Alkali Isomerization—Gas Chromatography With the Microreactor Apparatus[1]

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ABSTRACT

A simple and rapid method of simultaneously conjugating polyunsaturation and forming methyl esters from triglyceride oils, other esters and fatty acids has been developed by using the microreactor apparatus (MRA). These reactions occur while a mixture of tetramethylammonium hydroxide and sample of fat is being injected into a gas chromatograph from the MRA reaction chamber. The speed of this microanalysis is limited only by peak elution and peak integration times. Analyses of soybean oil and six partially hydrogenated fats have been compared with those previously obtained by the alkali isomerization spectrometric method. The amount of conjugation that occurs during the reaction is a measure of the linoleic acid in the oil. This procedure should prove useful for rapid microscale analysis of conjugatable dienes in a variety of samples, including edible fat products.

INTRODUCTION

While esterifying fatty acids with tetramethylammonium hydroxide (TMAH) (1) in the microreactor apparatus (MRA) (2) for subsequent gas liquid chromatography (GLC), it was observed that linoleic acid gave methyl ester peaks corresponding to conjugated dienes. This observation forms the basis for developing the new MRA-alkali isomerization-GLC procedure of determining linoleic acid, other conjugatable dienes and nonconjugatable dienes in triglyceride esters, simple esters and fatty acid mixtures. Compared to the conventional alkali isomerization spectrometric method (3) there are the advantages of ease, microscale capability, speed and versatility. However, this new procedure is not suitable for samples containing large amounts of triene and is not proposed as a replacement for the more accurate alkali isomerization spectrometric method. It is suggested as a technique for the analyst having a gas chromatograph and needing to determine linoleic acid in oil which contains a small amount of triene.

In brief, the procedure consists in drawing TMAH and a sample of fat into a micro syringe, introducing the solution into the MRA and injecting the reaction mixture from the MRA into the injection port of a gas chromatograph at 250-300 C with helium gas flow. Two ways of calculating conjugatable dienes are employed, one which has indigenous methyl palmitate as internal standard and the other which measures the areas under the conjugated diene and nonconjugated diene peaks.

EXPERIMENTAL PROCEDURES

Materials

The refined soybean oil analyzed was purchased commercially. Soybean fatty acid and soybean methyl ester macrosamples were made by saponifying, acidifying and esterifying. Also analyzed were six partially hydrogenated fats previously studied by the alkali isomerization spectrometric method and subsequently stored in a zero degree room (4).

cis,cis-9,15 Methyl octadecadienoate and a mixture of *cis,cis*-9,12 and *cis,cis*-12,15 methyl octadecadienoates were purified and separated as described (5).

Linoleic acid, methyl linoleate and methyl palmitate from The Hormel Institute, Austin, Minn., showed 99.9+% purity as determined by gas chromatography.

TMAH was purchased from Aldrich Chemical Co., Milwaukee, Wis., as a 20% solution in methanol.

Analytical Procedure

The microalkali isomerization was performed by drawing 20 µl of TMAH solution, 1-6 µl of lipid sample and an additional 20 µl of TMAH solution into a 100 µl syringe, consecutively. Sample and TMAH were mixed by injecting into the MRA (2) reaction chamber. An MRA reaction temperature of 250-300 C and an injection time of 3 min were used to transfer the sample to the gas chromatograph from the MRA.

A 10% mixture of methyl palmitate (internal standard) in methyl linoleate was used to determine the amount of conjugation in pure methyl linoleate.

Transesterification and esterification reactions to obtain a chromatogram of the methyl esters were performed in the MRA as previously described (6,7).

A gas chromatograph with thermoconductivity detector (Aerograph Model 350) was used for all runs with the injector port at 250 C, the column temperature programmed from 60 to 190 C at 10 deg/min, a 10 ft x 1/4 in. column filled with 10% EGSS-X on 100/120 mesh Chromosorb W (Applied Science Lab., State College, Pa.), a flow rate of 60 cc/min helium and X1 attenuation. Total time for GLC operations ranged from 40 to 60 min.

The *trans,trans* peak from the conjugation of linoleic acid (see Results and Discussion) was collected by placing the glass reaction chamber of the MRA at the collector port of the gas chromatograph. Ozonization pyrolysis of the collected sample to determine double bond positions was then performed in the MRA as described earlier (8).

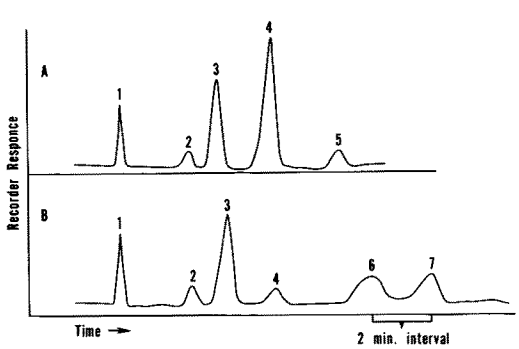

FIG. 1. Typical chromatograms before (A) and after (B) isomerizing in the microreactor apparatus with tetramethylammonium hydroxide. Portion of recording shown is after reaching temperature of 190 C. Gas chromatographic conditions given in text. Significant peaks are the methyl esters of 1) palmitate, 2) stearate, 3) monoene, 4) diene, 5) triene, 6) conjugated *cis,trans* diene and 7) conjugated *trans,trans* diene.

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TABLE I

Alkali Isomerization Gas Chromatographic (AI GLC) and Alkali Isomerization Spectrometric (AI S) Analyses on Six Partially Hydrogenated Fats

Sample	Total diene, % Gas chromatography		Linoleic acid, % AI GLC[a]		AI S	Conjugation, % AI GLC[a]	
	Literature,[b]	Present	1	2	Literature,[b]	1	2
	a	b	c	d	e	f	g
Liquid oil A	37.4	36.4	33.7	31.9	32.2	92.5	87.6
Liquid oil B	41.8	43.7	37.8	34.4	38.5	86.4	79.0
Shortening C	27.4	24.3	22.1	21.3	24.1	91.1	87.6
Shortening D	11.8	4.1	3.6	3.1	6.6	88.4	75.6
Shortening E	25.5	24.6	22.8	22.4	19.7	92.3	91.0
Shortening F	10.4	9.0	8.4	8.0	7.0	92.6	89.1

[a]Method of calculating: 1, Methyl palmitate peak used as a standard (method 1 in text). 2, Conjugated and nonconjugated peaks used (method 2 in text).

[b]Reference 4.

Calculations

An electronic integrator system (Infotronics, Houston, Tex.) allowed for base line correcting and determining areas under peaks.

Each reaction was carried out in triplicate and the average integral was used for calculating per cent conjugations.

Conjugation was calculated by two methods. Method 1 required two chromatograms of methyl esters, one before (Fig. 1A) and one after (Fig. 1B) isomerization. A ratio of methyl palmitate (internal standard) to diene calculated from Figure 1A was used to obtain the unknown total diene integral of Figure 1B. The measured unconjugated diene peak integral from Figure 1B completes the necessary information for calculating the amount of conjugation.

Referring to Figure 1A,B and indicating peak numbers as subscripts for the peak areas, the calculations of method 1 are:

$$X/B_1 = A_4/A_1$$

or,

$$X = A_4B_1/A_1$$

$$\% \text{ Conjugation} = 100 - (B_4/X)100$$

Substituting for X and dividing:

$$\% \text{ Conjugation} = 100 - (A_1B_4/A_4B_1)100$$

X is the total diene peak integral after conjugation.

Method 2 required only the chromatogram of methyl esters after isomerization. Integrals of the conjugated and nonconjugated peaks provided the basis for calculating the amount of conjugation.

Again, referring to Figure 1 and indicating peak numbers for subscripts, the calculations for method 2 become:

$$\% \text{ Conjugated diene} = 100 \times (B_6 + B_7)/(B_4 + B_6 + B_7)$$

RESULTS AND DISCUSSION

Figures 1A and 1B are the chromatograms resulting from a typical experiment. Figure 1A is the methyl ester chromatogram obtained by microtransesterification (6) in the MRA and Figure 1B is the chromatogram after conjugating and transesterifying the soybean oil with TMAH. It is obvious in Figure 1B that the diene peak (A_4) has practically disappeared, a small unconjugated peak (B_4) remains and conjugated peaks of *cis,trans* or *trans,cis* (B_6) and *trans,trans* (B_7) are formed.

In Table I are compared the per cent total diene, linoleic acid and conjugation of dienes in six partially hydrogenated fats obtained by alkali isomerication GLC and alkali isomerization spectrometric techniques (9). All samples (columns a and b) showed minor differences in amount of total diene except for shortening D in which the diene was the lowest. Differences are also small in amount of linoleic acid content due to methods of calculating conjugation (columns c and d) and due to analytical techniques (columns c and/or d vs. e).

The third section of Table I shows that the amount of conjugation varies with the method of calculation. A larger percentage of conjugation is always noted when the palmitate peak is used as the standard (method 1 under Calculations, column f). The lower amount of conjugation (method 2 under Calculations, column g) is in agreement with Jackson et al. (9) for the alkali isomerization spectrometric method. These authors noted that at elevated reaction temperatures (234 C) the conjugated diene peaks were as much as 8% lower than total diene, and they speculated that conjugated dienes were polymerizing. Another factor favoring calculation by method 1 is the possibility of nonconjugated triene adding to the conjugated diene peaks. In the partially hydrogenated fats listed in Table I, the triene was small and varied from 0.1% to 3.1% when MRA was used for transesterification and analysis.

Although the evidence appears to recommend method 1 calculation, as mentioned previously, it requires two chromatograms of methyl esters to be run, one before isomerization, one after. The results with pure linoleic acid and a 10% mixture of methyl palmitate in methyl linoleate of 96.6% and 95.5% conjugation, respectively, favor method 2 calculation since less experimental time is involved. Therefore, the choice of method for calculating results must be based on prior knowledge of the sample, time allotted for determinations and desired agreement with alkali isomerization spectrometric technique.

The effectiveness of TMAH isomerization for a variety of fatty material was demonstrated with refined soybean oil, soybean fatty acids and soybean methyl esters; they gave values of 95.2%, 96.1% and 96.3% conjugation, respectively.

The chromatogram resulting from the reaction of TMAH and a diene mixture of *cis,cis*-9,15, *cis,cis*-9,12 and *cis,cis*-12,15 methyl octadecadienoates showed a peak for *cis,cis*-9,15 methyl octadecadienoate and two conjugated diene peaks: *cis,trans* or *trans,cis* and *trans,trans*. Conjugation of the *cis,cis*-12,15-diene illustrates isomerization of pentadiene structures at positions other than 9,12 of linoleic acid. The nonreaction of the *cis,cis*-9,15-diene is also found with the alkali isomerization spectrometric method (10).

Figure 2 shows the results of ozonizing the collected *trans,trans* peak from the TMAH conjugation of linoleic

acid. The large number of peaks indicate that the double bond has been scattered throughout the molecule by TMAH at the elevated temperatures. However, scattering of the double bond does not interfere with use of TMAH isomerization for determining total conjugation or linoleic acid content of specific vegetable oils.

TMAH isomerization can also be performed in the injector port of the gas chromatograph at a temperature of 250 C. The injector port reaction is probably the method of choice if an ester analysis has already been performed (method 1) or a standard peak comparison is not necessary (method 2). A definite advantage of the MRA is the rapid transesterification of oils to obtain the reference chromatogram for calculations. Other advantages of MRA over the injection port technique includes those inherent to the MRA, such as its capability of handling microsamples isolated from thin layer chromatographic plates or collected from preparative GLC by direct condensation.

Compared to the conventional alkali isomerization spectrometric method the MRA isomerization GLC procedure handles microsamples easily through its isomerization and GLC analysis steps and has no dilution steps required for spectrophotometry. Like the more accurate and more precise alkali isomerization spectrometric method, the MRA GLC procedure can be performed on a variety of materials–triglycerides, monoesters and fatty acids–with equal ease. This procedure should find important applications in the analysis of hydrogenated fats, surveys of new oilseed crops, production of conjugated fatty acids and similar areas of research and analysis where knowledge of the double bond structure of dienoic fatty acids is required.

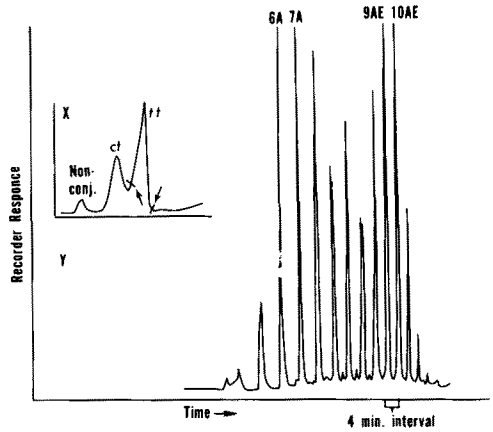

FIG. 2. Ozonization pyrolysis of the collected *trans,trans* conjugated diene peak from the tetramethylammonium hydroxide microreactor apparatus isomerization of linoleic acid. X, insert showing chromatographic result of linoleic acid isomerization. Start and stop collection points noted by arrows on *trans,trans* diene peak. Gas liquid chromatography (GLC) conditions given in text. Y, Aldehyde (A). Aldehydic ester (AE). GLC conditions: flame ionization detector, 4 ft x 1/8 in. column packed with 10% OV-17 on 80/100 Chromosorb WHP (Supelco Inc., Bellefonte, Pa.), 38 cc/min helium flow, temperature programmed from -40 to 280 C.

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