Fatty Acid Methodology for Heated Oils[1]

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Abstract

Criticisms have been voiced concerning the methods employed for determining the fatty acid composition of vegetable oils used in processing of fried foods. In the present study several different vegetable oils were heated under standardized conditions, at frying temperatures and under air, for various periods of time, and then subjected to analyses for fatty acid composition. The methods employed were: UV spectrophotometry; gas-liquid chromatography (GLC) employing either normalization of the peak areas, direct standards or internal standards; and the enzymic, lipoxidase procedure. The present findings confirm that the drop in iodine value is a good approximation of the reduction of polyunsaturated fatty acids in heated oils. In evaluating liquid nonhydrogenated vegetable oils that had been subjected to heat abuse under standard conditions, all of the analytical methods for the estimation of fatty acid composition, with the exception of normalization of GLC peak areas, give substantially the same results. With hydrogenated vegetable oils, the analytical methods provide values which differ significantly among themselves. Evidence was also presented to show that the quantity of material retained on the gas chromatographic column is directly related to the loss of polyunsaturates for oils heated under controlled conditions. For evaluation of oils from frying operations, the preferred method is the GLC method utilizing internal standards if the purpose is the determination of total polyunsaturated fatty acids. However, if the study being made involves a question of nutritional value, then the measurement of the essential fatty acids by the lipoxidase enzymic procedure is the more valid method for evaluation of heated oils.

Introduction

The literature contains numerous studies evaluating the effects of heat and atmosphere (1–31) on the quality of vegetable frying oils. Some of these studies have claimed production of nutritionally undesirable or toxic compounds as the result of heating of vegetable oils (19–31). However, the experimental program in most of these studies had no relationship to reality other than holding the oils at frying temperatures. These studies are characterized by the absence of food being fried and in some cases deliberate aeration during the extended heating periods. Frequently they involved the use of temperatures above 400 F and separation and concentration of suspect individual components. Such conditions are atypical of commercial frying operations and without practical significance for determining the nutritional properties of current frying oils. These studies have been criticized by many investigators who have shown that under the heating conditions used in commercial operations the level of undesirable components produced is insignificant, or that compounds which are produced are unabsorbable when fed in a natural diet (2–18).

Commercial frying involves both the replenishment of the frying oil to replace oil absorbed by the food and the evolution of steam, generated through release of water by the food. These two factors enhance the life of the oil in use by dilution and by shielding it with an inert atmosphere as well as by distilling off objectionable degradation products.

Melnick (2) demonstrated that oils containing a relatively small quantity of oxidative polymers would possess objectionable flavor, whereas a significant concentration of thermal polymers may be present without any organoleptic indication. From the work of Crampton and Common (19,20), it was also concluded by Melnick (3) that formation of toxic cyclized monomeric acids are only possible at commercial frying temperatures in oils containing linolenic acid. The only edible oil containing a significant amount of this acid is soybean oil; but unhydrogenated soybean oil is rarely used as a frying oil because of its flavor instability and no commercial fryer could continue in business with products possessing the off flavors of polymers of the oxidative type. Therefore, the problem of concern in frying oils (if any) is related primarily to the presence of thermal polymers of the nonoxidative type.

The presence of thermal polymers is indicated by the presence of their precursor, conjugated acids (4,32), which also develop during oxidative polymerization but are instantaneously destroyed because of the ease with which they are oxidized (33).

Melnick suggested the change in iodine value as a simple control index of the development of thermal polymers since failure to find a drop in iodine value would indicate freedom from polymer formation (2). In Table I, studies from the literature reporting both iodine values and fatty acid composition values have been collated. It can be seen that in all instances with the samples abused at excessively high temperature under air or inert gas or under simulated and commercial frying conditions, the drop in iodine value corresponds fairly well with the decrease in the dienoic acids.

Thus it was inferred (at least in our laboratory) that change in iodine value could be used as a means for estimating the reduction in concentration of the dienoic acids of a frying oil until Thompson and coworkers reported a reduction in dienoic acids twice that predicted from the iodine value decrease in some samples used commercially for deep fat frying (1). To study the significance of these results with its implication of inaccuracy of some of the fatty acid composition studies reported by previous investigators, a comparative study of present-day fatty acid composition procedures was initiated.

Experimental Procedures

A commercially deodorized corn oil was selected for the initial study because it contains the highest proportion of polyunsaturated fatty acids among oils used as frying oils. Five hundred gram portions of this oil were heated continuously and quiescently for periods of 8, 16, 24, 32, 40, and 48 hr at 185 ± 5 C, in an uncovered stainless steel frying pan, with a surface area of approximately 110 sq cm. The length of time required to heat the oil to this temperature (typical of frying temperatures used in commercial operations) was between 15 and 20 min. Upon com-

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TABLE I
Previously Reported Relationship of Iodine Value and Dienoic Acid Content of Used Vegetable Oils

Sample preparation	Type of oil or fat	Initial IV (Wijs)[a]	Decrease in IV	Decrease in %[b] dienoic acids
CO_2 blown into sample at 600 F for 16 hr (4)	Cottonseed salad oil	114.0	4.6	5.4
Air blown into sample at 600 F for 1.5 hr (4)	Cottonseed salad oil	114.0	6.3	4.7
Air blown into sample at 180 C for 24 hr (25)	Unidentified oil blend	76	28	23
Quiescent heating at 225 C for 194 hr (30)	Cottonseed salad oil	109.8	36.4	37.1
Simulated restaurant type frying condition 182 C with with intermittent use (5)	Cottonseed salad oil	109.0	7.0	7.3
	Hydrogenated soybean oil	69.7	3.3	2.5
	Lard	64.4	8.7	5.6
Commercial manufacture of potato chips (4)	Corn oil	124.8	2.4	1.9
	Cottonseed salad oil	111.6	2.2	2.1
	Cottonseed salad oil	114.7	2.2	1.3
	Hydrogenated cottonseed oil	85.6	0.5	0.7

[a] Iodine value (Wijs) (34).
[b] Determined by alkali-isomerization UV spectrophotometric procedures (35,36).

pletion of a test, the oil was allowed to cool in the pan to room temperature and was then transferred to 4 oz bottles. An atmosphere of nitrogen was introduced and the bottles tightly sealed and stored at −4 C until analyzed.

Samples of four other oils commonly used in commercial frying were also heated in the laboratory 40 hr under the standardized conditions employed in heating the corn oil. Five independent methods were used for the determination of the fatty acid composition of both the fresh and heated oil samples.

Of the procedures utilized, the lipoxidase or enzymic procedure (which has long been in use in our laboratory) (37) is the most specific test for measuring essential fatty acids (EFA), providing values for only the polyunsaturated fatty acids with a *cis,cis* methylene interrupted double bond configuration (38,39). Linolenic acid when present, is measured as though it were linoleic acid by this procedure.

Beare, who has also actively used the enzymic procedure (40,41), recently reported the application of this procedure to heated oils (42) but did not show if the change in EFA could be related to values obtained by other methods as used in earlier publications.

A second procedure used was the UV Spectrophotometric method as developed by Brice and associates which utilizes alkali-isomerization with 11% potassium hydroxide in glycerin, under air, for a period of 45 min (35).

The gas liquid chromatographic analyses (GLC) were made utilizing a Beckman GC-2A with a thermal conductivity detector and both column and conditions in accord with those suggested in the official procedure (43). The samples for GLC were all esterified by a procedure similar to that reported by Luddy et al. (44), to use samples of smaller size and thereby conserve the trimyristin and triheptadecanoin employed as internal standards.

Sixty milligrams of oil was carefully weighed into a 2 ml volumetric flask, followed by the addition of 0.5 ml of 0.8% sodium methylate, and the flask flushed with nitrogen and stoppered. The flask was then placed in a water bath, maintained at 65–70 C for 5 min with vigorous agitation for the first 30 sec. After cooling, the contents of the flask were transferred quantitatively to a 30 ml separatory funnel using 3 ml of ethyl ether, added in 1 ml portions, and vigorously shaken for 1 min. The ether solution was washed with 1 ml portions of water until neutral (about five times), dried with anhydrous sodium sulfate, transferred to a vial, and the solvent evaporated at room temperature under a stream of nitrogen. The esters prepared in this manner are stable for several days if stored in a freezer.

For the determinations made utilizing internal standards, 3 mg of trimyristin or triheptadecanoin were esterified together with 57 mg of the oil by the above procedure. Area measurements of all the chromatograms were made using a compensating polar planimeter.

Three separate evaluation techniques were utilized with the GLC analyses: (a) straight normalization, the ratio of the areas of the individual components of the sample to the sum of the areas of all components in the sample; (b) direct or absolute standards, the comparison of the peak areas of the individual component esters of the sample with corresponding calibration curves prepared using pure esters; (c) internal standards. For the determinations made utilizing internal standards, the curve areas obtained were related to the internal standard through calibration curves of the ratio of the weight of the component to the weight of the standard versus the ratio of the peak area of the component to the peak area of the internal standard.

Results and Discussion

Physical constants and the fatty acid composition determined by all five procedures for the fresh unheated oils used in this study are shown in Table II.

It can be seen that regardless of the method used, the fatty acid compositions of liquid nonhydrogenated oils are relatively the same. Minor differences can all be attributed to known deficiencies in the different methods. For example, an overestimation of the linolenic acid value by the spectrophotometric procedure in the case of the soybean oil products magnifies somewhat the differences found for the calculated oleic acid and saturated acid values obtained with that procedure. The somewhat higher palmitic acid values of the normalized GLC curves can be attributed to well known factors of instrument response (45), and the direct standard GLC procedure is susceptible to the variations from nonreproducible sample injections of both standards and samples and the reactivity of the unsaturated methyl ester standards.

TABLE II
Analytical Characterization of Fresh Oils and Fats Used in the Present Study

Sample	Iodine value (Wijs)	Cold test at 0 C, hr	Melting point, °F (Wiley)	Method of assay[a]	Fatty acid composition (% of total fatty acids)					
					Saturated fatty acids			Unsaturated fatty acids		
					Palmitic	Stearic	Total[b]	Oleic	Linoleic	Linolenic
Corn oil	126.7	48	23.0	GLC-normalized	11.7	1.9	14.4	26.5	58.1	1.1
				Internal std.	11.0	1.5	13.3	25.0	58.5	1.1
				Direct std.	9.7	1.8	12.3	24.7	57.3	1.1
				Spec.	.....	....	14.4	25.7	59.1	0.9
				Enzymic	.....	....	.....	.....	58.7[c]	
Cottonseed salad oil	117.2	29	36.0	GLC-normalized	19.5	2.4	23.7	18.3	56.5	0.8
				Internal std.	18.7	2.3	22.8	18.0	57.0	0.7
				Direct std.	18.6	2.2	22.6	17.8	59.6	0.7
				Spec.	.....	....	21.7	21.0	57.2	0.0
				Enzymic	.....	....	.....	.....	56.2[c]	
Soybean salad oil	136.2	15	31.0	GLC-normalized	10.8	3.7	16.4	21.2	53.2	9.2
				Internal std.	9.5	3.7	15.1	21.5	53.0	9.2
				Direct std.	9.5	3.6	15.0	20.8	54.0	9.2
				Spec.	.....	....	17.7	17.5	54.7	10.1
				Enzymic	.....	....	.....	.....	63.8[c]	
Lightly hydrogenated winterized soybean oil	117.1	8	41.0	GLC-normalized	10.7	4.2	16.3	36.7	42.7	4.3
				Internal std.	9.3	4.3	15.0	36.3	42.5	4.5
				Direct std.	9.4	4.2	15.0	36.6	44.4	4.6
				Spec.	.....	....	13.7	42.0	39.2	5.1
				Enzymic	.....	....	.....	.....	38.5[c]	
Hydrogenated and compounded soybean oil shortening[d]	96.8	....	115.7	GLC-normalized	13.5	10.7	25.9	38.9	31.6	3.3
				Internal std.	12.7	10.3	24.7	37.8	32.0	2.9
				Direct std.	12.6	10.1	24.4	36.0	31.2	2.8
				Spec.	.....	....	23.0	45.5	27.9	3.6
				Enzymic	.....	....	.....	.....	27.4[c]	

[a] Spec., alkali-isomerization spectrophotometric method; Enzymic, lipoxidase procedure.
[b] GLC determinations include minor saturated components.
[c] Enzymic; represents value for the total *cis,cis* polyunsaturated fatty acids (EFA).
[d] SCI for this sample: 50F, 15.4; 70F, 12.9; 80F, 12.4; 92F, 10.8; 102F, 8.0.

The internal standard procedure utilizing triglyceride standards, as in this study, should theoretically compensate for all the potential variables from esterification through calculation, of all the major component acids.

With the hydrogenated oils, the methods used in this investigation provide values which differ significantly. These greater differences are caused, in part, by the differences in response to the fatty acid isomers developed during hydrogenation as indicated in earlier reports (37,46).

The changes in the fatty acid composition, particularly in linoleic acid content reflected by the different assay methods, and by iodine value obtained for the oils heat-abused in the laboratory are summarized in Table III.

With the exception of the normalized data, reasonable agreement is obtained for the changes in composition by the various methods for all the non-hydrogenated oils. Similar differences among the values obtained by the different assay methods were again found with the hydrogenated oils after heating as was indicated in Table II for these oils before heat abuse.

As Kilgore and Luker (47) and Thompson et al. (1) pointed out, normalization of GLC data causes the values for the individual component fatty acids to become inter-related. Analysts using such an approach will erroneously report increases in saturated acids in heated fats if they fail to compensate for this analytical limitation or fail to cross-check their findings with results by other methods. To circumvent this problem, arising from normalization of GLC data for fatty acid composition, Kilgore assumed that the saturated fatty acids remained constant in the oils and recalculated the values obtained for unsaturated acids using as a correction factor the ratio of the concentration of saturated acids in the oil before and after heating.

It was observed, while studying the chromatograms of the heated corn oils (Table III), that while a total area of the chromatogram decreased with increasing heating time of the oil, the areas of the individual acids with the exception of linoleic and oleic acids remained relatively constant. Therefore, the peak areas for all components except linoleic acid, linolenic acid, oleic acid and some minor breakdown products obtained for the series of corn oil samples regardless of heating time, were averaged. The minor variations from the average, found for individual peak areas for a particular determination, is assignable to minor variations in the sample volume injected because each peak area remained proportional to the variations in the areas of all the other constant components.

An adjusted total area for the chromatogram of the unheated corn oil was calculated, incorporating the average peak areas for all the stable components and the areas of linoleic and oleic acids originally present in the unheated oil. The peak areas for linoleic acid, linolenic acid, oleic acid and minor breakdown products found for each of the heated corn oils were individually applied against the adjusted total area to obtain the values from which the column of corrected normalized values were obtained.

The decrease in the linoleic acid values of the corn oil sample, after correction, agrees with the decrease in EFA shown by the enzymic procedure. It can be seen in Table IV, that the apparent linolenic acid content of the corn oil remained constant with progressive heating. Since linolenic is more reactive than linoleic acid and the heated samples do show a progressive decrease in concentration of the latter acid but not of the apparent linolenic acid, it must be concluded that no linolenic acid as such was actually present in this oil sample. The enzymic procedure gives a direct measurement of the change in the linoleic acid content of this oil.

In Table III, an attempt was made to estimate the quantity of material derived from the test oils retained on the GLC column. The difference in an adjusted total area of a chromatogram for a heated oil and the adjusted total area of the unheated oil is assumed to be a measure of the materials retained on the chromatographic column under the normal operating conditions for determining fatty acid composition (43). It would appear that this retained material is a reaction product (polymer, peroxide,

TABLE III
Changes in Composition of Heat-Abused Oils by Various Methods

Sample	Heated under air, 185 C, hr	Decrease in IV (Wijs)	Decrease in EFA,[a] %	Per cent decrease in linoleic acid content				GLC normalized corrected/ constant components	Non-eluted material in GLC corrected for constant components, %	Non-eluted *material* (internal standards GLC), %	Urea non-adducting material, %
				Spec.[b]	GLC internal standard	GLC direct standards	GLC normalized				
Corn oil	8	2.4	2.2	2.0	.....	.....	1.4	2.0	1.8	.....	.....
	16	4.2	3.1	4.1	.....	.....	2.6	3.1	2.1	.....	.....
	24	5.2	5.9	7.5	.....	.....	3.7	5.2	3.9	.....	.....
	32	7.9	8.9	10.6	.....	.....	5.4	11.8	11.1	.....	.....
	40	11.5	12.9	13.9	12.8	11.3	6.3	12.3	12.1	14.9	14.3
	48	18.6	21.9	22.0	21.8	24.5	10.2	20.2	21.2	26.1	.....
Cottonseed salad oil	40	34.4	36.5	38.5	38.2	37.8	22.8	36.2	39.2	41.2	40.9
Soybean salad oil[c]	40	33.9	36.8	37.8	33.6	32.4	20.5	35.4	36.8	36.5	36.7
Lightly hydrogenated winterized soybean oil[c]	40	26.7	24.2	23.8	27.3	28.8	19.0	28.2	32.3	34.0	32.6
Hydrogenated and compounded soybean oil shortening[c]	40	21.0	15.6	16.4	17.2	15.8	12.5	18.0	22.9	23.8	22.6

[a] Determined by enzymic procedure (37).
[b] Spec., alkali-isomerization spectrophotometric method (35).
[c] Values for soybean oils include any linolenic acid present.

etc.) of linoleic acid because its increase in the corn oil samples, with heating time, approximates the decrease in the linoleic acid content by the more direct procedures.

The corn oil sample did, however, show less change in linoleic acid than the other oil types, probably because of trace quantities of silicone in it. The authors were unable to obtain a commercial domestic corn oil completely free of this antifoaming agent. Comparison of the data for this corn oil with the recent findings by Beare et al. (42) (who used essentially the same heating conditions), disclosed that the level of degradation found by Beare could be attained only by heating the corn oil of the present study three times as long. Because of the general use of silicone additives, the commercial corn oil available in the U.S. market can be expected to have regularly a stability superior to that of the corn oil used by Beare.

The only other means of obtaining fatty acid composition results by GLC, which would both be independent of the material not eluted from the column and the variations intrinsic in sample injection, is through use of internal standards. The latter technique, as discussed by Dal Nogare and Juvet (48) and McNair and Bonelle (49), relates each component in the test system to a known amount of an added component of the same general class of compounds. The internal standard provides a peak completely separate but close to the major peaks of the chromatogram. This technique has been employed in studies of polymerized oils by Zielinski (50). Use of the triglycerides for internal standards, as described above, reduces potential errors from the esterification step, and also eliminates the need for excessive precautions in handling oxygen susceptible methyl esters. This procedure is regarded to be superior to previously used GLC evaluation techniques, particularly in assays of hydrogenated or heated oils where isomers or degradation products are present.

A reasonably good estimate of the noneluting material can also be obtained by subtracting from 100% the sum of all the fatty acid components of an oil analyzed by GLC using internal standards. For this purpose, a second chromatogram of the samples without the presence of an internal standard was normalized to provide data for correction or confirmation of the absence of a contributory peak at the position of the internal standard employed. The normalized chromatogram was also used for obtaining values for minor and unidentified components to prevent their inclusion in the value for the noneluted material.

Firestone et al. (51) isolated the polymer fraction from heated oils by the procedure of urea adduction wherein the urea nonadducting materials are the isolated polymers. He has proven the reliability of this technique by comparison with the determination of dimers and trimers by means of micromolecular distillation and sublimation of heated oils (52,53).

Except for the hydrogenated oil samples in Table III, the values for urea nonadducting materials (51,54), isolated from the oils heated for 40 hr, agree fairly well with the results obtained by the different and unrelated techniques other than those derived from normalization of GLC curves. The hydrogenated oils contained higher quantities of apparent polymer and related products (by the methods of urea adduction and measurement of materials retained on the GLC column) than would be deduced from other methods used. The greater the degree of hydrogenation the greater the quantity of *trans* isomers developed. These have been reported to have a faster relative rate of reaction in dimer or polymer formation (55). Hence, the greater will be the differences in the values obtained by the different analytical procedures.

TABLE IV
Fatty Acid Composition of Corn Oil Determined by GLC and Calculated Using Average Peak Areas for All Other Components[a]

Corn oil, hr	Per cent fatty acids in total fatty acids		
	Apparent linolenic[b]	Linoleic	Oleic
Unheated	0.82	57.8	26.3
Heated			
8	0.82	55.8	26.5
16	0.82	54.6	27.3
24	0.82	52.6	27.4
32	0.82	47.0	25.8
40	0.82	45.5	26.0
48	0.82	37.6	24.8

[a] Saturated acids remain constant at 14.2%.
[b] Component possessing retention time of linolenic acid.

TABLE V
Evaluation of Commercial Frying Oils

Operation providing oil sample	State of oil	% FFA[a]	IV (Wijs)		% EFA[b]		% Total linoleic acid[c]	
			Found	Change	Found	Change	Found	Change
Roasting of peanuts with lightly hydrogenated cottonseed oil	Fresh	<0.05	86.5	....	20.0	....	25.8	....
	Heated	0.9	85.5	1.0	19.1	0.9	25.0	0.8
	Heated	1.0	85.2	1.3	18.9	1.1	24.5	1.3
Chicken frying with an isolated stable fraction of hydrogenated domestic vegetable oils	Fresh	<0.05	78.2	....	1.2	....	9.5	....
	Heated	0.6	76.2	2.0	0.1	1.1	8.0	1.5
Miscellaneous restaurant fryings with hydrogenated soybean oil	Fresh	<0.05	81.1	....	10.1	....	17.6	....
	Heated	3.5	76.2	4.9	7.6	2.5	13.0	4.6
	Heated	4.5	66.6	14.5	3.9	6.2	7.0	10.6

[a] FFA (free fatty acids) (56).
[b] EFA, by enzymic (lipoxidase) procedure.
[c] GLC, with internal standard.

Table V contains results from representative samples of discarded oils from different commercial frying operations; a peanut processor, a chicken processor and a restaurant chain.

Analyses of like samples of the oils before heating, were obtained for comparison. Fairly good agreement was found between values for all samples with respect to the change in iodine value and the decrease in per cent linoleic acid content obtained by the GLC method using internal standards. However, the change in EFA values only showed agreement in the case of oils used in the large volume fryers where the oil is used until a free fatty acid value of about 1.0% is obtained. The oil from the restaurant frying operation, used intermittently with a large variety of food products, showed much less change in linoleic acid content by the enzymic method. The decrease in iodine value in this commercially abused oil is therefore attributed not only to loss of essential fatty acids but also to loss in linoleic acid isomers. The absolute values for EFA in both the used and fresh oils were substantially less than the GLC linoleic acid values because of the specificity of the enzymic method for measuring fatty acids with the *cis,cis* methylene double bond configuration; this assay procedure still remains the method of choice in estimating nutritional value of both the fresh and used oils.

In addition to the tests summarized here, we conducted studies to measure the urea nonadducting material and the quantity of material which resisted elution from the GLC column. Suffice it to say, our findings by these two techniques on these commercially used frying oils were so variable as to require further work in this area. To what degree the extractives in the frying oils, due to unknown products derived from the foods and frying oils being heated, and/or to unknown reaction products between heated foods and oils, interfere or modify the measurements of urea nonadducting material and measurements of material which resist elution from the GLC column is impossible to define at this time.

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