The octadecatrienoic fatty acids of partially hydrogenated soybean oil (PHSBO) were concentrated, isolated and analyzed. The results indicated that the 18:3 acids present in PHSBO are composed of four isomers. The isomer present in the largest amount (2.7%) is the all cis isomer, c9,c12,c15-18:3 linolenic acid, and comprises 68.60% of all the isomeric 18:3 acids of PHSBO.

The remaining three 18:3 isomers found were t9,t12,c15-18:3, t9,c12,c15- and c9,c12,t15-18:3, which in total accounted for 1.2% of the total fatty acids of PHSBO.

Soybean oil is a major vegetable oil in the world market. However, one disadvantage in its use as an edible oil is its relatively high (7-8%) linolenic acid content. Linolenic acid (c9,c12,c15-18:3) is highly susceptible to autoxidation, which results in the production of undesirable flavor and odor components in soybean oil.

It is common practice to subject liquid soybean oil to partial and selective hydrogenation which serves to lower the linolenic acid content to ca. 3%, thereby improving the oxidative stability of the oil. Aside from functioning to saturate the double bonds, partial hydrogenation also produces geometric and positional isomers of the unsaturated fatty acids present in the oil (1,2).

Linolenic acid is now thought to function as an essential fatty acid in the human diet (3); it may have some further function in nervous tissue (4) and acts as a prostaglandin precursor (5). Soybean oil is a natural source of linolenic acid. Other dietary sources of linolenic acid are less readily available.

It is therefore of practical importance to know the amount of linolenic acid (c9,c12,c15-18:3) as well as the quantity and types of trienoic isomers formed from linolenic acid via partial hydrogenation of soybean oil (PHSBO).

In the present study the content and types of trienoic acids present in a representative sample of PHSBO were determined. This (PHSBO) is typical of that produced for use as both a salad and a cooking oil (1,2).

**MATERIALS AND METHODS**

Soybean oil. A representative sample of partially hydrogenated soybean oil in the form of a liquid salad oil was purchased from a commercial grocer.

Preparation of free fatty acids. Free fatty acids were prepared by heating 10 g of partially hydrogenated soybean oil (PHSBO) in 100 ml of 2.5% potassium hydroxide in ethanol under reflux conditions. After heating for 2 hr the solution was acidified to pH 2 with cold 1% H2SO4 in water (v/v). The free fatty acids were extracted using 3- to 100-ml portions of hexane:ethyl ether (50:50). The organic extract was washed with water, dried over sodium sulfate and the solvent removed under vacuum with a rotary evaporator.

Methylation. Methyl esters were prepared from free fatty acids using boron trifluoride-methanol according to AOCS method Ce 2-66 (6).

Hydrazine reduction. Methyl esters (3-5 mg) were dissolved in 10 ml of 96% methanol. The solution was heated to 50 C and oxygenated (ca. 1-2 ml/min) for the time of the reaction. One ml of anhydrous hydrazine was added, followed by heating for 2 hr. The reaction was terminated by an addition of 3N HCL to pH 4-5 (7). The methyl esters were extracted with 3 × 7 ml hexane as described in the preparation of fatty acids.

Fractional crystallization. A 5% solution of fatty acids in acetone was cooled at −40 C in an acetone/dry ice bath.
for 30 min. The -40 C filtrate was collected along with two cold acetone rinses (-67 C) of the -40 C crystals, and again cooled to -54 C. The -54 C filtrate and acetone rinses were collected, combined, solvent evaporated and resultant fatty acids recovered.

Reverse phase high performance liquid chromatography. The system consisted of a dual piston Tracor 995 isocratic pump (Tracor Instrument, Austin, Texas); a Rheodyne loop injector (20 l) (model 7120); a Waters R401 differential refractometer (Waters Assoc., Milford, Massachusetts); and a Hewlett Packard 3390A reporting integrator (Hewlett Packard, Palo Alto, California). The column was a stainless steel column of octadecyl bonded spherical silica (LC-18, Supelco, Supelco Park, Bellefonte, Pennsylvania), 25 cm × 4.6 mm and 5µ particle size.

Elution with methanol as the mobile phase at 0.7 ml/ min effected separation of methyl esters according to degree of unsaturation. The trienoic methyl esters were separated with acetonitrile:water (4:1) at 2.0 ml/min as the eluting solvent.

Recovery of the methyl esters from the acetonitrile: water eluant was achieved by adding a volume of both CH2Cl2 and H2O (equivalent to the volume of ACN:H2O) to the eluate. The organic phase was removed and the remaining aqueous phase extracted twice more with CH2Cl2. Recovery of the methyl esters from the combined organic phases was as outlined in preparation of free fatty acids.

Argentation thin layer chromatography. Thin layer chromatography was carried out on glass plates coated with 10% silver nitrate impregnated silica gel (10µ) of 0.5 mm thickness (Supelco Redi Coats-AG). The plates were activated at 110 C for 30 min and stored over saturated calcium chloride (8). Methyl esters were applied in solution with hexane (1 mg/ml). The plates were then serially developed in chloroform 4 times, sprayed with 0.2% dichlorofluorescein in EtOH and viewed under UV light (254 nm) (9).

The methyl esters were recovered by scraping a band into 40 ml of 1% aq. HCL. Hexane (20 ml) was added, the mixture was warmed for 1–2 min under reflux conditions, cooled and extracted with diethyl ether:hexane (1:1). The combined extracts were rinsed twice with water (20 ml) then saturated NaCl solution (to remove any remaining dye) and finally with water. The organic extract was dried, filtered and evaporated in the usual manner.

Gas liquid chromatography. Analytical gas liquid chromatography (GLC) of fatty acid methyl esters (FAMEs) was carried out with a Hewlett Packard 5790 gas chromatograph fitted with a flame ionization detector (FID) and Hewlett Packard 3390A integrator. The column used was a +75% cyano propyl coated fused silica capillary column: SP2560 (Supelco Inc.), 100 m × 0.25 cm, with a 0.2 µm film thickness. The gas chromatographic conditions were a split mode inlet system at 1:100 split ratio, hydrogen as the carrier gas at 19 cm/sec linear gas rate and column pressure of 19 psi. Injector and detector temperatures were 250 C.

The concentration of sample injected onto the column was based on the concentration of a single component of interest; the concentration range for a single component was 0.5–2.0 µg/µl. The sample concentration ranged from 2.0–5.0 µg/µl. The volume injected ranged from 2.0–4.0 µl.

The equivalent chain length values (ECL) of the unsaturated fatty acids were calculated according to the method described by Miwa et al. (10) and modified by Scholfield (11). RESULTS AND DISCUSSION

The GLC fatty acid profile of PHSBO fatty acid methyl esters is illustrated in Figure 1. Several unknown peaks designated A, B, C and a major peak D appear in the area associated with trienoic FAMEs. Component D, which is 2.7% of the total oil (by GLC), has the retention time of pure methyl linolenate (e9,c12,c15-18:3).

Fractional crystallization at -40 C was used to remove from solution a major portion of the 16:0 and 18:0 saturated fatty acids. Recrystallization of the -40 C filtrate at -54 C resulted primarily in removal of monoenoic fatty acids. The -54 C filtrate consisted of a mixture of mono-, di- and trienoic 18 carbon FAME of relative proportions 40.6%, 55.6% and 3.7%, respectively, as monitored by GLC.

Reverse phase high performance liquid chromatography (RP-HPLC) of the FAMEs resulting from fractional crystallization effected separation of these methyl esters based on equivalent carbon number or unsaturation. This technique allowed the isolation of the trienoic acid fraction from the mono- and dienoic acids (Fig. 2). Subsequent GLC analysis indicated the presence of four components (peaks A, B, C and D, Fig. 3) located in the trienoic acid region and which are identical in retention time to those previously detected in the PHSBO. The relative area percent of each component peak is given in Table 1.

Scholfield (11) has reported elution orders and equivalent chain length (ECL) values for the geometric isomers of methyl linolenate separated on a 100-m glass capillary Silar 10C column (75% cyano propyl) (Table 2). The Silar 10C coated column is only slightly less polar.

### TABLE 1

| Component Peaks in the Isolated 18:3 FAMEs of PHSBO |
|-----------------|---|---|---|---|
| Peak | A | B | C | D |
| Relative Area% | 15.44 | 4.65 | 10.10 | 68.60 |
| Relative Area% | 0.78 | 2.50 | 3.70 | 0.91 |

x. Average of 3 runs (GLC); s%, relative standard deviation.

FIG. 3. Gas liquid chromatogram of the 18:3 isomers of PHSBO. Column, SP2560; temperature, 170 C; sample volume, 3 µl; sample concentration, 2 mg/ml; further conditions in Fig. 1.