The effect of water hardness is more pronounced in the case of foam than detersive effects. Figure 5 shows a change from 50 ppm water hardness as $CaCO_3$ to 200 ppm produces a delta reflectance of approx 0.5 units after three soilings and washings. Progressing from 200 ppm to 300 ppm a delta Rd value of 4.5 units is indicated. Figure 6 again shows the same relationship existing to the towel test as previously shown.

The relationship of detergency to structure of straight chain alkyl benzene sulfonates is shown in Figure 7. These materials were formulated into a typical heavy-duty laundry composition at a 25% active ingredient level. It will be noted that both phenyl position and chain length are responsible for detergency effectiveness. These relationships are more fully discussed in a paper by Rubinfeld et al. (16).

Figure 8 was a comparison between a bundle test and the screening test with one half the amt of airborne soil and one half the water content. In other words 2 g of particulate were added to 500 ml of a 10% sebum emulsion. The Rd scale on the bottom chart is one half of the scale on the upper chart. In this comparison, both redeposition and detergency were measured in two products, A and B. Product A contained a different redeposition agent than product B. Both tests show approx the same reflectance difference between the two products for redeposition and detergency. However, the screening test show the fabrics to have a reflectance in the upper forties after three washes, while in the bundle test, the clothes never go below 80% reflectance after 14 washes. Since the bundle test is composed chiefly of t-shirts, sheets, pillowcases, etc., one would expect the presence of very little particulate. A modification of this test will be presented at a later date, in which the particulate is omitted from the soiling mixture. This type of procedure more closely parallels so-called bundle testing.

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Analyses of Fatty Acid Isomers in Two Commercially Hydrogenated Soybean Oils[1]

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Abstract

A conventional shortening and a hydrogenated winterized oil have been investigated to determine their composition of natural and isomeric fatty acids. Two solvent systems were applied in countercurrent distributions: the acetonitrile pentane-hexane system for separation of monoenoates from dienoates and the methanolic silver nitrate pentane-hexane system for separation of geometric isomers. While *cis* and *trans* monoenoates were well resolved, the separation of *cis,cis* from *cis,trans* dienoates was complicated by the presence of positional isomers. The fractions isolated were oxidatively cleaved, and the esters of the resultant acids were quantitatively analyzed by gas-liquid chromatography.

Although the amounts of saturated components of the two fat products were similar, the percentage of *trans* isomers of the shortening was more than twice that of the winterized oil. The amount of oleic acid (*cis*-9-octadecenoic) was 19.6% for the shortening and 25.4% for the winterized oil. The shortening contained 13.3% linoleic acid (*cis,cis*-9,12-octadecadienoic), whereas the winterized oil contained 30% linoleic acid.

Although our primary interest was in the estimation of *cis*-9-octadecenoic and *cis,cis*-9,12-octadecadienoic acids, the completeness of cleavage data makes it possible to estimate all geometric and positional monoenoate and dienoate isomers in the two fat products.

Introduction

Hydrogenating fats produces many isomeric fatty acids because double bonds migrate and geometric isomers form. To increase our understanding of these complex changes, the composition of the unsaturated fats in two commercial products was studied and compared.

The complexity of the fatty acids formed on partial hydrogenation of methyl linolenate was described in previous studies (11,12,14) in which crystallization and countercurrent distribution (CCD) with acetonitrile and pentane-hexane and argentation CCD were used as fractionation tools. Kuemmel (8) in 1962 described the use of alumina chromatography of mercury aducts of fatty acid esters to perform quantitative separation of saturates, monoenoates, and polyenoates.

The use of silver nitrate for the separation of geometric isomers was initiated by Nichols (10) in 1952, but only recently has it been applied to mixtures of model compounds (4). An argentation CCD system has been described (13) and utilized to study partially hydrogenated methyl linolenate (12,14).

The effectiveness of this CCD system for the analysis of two commercially hydrogenated fat products is reported here. By applying various procedures to

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TABLE I

Composition of Esters of Commercial Fat Products

Item	HWVO	HSBO
GLC analysis, %		
Palmitate	10.0	9.7
Stearate	3.0	4.5
Monoenoate	45.6	63.0
Dienoate	39.4	22.8
Trienoate	2.0	0.0
Iodine value	108.5	87.8
Conjugated diene, %	0.6	0.4
Alkali conjugable diene, %	34.5	13.9
Lipoxidase conjugable, %	34.3	12.6
trans, % (Isolated)	15.1	36.8
Azelaic acid on cleavage, mole %	71.7	45.3

the fractions, such as oxidative cleavage, infrared and ultraviolet spectrophotometry, alkali and lipoxidase isomerization methods, and gas-liquid chromatography (both packed [GLC] and capillary [CGLC] columns), an estimate can be made of the amount of "natural oleic acid" (*cis*-9-octadecenoic) and of "natural linoleic acid" (*cis,cis*-9,12-octadecadienoic) present in the commercial fat products.

Experimental

Materials

Two commercial fat products were studied: a hydrogenated winterized vegetable oil (HWVO) derived largely from soybean oil and a hydrogenated soybean oil shortening (HSBO). These two fats were transesterified in methanol containing sodium methoxide, and the recovered esters were distilled. Analyses of these esters are shown in Table I.

Analytical Procedures

The fatty acid composition in Table I are area percentages obtained by gas chromatography on a packed polyvinyl acetate column with an argon radium D detector. National Institutes of Health standard mixture D (5), when analyzed by this method, gave values for the component esters varying by +2.3 to −3.2 from the weight percentages. Iodine values and conjugated diene were determined by official AOCS methods (2). Alkali isomerizations were run 4¾ hr at 180C to conjugate *trans*-containing dienoic esters (6). Lipoxidase isomerizations were by MacGee's procedure (9). Isolated *trans* bonds were measured in CS_2 solution at 10.4 μ from a baseline drawn tangent to the infrared absorption curve at approximately 9.3 and 10.75 μ. Methyl elaidate was used as the standard.

Azelaic acid (Table I), as well as other dibasic acids in these samples and subsequent fractions, was determined by saponifying a small part of the esters; the recovered acids were oxidatively cleaned with periodate-permanganate (7). Cleaved monobasic and dibasic acids were converted to their methyl esters with 10% boron trifluoride as the catalyst. The mix-

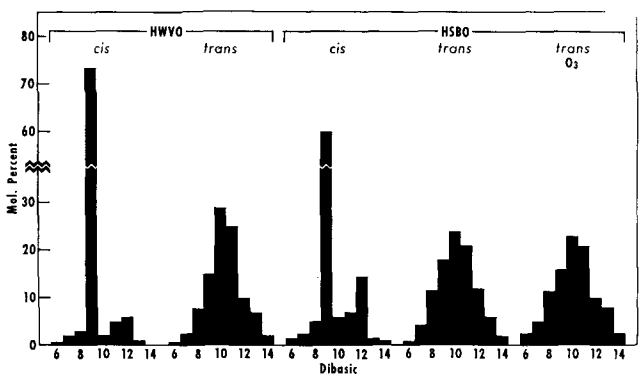

FIG. 1. Dibasic acids from cleavage of *cis* and *trans* monoenes from a hydrogenated winterized vegetable oil (HWVO) and a hydrogenated soybean oil (HSBO).

TABLE II

Composition of Fractions from CCD (Pentane-hexane acetonitrile system)

Fraction	HWVO	HSBO
Monoenoate fraction, %		
Palmitate	17.9	13.3
Stearate	4.1	5.5
Monenoate	78.0	81.1
trans (Isolated)	19.1	38.7
Dienoate fraction, %		
Dienoate, GLC	100.0	100.0
trans (Isolated)	11.2	26.8

ture of monobasic and dibasic esters was analyzed by GLC on two substrates in an Aerograph (16) dual column temperature programming instrument. Packings were 25% DEGS and 25% G.E. silicone SF 96 on 60 to 80 mesh Chromosorb W. The columns were ¼ in. diameter aluminum tubing, 6 ft long, and the helium rate was 95 to 100 ml per minute. A part of each sample of the mixture of monobasic and dibasic acids was also steam-distilled to remove readily volatile monobasic acids. This precaution was necessary because coincident elution of long-chain monoesters and short-chain diesters occurs with either polar or nonpolar substrates on GLC analysis. As a check on the periodate-permanganate cleavage procedure, one monoenoate fraction (*trans* monoenoate of HSBO) was also cleaved by Ackman's (1) ozonization method, and the final esters were analyzed by GLC.

Separation of Monoenoates and Dienoates

The CCD separation procedures used with the esters were quite similar to those described for partially reduced methyl linolenate (12,14). Samples (40 ml) of the methyl esters were distributed using 40 ml of acetonitrile lower phase in each tube and 10-ml portions of pentane-hexane upper layer. Samples were collected by the single withdrawal technique. To obtain sufficient dienoate for the subsequent argentation CCD, it was necessary to make four separations of the conventional HSBO and to combine the dienoate fractions. Analyses of the monoenoate and dienoate fractions used for argentation CCD are shown in Table II.

A calculation of fatty acid composition made by GLC analysis of all CCD fractions and summation of the composition of each fraction agree well with GLC data in Table I, but the CCD calculation, which is more sensitive for minor components, shows about 0.8% trienoate in HWSO.

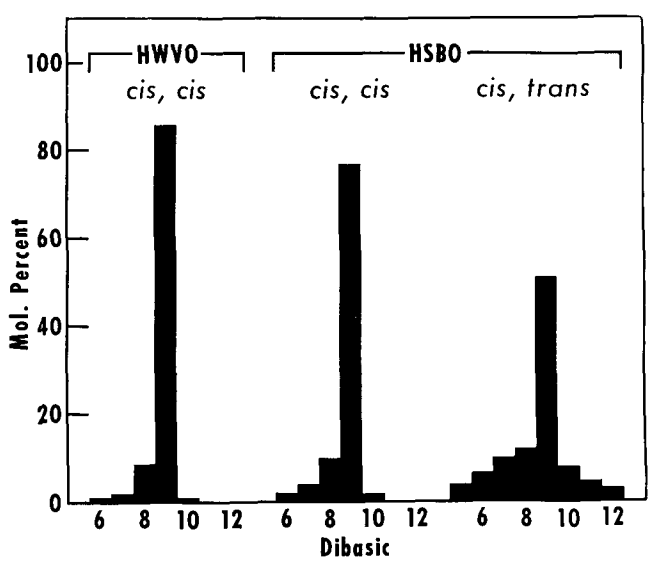

FIG. 2. Dibasic acids from cleavage of *cis,cis* and *cis,trans* dienes in two different oils.

TABLE III

Composition of Fractions from CCD for Oxidative Cleavage (Argentation system)

Fraction	HWVO		HSBO	
Monoene fractions, %	*cis*	*trans*	*cis*	*trans*
Palmitate	0	50.1	0	16.2
Monoenoate	100.0	40.9	100.0	83.8
trans	0	46.6	0	76.9
Azelaic acid on cleavage, mole	73.7	15.0	59.6	17.9
Diene fractions, %	*cis,cis*		*cis,cis*	*cis,trans*
Conjugated diene	6.7		8.4	10.9
Alkali conjugable diene	92.1		83.7	65.0
Lipoxidase conjugable	88.2		82.6	0.5
9,12-Dienoate, capillary GLC	87.0		81.0	1.3
trans	0		13.0	95.7
Azelaic acid on cleavage, mole	85.7		76.9	51.1

TABLE IV

Summary of Unsaturation in Fat Products; Comparison with Original Ester Analyses

Ester	HWVO	HSBO
Unsaturation at carbon 9		
cis Monoenoate	25.4	19.6
trans Monoenoate	1.7	5.4
cis,cis Dienoate	30.0	12.8
Mono-*trans* dienoate	.....	3.1
Experimental total	57.1	40.9
Original ester total	62.4	38.9
Unsaturation of all other carbons		
cis Monoenoate	9.1	13.3
trans Monoenoate	9.5	24.7
cis,cis Dienoate	5.0	3.9
Mono-*trans* dienoate	.....	3.0
Experimental total	23.6	44.9
Original ester total	24.6	46.9

Argentation CCD

Geometric isomers were separated by argentation CCD to obtain fractions for oxidative cleavage and double bond locations. The separation of components between 40-ml portions of 0.2 *M* silver nitrate in 90% methanol (10% water) and 10-ml portions of pentane-hexane was monitored by an automatic refractive index recording unit (3). With the monoenes, single-tube withdrawal operation and collection of saturates were begun after recycling to 650 transfers. Elution of *cis* monoenoates was completed after 960 transfers. The *trans* and *cis* monoenoates gave well-resolved peaks, which after distillation were saponified for subsequent cleavage and double bond location.

Because of the wide range of partition coefficients of the dienoic esters, it was not possible to recycle them; consequently, separation of the dienoic fractions was less complete. Analyses of fractions chosen for cleavage appear in Table III and results of oxidative cleavage and dibasic acid analysis in Figures 1 and 2.

Results and Discussion

Composition of Unfractionated Materials

Analyses of the esters derived from the two hydrogenated products showed similarities and differences. Although GLC (Table I) indicated about the same percentage of saturates, HWVO gave lower monoenoate but higher dienoate, as well as some trienoate. The melting range for HWVO acids was 20–25C and for HSBO acids, 27–32C; moreover, the iodine value of HWVO acids was higher. The salient differences were lower *trans* (as elaidate) and a higher isomerizable dienoate for HWVO. The HWVO on cleavage gave 71.7 mol % azelaic acid, whereas the HSBO gave only 45.3 mol %. Lipoxidase isomerization, a measure of *cis,cis* methylene interrupted diene, was in good agreement with alkali isomerization for both products.

From the acid compositions in Tables I and II and from the cleavage data illustrated in Figures 1 and 2, we can calculate the amount of various fatty acids in the fats. From HWVO as shown in Table II, a monoene fraction was obtained containing 19.1% *trans* esters but which also contained 22% saturated esters. Then HWVO monoene contains $19.1 \times 1/(1.00 - .22) = 24.5\%$ *trans* esters and $100 - 24.5$ or 75.5% *cis* esters. From Table III and Figure 1 *cis* monene has 73.7% of the double bond in the 9,10 position. Then 75.5×0.737 or 55.6% of the monoene is *cis*-9-octadecenoate. Since HWVO contains 45.6% monoene, it follows that 45.6×0.556 or 25.4% *cis*-9-octadecenoate(oleate) is present in the HWVO esters. By a similar calculation HWVO is seen to contain $0.755 \times (1 - 0.737) \times 45.6$ or 9.1% *cis* monoene with unsaturation at all positions other than the ninth carbon. The *trans* monoenes have 15% of the double bonds in the 9,10 position so HWVO has $0.245 \times 0.15 \times 45.6$ or 1.7% *trans*-9-octadecenoate(elaidate) and $0.245 \times (1 - 0.15) \times 45.6$ or 9.5% *trans* monoene with unsaturation at all other carbons. Similar calculations for the amount of *cis* and *trans* isomers at each double bond position can be made to provide a complete analysis of the monoenes in terms of double bond position and geometric configuration.

Similar calculations can be made for the dienoic esters. The diene fraction obtained from HWVO by acetonitrile CCD contained 11.2% *trans* bonds (Table II). Because this value is small and because no evidence for *trans,trans* esters was found by argentation CCD this fraction is assumed to represent 11.2% mono-*trans* dienoates. Then $100 - 11.2 = 88.8\%$ of the dienoate fraction that is the *cis,cis* form. From Table III and Figure 2 *cis,cis*-dienoate has 85.7% of the double bond nearest the carboxyl in the 9,10 position; from Table I, HWVO contains 39.4% diene. Therefore, HWVO has $0.888 \times 0.857 \times 39.4$ or 31.2%

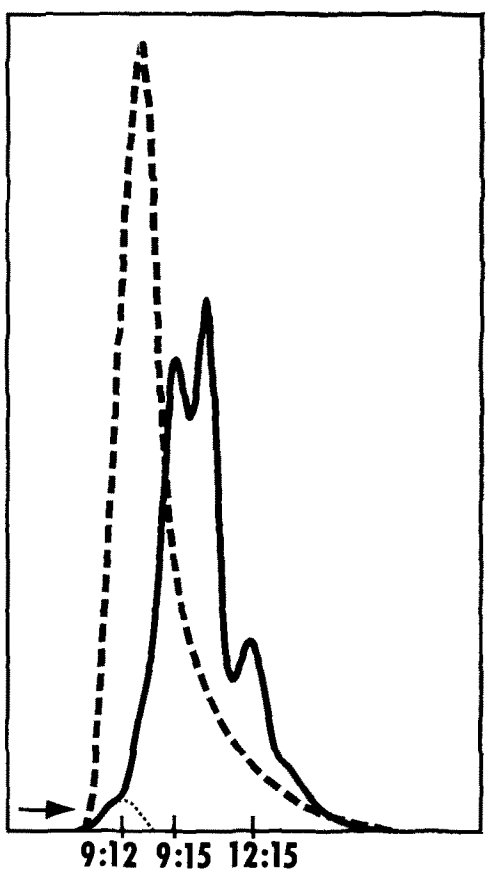

FIG. 3. Superimposed capillary chromatograms for *cis, trans* HSBO (solid line) and for *cis,cis* HWVO (broken line) diene fractions.

cis,cis-dienoate with double bond nearest carboxyl in 9,10 position. In argentation CCD of HWVO diene no fraction with more than 54% *trans* bonds was isolated so no suitable fraction was available for location of double bond position in mono-*trans* dienes.

Calculations were made in the same way for HSBO and results for both fractions are summarized in Table IV.

Compositions were also calculated as above, but the values for *cis* and *trans* monoenes and *cis,cis* and mono-*trans* dienes are based on analysis of argentation CCD fractions and summation of the values for all fractions. These compositions check closely those given in Table IV. Argentation CCD does show the presence of small amounts of slow-moving dienes of the 9,15 type amounting to about 1.7% in HWVO and less than 0.1% in HSBO.

From the saturated ester and cleavage data in Table I the terminal unsaturation in the original ester at carbon 9 may be readily calculated.
For HWVO:

Unsaturation at carbon 9 = (71.7) (0.87) or 62.4%

Unsaturation at all other carbons = (100.0 − 71.7) (0.87) or 24.6%

Similarly, we may calculate the unsaturation for the HSBO original ester. Table I shows 45.3 mol % azelaic acid after cleavage at a saturate value of 14.2%, and includes all calculated values:

Terminal unsaturation at carbon 9 = (45.3) (0.858) or 38.9%

Terminal unsaturation at all other carbons = (100.0 − 45.3) (0.858) or 46.9%

These calculated percentages agree with the sum of the unsaturation for all isomers as shown in Table IV. The slightly high original ester totals for HWVO are caused in part by exclusion of any value for mono-*trans* dienoates or for linolenate from the summations.

The values for 9,12-dienoates by capillary GLC in Table III were obtained with a 200 ft x 0.01 in Apiezon L column. The type of separations obtained is illustrated in Figure 3, which depicts two superimposed CGLC chromatograms. The solid curve corresponds to the *cis,trans*-dienoate fraction from HSBO containing 95.7% *trans,* and the broken curve is for a *cis,cis*-dienoate fraction containing no *trans*. Emergence positions for a standard *cis,cis* mixture of approximately equal parts of 9,12-, 9,15-, and 12,15-dienoates are indicated on the abscissa. Peak positions for the samples involved were reproducible. The *cis,cis* fraction contained only 9,12-dienoate. The *cis,trans* fraction contained a very small amount of *cis,cis*-9,12-dienoate; a slightly larger amount of *cis, cis*-12,15-dienoate; and two other unknown components, probably isomeric *cis,trans* dienoates.

Conjugated diene was appreciable for both the *cis, cis*-dienoate and the *cis,trans*-dienoate fractions (Table II), as contrasted with the small amount in the original shortenings. Alkali and lipoxidase isomerizations and CGLC analysis for *cis,cis*-9,12-dienoate were in good agreement for both hydrogenated products. Lipoxidase isomerization for the HSBO dienoate was negligible at a *trans* value of 95.7% and increased with decreasing *trans* content.

Although our chief interest was to estimate the amounts of "natural" oleic and "natural" linoleic acid in the two fat products, we can use our cleavage data (Fig. 1) to calculate the amount of any *cis* or *trans* isomeric monoenoate present in them merely by inserting the amount of dibasic acid. For example, the percentage of dodecane dicarboxylic acid (C-12) for the *cis* and *trans* monoenoate fractions for HWVO is 6.0 and 11.0%, respectively. Inclusion of these values in our calculations gave 2.1 and 1.2% for the *cis* and *trans* monoenoate at carbon 12:

cis Unsaturation at carbon 12 = (0.060) (76.56) (0.465) or 2.1%

trans Unsaturation at carbon 12 = (0.110) (23.44) (0.456) or 1.2%

Similar calculations may be performed to establish the positions of the unsaturated bonds nearest the carboxyl for *cis,cis*-dienoate and for *cis,trans*-dienoate.

The amount of natural linoleic acid (*cis,cis*-9,12-ocatadecadienoic acid) in the human diet is important to know because of its reported role in atherosclerosis and as an essential fatty acid. The values for linoleic acid reported here must not be considered as final. As demonstrated, the usual GLC analysis does not distinguish isomeric octadecadienoates from true linoleic acid. Alkali isomerization is more specific and measures principally pentadiene systems regardless of their relative position in the molecule, and to some extent irrespective of the configuration of double bonds. Lipoxidase isomerization has the advantage of measuring only *cis,cis*-pentadiene systems but, for example, includes 12,15-dienoates along with linoleic acid. CGLC does distinguish linoleic acid from the 9,15 and the 12,15-isomers but still probably gives a maximum value for linoleic acid. All these measures of true linoleic acid become more specific when preceded by argentation CCD and when combined with a knowledge of the percentage of azelaic and other dibasic acids after double bond cleavage. Although the present procedures are obviously too involved for routine application, simplifying and developing the general procedure may result in a practical method for determining natural linoleic acid.

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