the GC-CNP procedure. Eight determinations were tabulated over a two-week period to provide the statistical data in order to establish the accuracy and reproducibility of the method. These data were generated to include variations in sample preparation, chromatography and general day-to-day variations. The results of the statistical evaluation are in Table IV. The reproducibility, in general, is very good with the coefficient of variation typically ∿2.5%.

The secondary standard that was prepared was determined by normalization in the same manner as any glyceride sample. This was determined daily over a two-week period to establish its composition and statistical variation (Table V). Once again, the reproducibility was very respectable.

The average quantitative results obtained from eight separate determinations on a typical processed soybean oil are reported in Table VI. The data shows soybean oil is composed primarily of C52T and C54T components.

Interferences. The interferences in this method occur when two different types of components have the same relative retention time. A good example would be a sample which contains a mixture of palm oil and coconut oil. The low-MW triglycerides of coconut oil coelute with the internal standard and the diglycerides from the palm oil. While the fingerprint chromatogram clearly indicates the presence of coconut oil, exact quantitation is not possible. In general, a diglyceride elutes at a carbon number which is about two units lower than the corresponding triglyceride. For instance, a C36D and a C38T have the same retention time. Unidentified components are assigned response factors as a function of their RRT, quantitated, and summed into a class labeled "other."

Very few other problems have been encountered with

TABLE VI

CNP of a Typical Processed Soybean Oil (N=8)

Component	Mean (%)	Std. dev. (±)
C16A	–	–
C18A	–	–
C16M	–	–
C18M	–	–
C32D	0.3	–
C34D	1.6	–
C36D	0.7	–
C46T	0.2	0.07
C48T	0.3	0.09
C50T	2.9	0.15
C52T	25.4	0.54
C54T	63.6	0.87
C56T	2.9	0.30
C58T	1.0	0.12

glyceride samples and fats extracted from common food products.

Comparative studies using packed column method. The GC-CNP method is a very versatile and informative method. In a single analysis, results can be obtained for fatty acids, mono-, di- and triglycerides, and PGME. Prior to the development of this method, separate methods existed for the determination of these components. To establish the accuracy of the GC-CNP determinations the following methods were compared with GC-CNP: (a) mono-, di-, triglycerides by column chromatography (CC-MDT); (b) monoglycerides by periodic acid titration.

CC-MDT vs GC-CNP. The method previously used to analyze for mono-, di- and triglycerides was a column chromatographic (CC) separation followed by a gravimetric determination of the column fractions (5). A series of samples was analyzed by both the CC-MDT method and the GC-CNP method to show a correlation between the results.

To verify the composition of the column chromatographic fractions, each fraction was collected and analyzed by the GC-CNP method. It was found that cross contamination had occurred, e.g., the diglyceride fraction contained both mono- and triglycerides, besides the diglycerides. The GC-CNP analysis of the fractions was used to mathematically correct each column fraction to its actual composition. The corrected values were then compared to the GC-CNP results.

Table VII shows the comparison of 16 samples of processed soybean oil. The correlation is excellent. On the average, mono- and diglyceride results agree remarkably well. The only discrepancy arises from the inability of the CC-MDT method to measure fatty acids or nonglyceride components. The GC-CNP can measure these materials and the difference is reflected in the triglyceride value.

Because of its higher mono- and diglyceride content, the GC-CNP standard magnified the differences in values obtained between the CC and GC methods. The data are shown in Table VIII. Once again, the individual CC fractions were not completely resolved as shown by the GC analyses.

When the CC analysis is corrected for the carry-over that occurs, the GC and CC results agree. The mathematical correction verifies the accuracy of the GC-CNP method while confirming a determinant error in the CC method. These comparative studies show that the GC-CNP method offers a significant improvement over the CC method. In addition to this, there are other general considerations that should be mentioned.

The advantages of GC-CNP are: quantitation of free fatty acids; constant monitoring of the analysis; detection of anomalies in a given sample; analysis for any level of mono-, di- and triglyceride (the column method requires different conditions for mono levels > 5%); reporting of results as relative %, or absolute %; quantitation of "non-normal" material by difference; quantitation of samples with the aid of an interface to a computer system; identification and quantitation of individual glyceride components by carbon number; elimination of increasingly hard to obtain solvents and column packing materials required for CC; lower sample size requirements (∿40 mg vs 3 g); shorter turnaround time (45 min vs 8 hr); greater daily output.

CNP vs periodic acid titration. The periodic acid titration (6) is an established method for the determination of 1-monoglycerides. Therefore, it was a good method to use

TABLE VII

Comparison of CC-MDT and GC-CNP Analyses

	Mono (%)		Di (%)		Tri (%)		Other (%)	
Sample no.	CC	TC	CC	TC	CC	TC	FA	Unident.
1	1.8	1.9	5.7	6.0	92.9	90.6	0.1	1.4
2	1.4	1.5	5.0	5.2	93.5	92.5	–	0.8
3	1.7	1.7	6.5	5.6	92.0	91.3	0.1	1.3
4	1.6	1.7	5.1	5.4	93.4	92.6	0.1	0.2
5	2.2	2.2	6.2	6.3	91.3	91.0	0.1	0.4
6	1.9	1.8	5.6	5.8	92.6	90.2	–	0.6
7	1.6	1.7	6.8	7.0	91.7	90.1	–	1.2
8	2.0	2.4	6.5	6.4	91.2	90.5	0.1	0.6
9	1.8	1.8	5.3	5.5	93.0	90.9	–	1.8
10	1.5	1.6	4.9	5.2	93.7	91.7	0.1	1.4
11	1.5	1.5	4.9	5.1	93.6	91.6	0.1	1.6
12	1.8	1.9	5.4	5.5	92.9	90.7	0.1	1.8
13	1.5	1.5	4.8	6.4	93.9	90.1	0.1	1.9
14	1.9	1.8	5.8	6.0	92.5	90.3	0.1	1.8
15	1.9	1.8	5.6	5.8	92.6	90.2	0.1	2.1
16	1.8	1.9	6.3	5.6	92.0	90.3	0.1	2.1
Average	1.7	1.7	5.4	5.6	93.0	91.3	0.1	1.3

[a]These components cannot be measured by GC.

TABLE VIII

GC-CNP of CC-MDT Fractions

Standard	CC (%)	Mono (%)	Di (%)	Tri (%)	Corr. CC (%)	GC-CNP (%)
FA	–	0.2	1.0	0.5	0.5	0.5
Mono	15.0	96.3	19.9	0.2	17.6	18.9
Di	14.9	0.4	75.6	17.9	23.8	23.5
Tri	69.4	0.6	1.6	80.5	56.2	55.9
Other	–	2.5	1.9	0.9	1.9	1.3

as an independent check on the determination of monoglycerides by GC-CNP. A total of 10 samples was compared by the two methods (Table IX). Eight of the samples were soybean shortening which yielded an average value of 1.66% mono- by titration 1.7% mono- by GC-CNP. The GC-CNP secondary standard gave comparable results on samples with higher monoglyceride levels. The accuracy of the GC method is confirmed by the monoglyceride titration. Because the periodic acid titration does not determine 2-monoglycerides, values obtained by GC-CNP may be larger for oils high in 2-monoglycerides.

TABLE IX

Comparison of Monoglyceride Determination by GC-CNP vs Periodic Titration

	Monoglyceride (%)	
	Titration	GC-CNP
Soybean shortening		
1	1.63	1.7
2	1.66	1.7
3	1.65	1.6
4	1.64	1.8
5	1.54	1.6
6	1.83	2.1
7	1.67	1.7
8	1.65	1.7
CNP standard		
1	19.10	19.0
2	19.10	19.0

Capillary Column Separation of Glycerides

Background. The concept of high resolution using capillary columns was first introduced shortly after the introduction of GC in the late 1950s; its theory was well developed long before its widespread practical use which began around 1975.

Many technical difficulties plagued the early investigators of the technique, and many years were spent learning to manufacture, deactivate and coat glass capillary columns. Early laboratory-made columns were fragile, thermally unstable, and had highly irreproducible coating efficiencies from column to column. Early instrumentation also was poorly designed. Injection ports for delivering small amounts of analyte on the column were designed along with reduced dead volume detectors and column fittings. During the 1960s and early 70s, most of these problems were gradually solved. Today, capillary columns are commercially available which are stable, reproducible and reasonably affordable. New instrumentation allows effective use of these columns and modern electronics permits integration of the rapidly eluting components.

We will now turn our discussion to the implementation and application of these recent advances for the rapid separation of intact mixed glycerides. The improvements presented herein allow what previously was a week's worth of separations to be completed in a day, leaving the state-of-the-art equipment available for nonroutine problem solving.

Capillary instrument selection and description. The instrument selected for capillary work in our laboratory is the Hewlett-Packard Model 5880A gas chromatograph which was selected for various reasons. The inlet system (injection port) is highly flexible and can be used in the split, splitless and regular injection modes. It also can be adapted with a special, inexpensive liner and isolation valve to allow direct injections on capillary columns. The work presented here was performed in the split mode. The inlet system consists of a pressure regulator located downstream from the column inlet to eliminate lubricant contamination from the regulator's diaphragm device. The column inlet pressure is controlled with the regulator and is independent of the flow controller used to adjust the split vent flow. Contamination from septum volatiles is reduced by a slow septum purge controlled by a needle valve and appropriate traps are used to filter the carrier gas. The split liner contains a small amount of packing (coated with a methyl silicone phase) to provide good thermal mixing of the vaporized sample before splitting, thus minimizing discrimination effects.

The column oven is well controlled and highly reproducible in the temperature programming mode. Cooling capability of the oven is unmatched, being able to cool from 200 to 100 C in 65 sec. The microprocessor controlled printer/plotter allows one to plot the oven temperature and is used to check the performance of the oven. This feature is unique to Hewlett-Packard gas chromatographs, and is useful when working under FDA Good Laboratory Practices (GLP) guidelines. Inlet system and detector temperatures also can be plotted.

The detector design has minimal dead volume, high sensitivity, and a fast signal output of 40 Hz. Detector dead volume can be totally eliminated by inserting the end of the column up the jet to within 1-2 mm of the flame.

Special column fittings totally eliminate dead volume at connections and use either convenient silicone rubber O-rings or graphite furrels for work above 250 C.

The chromatograph has a built-in analog to digital convertor with adjustable data acquisition rates for accurate output of fast peaks to the data system. The Hewlett-Packard 3351B data system links directly to the chromatograph for integration and data processing. The HP-33513 has 32K memory and can integrate data from 15 instruments simultaneously. BASIC programming can be used for additional calculations and data formating, and two terminals with high-speed thermal printers provide rapid notebook-size reports.

Rapid separation of glycerides. The previously used conventional, packed column with on-column injection provided good separation and accurate results, but required 35-40 min, as seen Figure 1. The degree of resolution is readily judged by observing the separation in the diglyceride region. A rapid separation on a short capillary column of

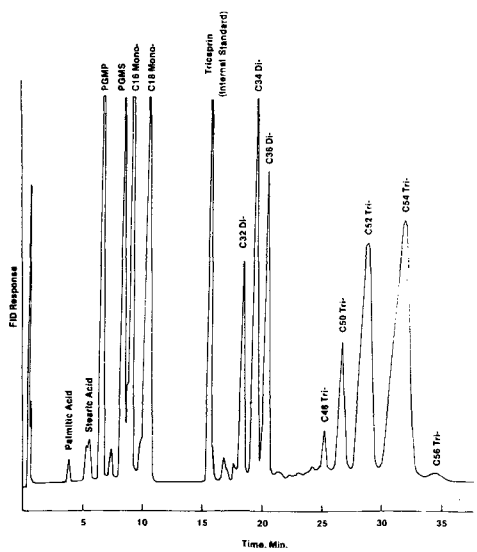


FIG. 1. Gas chromatographic separation of free fatty acids, mono-, di- and triglycerides using a 1/8" X 1.5', SS, 3% OV-101 on 80/100 Gas Chrom Q packed column.

the same standard mixture is presented for comparison in Figure 2. Baseline separation of the diglycerides and even partial separation of the positional isomers is achieved in less than 10 min using a rapid temperature programming rate of 20 C/min.

The capillary column used for the rapid CNP separation is coated with CP-SIL 5, a high temperature, nonpolar methyl silicone phase. It is the most thermally stable commercial column available with an upper temperature limit of 355 C. This column may also be used with a slow temperature program rate (ca. 2-4 C/min) to baseline resolve the mono- and diglyceride positional isomers.

Using the conditions listed in Table X, the secondary reference standard was determined seven times (Table XI). Excellent agreement between the known and determined values was obtained while precision was significantly improved over the packed column separation, as shown in Table XII. The rapid separation of a typical processed soybean oil is shown in Figure 3. The quantitative results in Table XIII confirm the identity of the source oil to be soybean (compare to Table VI).

SEPARATION OF DERIVED FATTY ACID METHYL ESTERS

Background

Numerous separations are currently in use for the analysis of fatty acid methyl esters derived from soybean oil and other lipids. The most frequently employed method is based on the separation of the esters according to the carbon number and degree of unsaturation using packed columns (7). Various liquid phases, such as DEGS, DEGS-PS, EGS, EGSS-X, BDS (Butane Diol Succinate) EGA (ethylene glycol adipate), Silar 5CP, Silar 10C, and FFAP (Carbowax) are commonly used to achieve such a separation. Columns packed with more polar cyano silicone stationary phases, such as SP-2340 and OV-275, allows better resolution of the fatty acid esters differing in the number and geometry of unsaturated groups (8,9). More recently (10), when a phase such as SP-2340 is coated on a 100-m glass capillary, fatty acid methyl esters of unsaturated fatty acids are resolved based on the position of the double bonds along the acyl chain.

The remainder of this paper reviews these separations as they apply to processed soy oil and extends the discussion to the use of efficient capillary columns to achieve equivalent separations in rapid fashion.

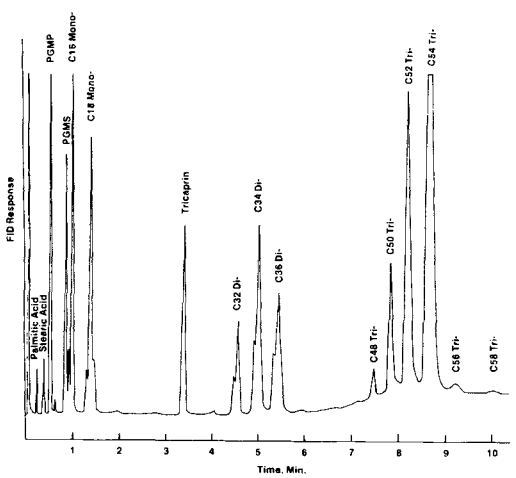


FIG. 2. Rapid gas chromatographic separation of free fatty acids, mono-, di- and triglycerides using a 0.5 mm × 6.0 m, W.C.O.T. CP[tm] Sil 5 glass capillary column.

TABLE X

Capillary Column Chromatographic Conditions for Separation of Intact Glycerides

Gas chromatograph: Hewlett-Packard 5880A		
Column: 0.5 mm × 6 m, glass W.C.O.T., CP Sil 5		
Temperatures:	Injection port	360 A
	Detector	360 C
	Column	200-355 C @ 20 C/min 355 C for 4 min
Flow rates and pressures:		
	Column inlet pressure	7 psi
	Split vent	200 ml/min
	Septum purge	2 ml/min
	Make-up carrier	30 ml/min
	Hydrogen	30 ml/min
	Air	40 ml/min
Sample size:	∿20-40 mg sample in 1 ml BSTFA 5 μl injected	

TABLE XI

Statistical Analysis of Secondary Reference CNP Standard on 6-m Glass Capillary Column

Component	Known (%)	Mean (%; n=7)	Std. dev.
C16A	0.2	0.2	0.0
C18A	0.5	0.5	0.0
PGMP	5.6	5.7	0.7
PGMS	5.6	5.7	0.6
C16M	7.3	7.3	0.8
C18M	9.5	9.6	1.1
C32D	3.9	4.0	0.2
C34D	9.0	9.1	0.4
C36D	7.6	7.5	0.4
C46T	0.1	0.1	0.0
C48T	1.1	1.1	0.1
C50T	4.9	4.9	0.2
C52T	14.9	14.8	0.7
C54T	26.9	26.7	1.4
C56T	1.3	1.1	0.1
C58T	0.3	0.2	0.1

TABLE XII

Separation of Intact Glycerides: Comparison of Precision Data for Packed vs Capillary Columns

	Relative Standard Deviation (%)[a]	
Component	Packed (n=8)	Capillary (n=7)
C16A	–	–
C18A	–	–
PGMP	35.7	12.5
PGMS	58.9	10.7
C16M	16.4	11.0
C18M	9.5	11.6
C32D	60.0	5.1
C34D	21.1	4.4
C36D	31.6	5.3
C46T	–	–
C48T	145.5	9.1
C50T	73.5	4.1
C52T	8.1	4.7
C54T	6.3	5.2
C56T	–	7.7
C58T	–	33.3

[a]See Table XI for known component concentrations.

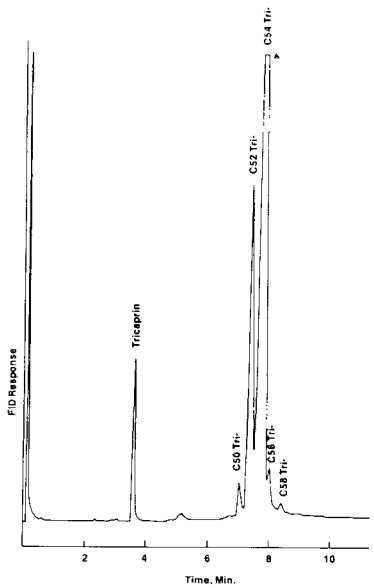


FIG. 3. Rapid chromatographic separation of typical processed soybean oil glycerides using a 0.5 mm × 6 m, W.C.O.T. CP^{tm} Sil 5 glass capillary column.

Derivatization

The gas chromatographic analysis of fats and oils is most easily accomplished if the fatty acids of the glyceride are converted to methyl esters. Although refluxing with sulfuric acid/methanol will convert almost any fatty material to its methyl ester, the procedure is cumbersome and time-consuming. The method of Radin et al. (11) using sodium methoxide reagent will perform the same task for most samples and is rapid and simple to use. This sodium methoxide procedure relies on the principle of transesterification to redistribute the fatty acid groups in relation to the concentration of the alcohols present.

Separation by Carbon Number and Degree of Unsaturation

Figure 4 shows the classical separation obtained on a standard mixture of methyl esters using a packed column. The esters are not separated with respect to double bond geometry; however, the separation is quite good between saturated and unsaturated esters as observed in the degree of separation between C-18 and C-18:1. The time required for the packed column separation is ca. 20 min and can be significantly reduced to less than 5 min using a short capillary column, as depicted in Figure 5. This glass capillary column is coated with Carbowax 20M and likewise does not separate the geometrical isomers. However, resolution between C-18 and -18:1 peaks is equivalent to that obtained on the packed column. Results using a standard mixture of methyl esters on both packed and short capillary columns are compared in Table XIV. Precision of the capillary results is greatly improved, as seen in the data presented.

Carbowax 20M may also be coated on fused silica capillary columns, but without the same degree of coating efficiency obtained on glass. A 12-m fused silica column is therefore required to separate the methyl esters in 8 min, as shown in Figure 6. Although the Carbowax 20M fused silica capillary column is not as efficient as the glass capillary, the separation time is still considered rapid. Fused silica columns are difficult to break and easily installed into the instrument. Coating efficiencies on fused silica surfaces will undoubtedly improve in the future, as much effort is

TABLE XIII

CNP of Typical Processed Soybean Oil Obtained on Short Capillary Column

Component	Value (%)
C16A	–
C18A	–
C16M	–
C18M	–
C32D	–
C34D	–
C36D	0.9
C46T	–
C48T	0.3
C50T	2.4
C52T	24.1
C54T	68.2
C56T	2.6
C58T	0.5

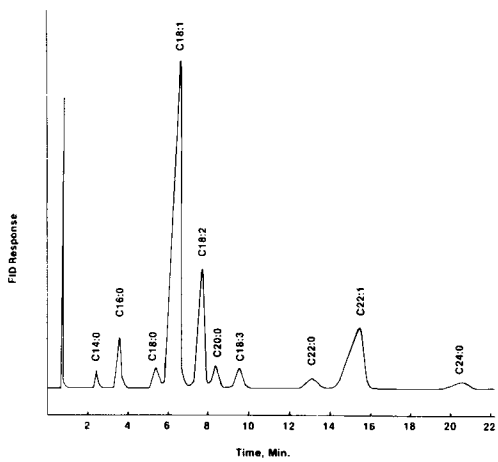


FIG. 4. Classical separation of standard fatty acid methyl esters using a 1/4" × 10', A1, 10% DEGS-PS on Gas Chrom Q packed column.

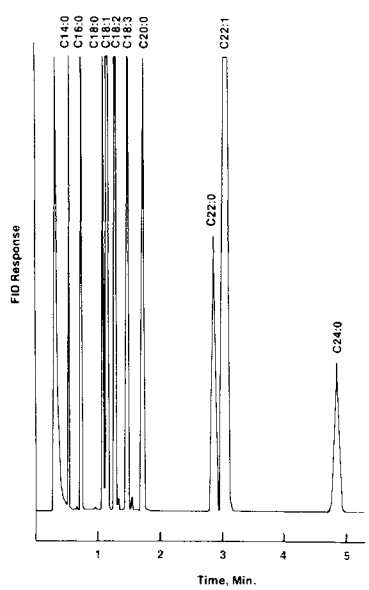


FIG. 5. Rapid separation of standard fatty acid methyl esters using a 0.25 mm × 5 m, W.C.O.T. Carbowax 20M glass capillary column.

TABLE XIV

Packed vs Capillary Separation of Fatty Acid Methyl Esters

Component	Concentration (%)			Retention time (min)	
	Known	Capillary (n=6)	Packed (n=6)	Capillary[a]	Packed[b]
C-14	1.0	1.1 ± 0.1	0.9 ± 0.05	0.47 ± 0.01	2.41 ± 0.01
C-16	4.0	4.0 ± 0.2	3.9 ± 3.4	0.67 ± 0.01	3.52 ± 0.02
C-18	3.0	2.9 ± 0.1	3.0 ± 0.06	1.03 ± 0.01	5.38 ± 0.03
18:1	45.0	44.5 ± 0.5	45.4 ± 0.41	1.09 ± 0.01	6.46 ± 0.05
18:2	15.0	16.2 ± 0.1	15.3 ± 0.25	1.21 ± 0.01	7.62 ± 0.05
C-20	3.0	3.2 ± 0.1	3.4 ± 0.20	1.40 ± 0.01	8.31 ± 0.06
18:3	3.0	2.9 ± 0.1	3.0 ± 0.14	1.66 ± 0.01	9.48 ± 0.07
C-22	3.0	2.9 ± 0.1	2.9 ± 0.22	2.79 ± 0.01	13.01 ± 0.10
22:1	20.0	19.3 ± 0.6	19.3 ± 0.36	2.97 ± 0.02	15.43 ± 0.12
C-24	3.0	2.8 ± 0.1	2.7 ± 0.18	4.78 ± 0.02	20.43 ± 0.16

[a]Injections made manually.
[b]Injections made by auto sampler.

being made in this direction.

Chromatograms presented in Figure 7 compare the separation of fatty acid methyl esters derived from a processed soybean oil on both packed and capillary columns. Chromatographic conditions for both separations are given in Table XV.

The rapid capillary separation by carbon number and degree of unsaturation provides information which is usually adequate for most plant quality control purposes. An auto injector, coupled with the speed of this separation, enables unattended analysis at a rate of 12 samples/hr.

Separation by Geometry

A 1/8" × 20' stainless steel column packed with 15% OV-275 on Chromosorb P-AW (DMCS) from Supelco, Inc., was used in the gas chromatography of the methyl esters derived from vegetable oils and shortenings. This GC column separates the methyl esters according to the number of carbons in the fatty acid chain, the number of unsaturated carbon-carbon bonds, and the geometry (*cis/trans*) of the unsaturated carbon-carbon bonds. With this type of separation, it is possible to obtain the same information as that from the separate GC-FAC, *cis-cis* lipoxygenase (polyunsaturated fatty acids or PUFA), iodine monochloride titration (IV) and IR-*trans* analyses presently being used.

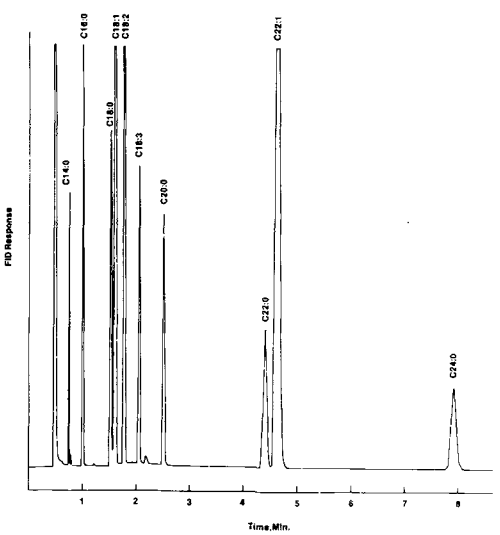


FIG. 6. Separation of standard fatty acid methyl esters using a 0.21 mm × 12 m, W.C.O.T. Carbowax 20M fused silica capillary column.

The chromatographic conditions for the separation are listed in Table XV. An example of the separation in the methyl esters prepared from a typical processed soybean oil is shown in Figure 8. The time required for the separation is ca. 35 min.

The GC-derived PUFA values from this study were calculated by summing the percentages of the methyl linoleate (18:2) and methyl linolenate (18:3). The comparison of many of these GC values, with the corresponding values obtained from the lipoxygenase method (12), are given in Table XVI. The results for six processed soybean oils and five soybean shortenings are tabulated and the average relative differences between the two methods are listed. The average difference between the GC-PUFA and lipoxygenase PUFA results are less than the combined standard deviations of the two methods; therefore, the GC-PUFA and lipoxygenase PUFA results are virtually identical. The reproducibility of the GC method for PUFA was determined by repeated measurements of the PUFA levels in Crisco and Puritan Oils. The reproducibility of the lipoxygenase PUFA method at the 68% PUFA level is ± 0.74 or 1.1 rsd. Therefore, the GC method shows a slight improvement in precision over the enzymatic method.

The chemical iodine value (IV) is a measure of the level of unsaturation. The results from this GC separation method may also give a measure of the level of unsaturation. One may calculate a pseudo IV number from the GC results. This approach has one major advantage over the present IV method (13), in that the GC method is more

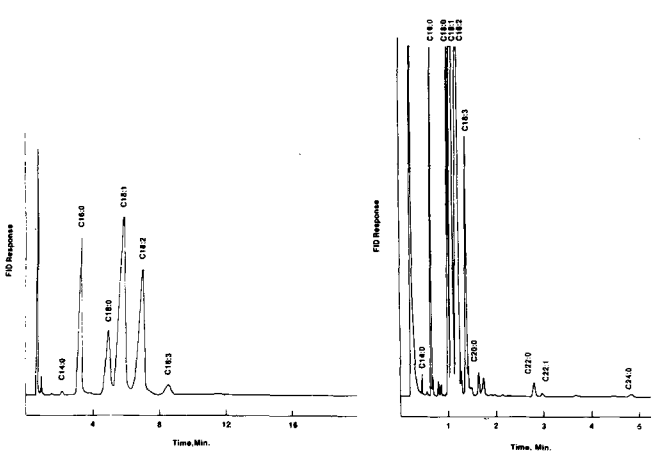


FIG. 7. Comparative chromatograms of derived fatty acid methyl esters from a typical processed soybean oil: (a) classical packed column separation; (b) rapid short capillary separation.

TABLE XV

Chromatographic Conditions for Classical Packed Column Separation of Methyl Esters

Instrument:	HP-5700 TCD
Column:	10' X 1/4"-A1, 10% DEGS-PS on Gas Chrom Q
Injection port temp:	300 C
Detector temp:	300 C
Oven temp (isothermal):	215 C
Carrier flow rate (He):	60 ml/min

Conditions for Rapid Separation of Methyl Esters

Instrument:	HP-5880A
Column:	5 m X 0.25 glass W.C.O.T. Carbowax 20M
Injection port temp:	300 C
Detector temp:	300 C
Oven temp (isothermal):	220 C
Column inlet pressure:	4 psi
Split vent flow rate:	200 ml/min
Aux. carrier flow rate:	30 ml/min
Hydrogen flow rate:	30 ml/min
Air flow rate:	400 ml/min

Conditions for Geometrical Separation of Methyl Esters by Packed Column

Instrument:	HP-5700
Column:	20' X 1/8", SS, 15% OV-275 on Chrom P-AW (DMCS) 100/120 mesh
Injection port temp:	275 C
Detector temp:	275 C
Initial oven temp:	220 C
Initial oven hold time:	32 min
Oven program rate:	32 C/min
Final oven temp:	230 C
Final oven hold time:	16 min
Column flow rate:	15 ml/min
Hydrogen flow rate:	30 ml/min
Air flow rate:	350 ml/min

Conditions for Rapid Separation of Methyl Ester Geometrical Iosmers

Instrument:	HP-5880A
Column:	27 m X 0.25 mm glass W.C.O.T. Silar 10C
Injection port temp:	270 C
Detector temp:	270 C
Oven temp (isothermal):	180 C
Column inlet pressure:	18 psi
Split vent flow rate:	100 ml/min
Aux. carrier flow rate:	30 ml/min
Hydrogen flow rate:	30 ml/min
Air flow rate:	400 ml/min

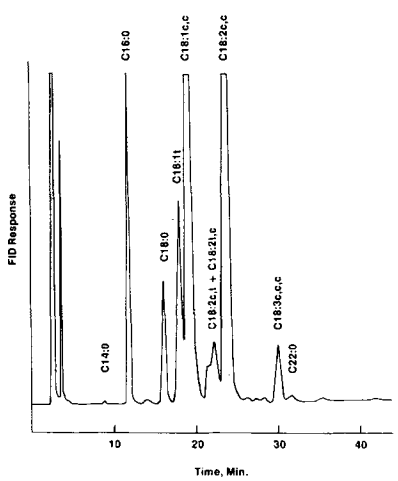


FIG. 8. Chromatogram illustrating the separation of *cis,trans* unsaturated fatty acid methyl esters derived from a typical processed soybean oil using a 1/8" X 20', SS, 15% OV-275 on 100/120 Chrom P-AW (DMCS) packed column.

TABLE XVI

Comparison of GC-PUFA and Lipoxygenase PUFA Results

Soybean oils	GC-PUFA (%)	PUFA-Lipoxygenase (%)
1	33.4	33.9
2	34.7	35.0
3	34.7	35.5
4	44.8	44.2
5	43.0	42.0
6	43.8	45.9
Shortenings		
1	25.6	25.8
2	22.6	22.0
3	15.3	14.8
4	20.6	20.0
5	6.1	7.1

Comparison of GC-IV with Chemical IV Numbers

Soybean oils	GC-IV (%)	Chemical IV (%)
1	109.2	112.3
2	110.0	112.4
3	110.0	113.1
4	115.2	118.2
5	116.2	118.6
6	112.3	116.5
7	116.1	117.2
8	115.4	118.1
Shortenings		
1	89.2	90.2
2	83.5	83.5
3	92.5	94.5
4	90.0	93.6
5	52.8	54.2
6	90.4	90.4
7	87.8	89.7
8	86.7	92.9
9	88.4	89.7
10	51.6	57.1

Comparison of GC-*trans* and Infrared-*trans* Methods

Shortening	GC-*trans* (%)	Infrared-*trans* (%)
1	17.5	17.1
2	19.5	19.6
3	31.4	32.2
4	22.6	22.4
5	11.1	12.1

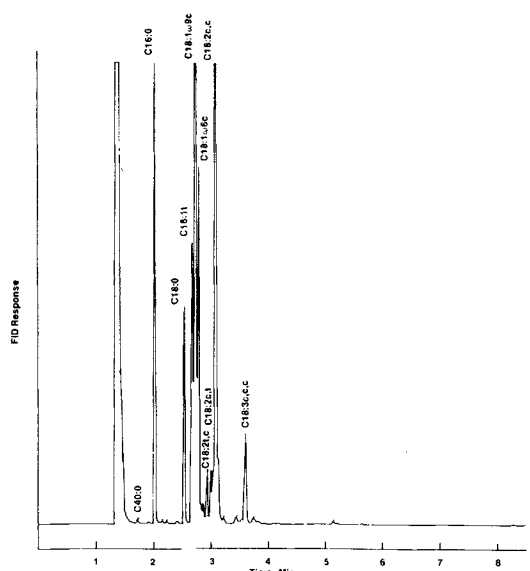


FIG. 9. Rapid separation of *cis,trans* unsaturated fatty acid methyl esters derived from a typical processed soybean oil using a 0.24 mm X 27 m, W.C.O.T. Silar 10C glass capillary column.

selective in its measurement of carbon-carbon unsaturation. The highly reactive nature of iodine monochloride (used in IV measurement by titrimetry) can cause a high, reproducible bias due to its reaction with functional groups other than the carbon-carbon double bonds. The results of this study indicate that this bias is only a severe problem when dealing with samples such as coffee oil extracts, which contain significant amounts of nonlipid material. Table XVI, which contains these data, shows that the agreement between the two methods is quite good. Differences between the chemical and GC-IV measurements are ∿3 IV units, in most cases. It should be noted, however, that there was a fairly reproducible difference of 2-4 IV units between GC and titrimetric measurements for soybean oils and shortenings in this study. The difference in IV results for soybean oils and shortenings from this work may be due to the nonspecificity of iodine monochloride in the chemical IV method, but we have no direct evidence to support this hypothesis. The comparison of GC-*trans* and IR-*trans* results for five shortenings is given in Table XVI. We are presently investigating the application of the GC method to the measurement of low *trans* samples.

Rapid Geometrical Separation

Figure 9 shows the best separation of soybean oil fatty acid methyl esters to date. Standard methyl esters can be separated isothermally (Table XV) using a 27-m glass Silar 10C glass capillary column on the basis of carbon number, degree and geometry of unsaturation in only 8 min. Accuracy and precision data (shown in Table XVII) are good using a fast data acquisition rate of readings/sec. Comparative studies against the classical GC-FAC, PUFA, IV, and *trans* methods are currently in progress. This separation is of particular interest because the total analysis time required to generate the above information by the classical methods is ca. 2.5 hr.

TABLE XVII

Separation of Fatty Acid Methyl Esters on 10-m Silar 10C Capillary Column: Accuracy and Precision Data

Component	Known conc. (%)	Mean conc. found (n=16) (%)
C-14	1.0	1.0 ± 0.1
C-16	4.0	4.1 ± 0.2
C-18	3.0	3.0 ± 0.1
18:1*t*	1.0	1.1 ± 0.1
18:1*c*	44.0	44.1 ± 0.5
18:2*c,c*	15.0	15.1 ± 0.1
C-20	3.0	3.0 ± 0.1
18:3*c,c,c*	3.0	3.0 ± 0.1
C-22	3.0	2.9 ± 0.1
22:1*t*	1.2	1.2 ± 0.1
22:1*c*	18.8	18.6 ± 0.6
C-24	3.0	3.0 ± 0.1

Separation by Geometry and Position

The separation of fatty acid methyl esters with respect to carbon number, degree of unsaturation, double bond

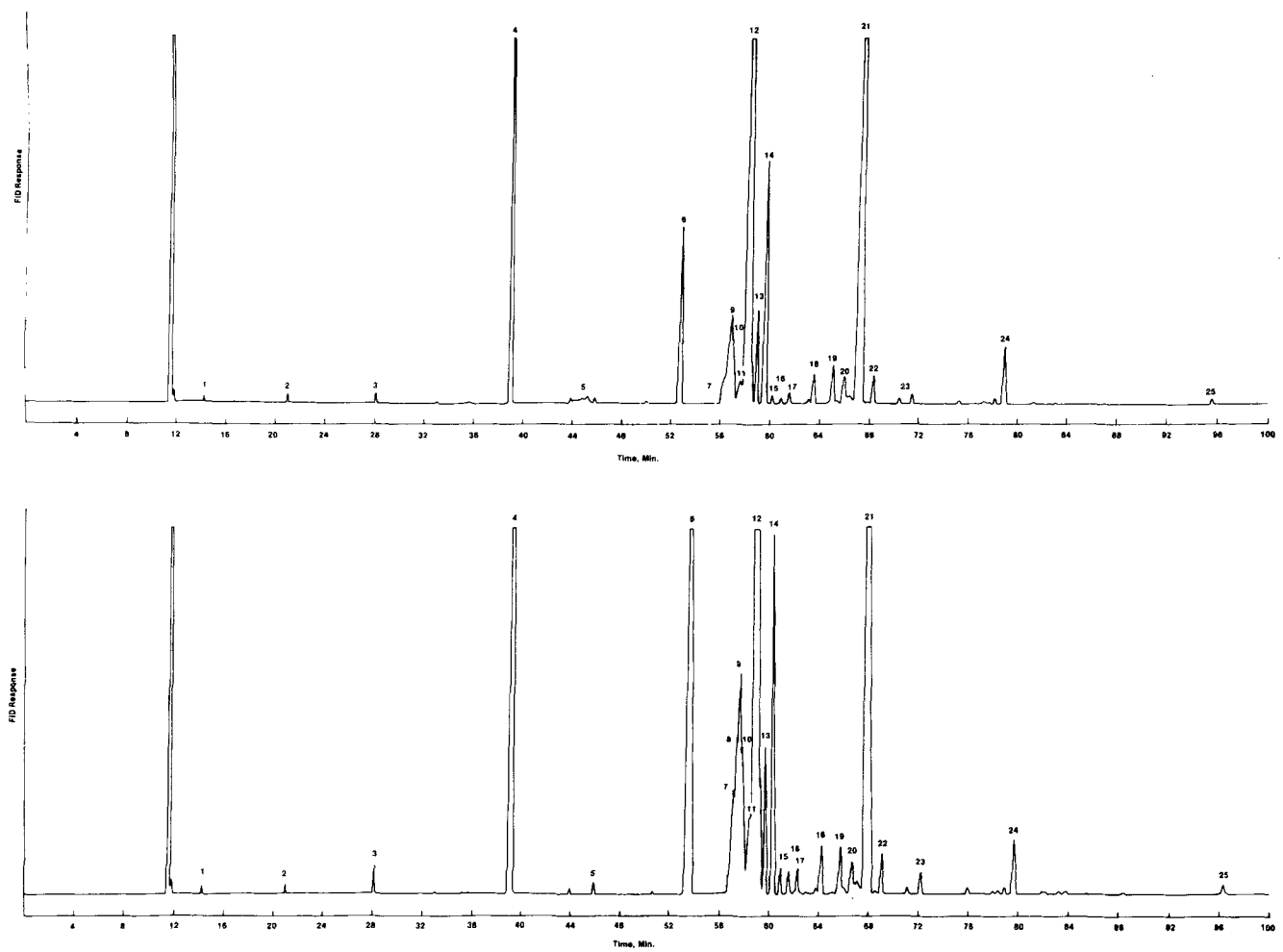


FIG. 10. Chromatograms illustrating the separation of derived fatty acid methyl esters with respect to double bond geometry and chain position using a 0.25 mm × 100 m, W.C.O.T. SP-2340 glass capillary column: (a) typical processed soybean oil; (b) shortening.

TABLE XVIII

Chromatographic Conditions and Peak Identities for Chromatograms Illustrated in Figure 10

Chromatographic conditions		Peak code	
		Peak no.	Identity
Instrument:	HP-5880A	1	10:0
Column:	100 m × 0.25 mm Glass W.C.O.T. SP-2340	2	12:0
Injection port temp:	300 C	3	14:0
Oven temp profile		4	16:0
Initial value:	150 C	5	17:0
Initial time:	0.00 min	6	18:0
Level 1		7	18:1ω10*t*
Program rate	0.50 C/min	8	18:1ω9*t*
Final value	177 C	9	18:1ω8*t*
Final time	0.00 min	10	18:1ω7*t*
Level 2		11	18:1ω10*c*
Program rate	0.20 C/min	12	18:1ω9*c*
Final value	184 C	13	18:1ω7*c*
Final time	0.00 min	14	18:1ω6*c*
Level 3	1.00 C/min	15	18:1ω5*c*
Program rate	1.00 C/min	16	Unknown
Final value	200 C	17	Unknown
Final time	20.00 min	18	18:2ω6*t,t*
Column inlet pressure:	30 psi	19	18:2ω6*c,t*
Split vent flow rate:	100 ml/min	20	18:2ω6*t,c*
Auxiliary flow rate:	30 ml/min	21	18:2ω6*c,c*
Air flow rate	400 ml/min	22	Unknown
Hydrogen flow rate:	30 ml/min	23	20:0
		24	18:3
		25	22:0

TABLE XIX

Comparison of Ag-TLC/O_3/GC and Capillary GC Results

Component	Soybean oil		Soybean shortening	
	Ag-TLC/O_3/GC	Cap. GC	Ag-TLC/O_3/GC	Cap. GC
Saturated	14.1%	15.8%	28.4%	32.3%
12:0	0.2	0.1	0.1	0.1
14:0	0.2	0.1	0.2	0.2
16:0	9.2	10.2	15.7	16.7
18:0	4.2	4.9	12.1	14.6
20:0	Trace	0.3	Trace	0.4
cis Mono	39.8%	39.9%	31.4%	30.1%
18:1ω10*c*	0.6	–	0.5	0.1
18:1ω9*c*	21.9	32.3	23.0	21.8
18:1ω8*c*	0.9	–	1.1	0.7
18:1ω7*c*	3.6	1.8	1.8	1.7
18:1ω6*c*	12.0	5.6	4.9	5.5
18:1ω5*c*	–	0.2	0.2	0.3
trans Mono	11.6%	6.6%	13.5%	10.3%
18:1ω10*t*	0.8	–	1.4	2.2
18:1ω9*t*	1.7	–	2.4	2.3
18:1ω8*t*	3.9	5.5	4.0	4.3
18:1ω7*t*	3.2	1.1	3.2	1.5
18:1ω6*t*	1.4	–	1.7	–
18:1ω5*t*	0.7	–	0.8	–
t,t Dienes	–%	1.0%	–%	0.9%
c,t Dienes	3.0%	2.1%	1.7%	1.7%
c,c Dienes	29.4%	30.4%	20.3%	21.3%
Trienes	3.5%	2.0%	3.8%	1.1%

geometry and position of the double bonds along the acyl chain can be achieved using a 100 m × 0.25 mm glass capillary column coated with SP-2340. The quantitative aspects of this separation have been reported recently by Slover and Lanza (10). This complex separation is illustrated in Figure 10 for a typical processed soybean oil and shortening. Conditions for the separation, along with the identities of the peaks numbered in the illustrative chromatograms, are given in Table XVIII.

Despite the high efficiency of the column, *trans*-positional isomers remain difficult to separate and are only partially resolved; however, reasonable peak areas can be measured with the HP-3351B data system by dropping perpendicular lines from the peak valleys to the baseline. The normalized quantitative data obtained from this separation for both soybean oil and shortening was compared against values determined by a more complex method using ozone treatment on methyl ester fractions isolated

by argentation TLC followed by gas chromatographic analysis (Ag-TLC/O_3/GC) of the ozonolysis products.

The results of the comparative study are reported in Table XIX. In general, the results compare well, particularly for the shortening sample; however, improvement in the capillary separation is still desirable. Although the capillary analysis requires ca. 2 hr, and is much more time-consuming than any of the previous separations discussed, it is a great improvement over the 1.5-day Ag-TLC/O_3/GC procedure.

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Techniques for Flavor and Odor Evaluation of Soy Oil

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ABSTRACT

Means of evaluating soybean oil for flavor on a "now" basis and on a "predictive" basis are presented. Emphasis is placed on more recent objective methodology for measuring oil volatiles and using their correlation with flavor. Applications of a modified volatile technique for use with soy isolates or soy proteins is shown. The importance of sensory analysis and a summary of methodology currently being used are discussed.

INTRODUCTION

Techniques for flavor and odor evaluation of soybean oil are needed as much today as in previous years, particularly as new extraction and processing changes are evolved due to high energy costs. The consumer also has been conditioned over the years and has become more perceptive of flavor quality.

Taste or organoleptic evaluation will always be the final judgment on flavor and odor, however, there is also a need for more objective methodology to support organoleptic decisions and, at the same time, supply information specific enough to understand and offer a solution to flavor and odor problems.

In this review, techniques for evaluating the flavor and odor of soybean oil are presented on the basis of "now tests" and "predictive tests."

"NOW" TECHNIQUES

Chemical and Physical

Some of the more common techniques for evaluating oil quality related either directly or indirectly to flavor are: peroxide (1), thiobarituric acid test (TBA) (2), Kreis test (3), anisidine test (4), oxirane test (1), conjugated diene and triene (2), conjugable oxidation products (COP) (5), fluorescence (6), infrared spectroscopy (7), polarography (8), and gas chromatography (9-11).

Official status in the USA has been given to only two of these tests, the peroxide AOCS, Cd8-53 and the oxirane test Cd-9-57 (1).

The most frequently consulted of the "now tests" listed here are peroxide, anisidine, conjugated diene and triene, thiobarbituric and volatiles using gas chromatography.

Peroxide Value

The initial and primary products of lipid oxidation are hydroperoxides which are transitory and break down by further reactions. Because of their breakdown to non-peroxide materials, their correlation with flavor can vary considerably. This test lacks specificity, i.e., it does not distinguish between types of fatty acids undergoing oxidation and it does not measure secondary products formed which are responsible for flavor change. Its chief value, then, is a measure of oxidation in its early stage.

Anisidine Value

This test developed by Holm (4) is a measure primarily of α-β-unsaturated aldehydes, and has been shown to correlate well with oxidation and flavor in oils (12,13). Others (14) have questioned its value for oils. The test did show a correlation with flavor deterioration of fats in dried emulsions (15). Holm (4) also introduced the term oxidation value which = anisidine value + 2 times the peroxide value (OV = AV + 2 PV). Using this combination of tests, slightly higher correlations with flavor were obtained.

Research by J.L. Williams in our own laboratory concluded that this test was of little value in measuring oil quality in U.S. soybean oils (unpublished data).