Alteration of Soybean Oil Composition by Plant Breeding

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ABSTRACT

Experimental lines selected from the cross PI 90406 x PI 92567 are being used in an attempt to improve soybean (*Glycine max* [L.] Merr.) oil by altering fatty acid composition through plant breeding. Preliminary evidence shows that the concentration of linolenic acid in soybean oil is reduced by selection for high levels of oleic acid. Levels of polyunsaturated acids in "high oleic" selections are lower, to various degrees, but the concentration of saturated fatty acids is not different from that of the variety Dare, a representative southern commercial cultivar. In triglyceride from the "high oleic" selection, N70-3436, levels of palmitic, stearic, oleic, linoleic, and linolenic acid are 9.5, 2.0, 40.1, 43.3, and 5.1 mol %, respectively. The types of triglyceride structures observed in the experimental lines which were examined also are changed. The combined level of triolein, monooleyl-dilinolein, and dioleyl-monolinolein in seed from N70-3436 is doubled and constitutes ca. 50% of the oil.

INTRODUCTION

Edible vegetable oils are projected to constitute nearly 2/3 of the world supply of fat and oil produced in 1980 (1). The number of agronomic crops contributing to this supply are relatively few and their importance varies greatly (2). The type of vegetable oils used to prepare products such as salad dressing, margarine, dairy foods, vegetable shortening, and cooking oil also is limited by physical and nutritional properties as well as availability and price.

Recently there has been considerable expansion of research to provide the basis for genetic manipulation of oil content and fatty acid composition for several crops (2,3). One objective in soybean breeding is the improvement of oil flavor and stability by the reduction of linolenic acid (18:3) concentration. The level of 18:3 in soybean oil is lowered indirectly by selecting for lines with high oleic acid (18:1) content (4,5). Several lines with elevated 18:1 levels have been developed. We report the effect selection for high 18:1 has on the fatty acid composition and triglyceride (TG) distribution in three experimental strains of soybean.

MATERIALS AND METHODS

Soybeans, *Glycine max* (L.) Merr., var. Dare, and the experimental lines N70-3436, N70-3432, and N70-3001 developed by C.A. Brim at the Central Crops Research Station, North Carolina Agricultural Experiment Station at Raleigh, during the 1974 growing season were used in this study. The experimental lines were selected for high 18:1 from the F_3 generation of the cross PI 90406 x PI 92567. Standard analytical methods for lipid extraction, and determination of oil content and fatty acid distribution in the fractionated oil were performed as described by Wilson and Rinne (6). Fatty acid methyl esters were separated by gas chromatography (GC) using a Hewlett Packard 5750 with flame ionization detector (FID) and 3380A reporting integrator. The column (2.74 m x 3.17 mm) was packed with 15% diethylene glycol succinate (DEGS) on 80/100 mesh acid washed Chromosorb W. Column temperature was 195 C; the injection port and FID were held at 250 C. Helium flow was maintained at 35 ml/min. Methyl heptadecanoate (17:0) was used as an internal standard. Triglyceride molecular species were fractionated by argentation thin layer chromatography (TLC), and total TG fractions were hydrolyzed with pancreatic lipase (7). Lipids were extracted from TLC gels by successive centrifugation at 3000 *g* for 5 min in 12-ml glass centrifuge tubes with 5 ml $CHCl_3$:CH_3OH (2:1), $CHCl_3$:CH_3OH (1:2), and CH_3OH, respectively. All supernatant fractions were combined and reduced in volume nearly to dryness under vacuum at 30 C. Lipid extracts were stored at -20 C in $CHCl_3$:CH_3OH (2:1) containing 50 μg butylated hydroxytoluene (BHT) per ml as described by Wilson and Rinne (6).

RESULTS

Oil content and levels of TG, diglyceride (DG), and total polar lipids (TPL) for three experimental "high oleic" lines and the var. Dare are given in Table I. Among the three lipid fractions isolated, TG accounts for the bulk of the lipid extract. The fatty acid distribution in TG for each line is given in Table II. The concentration of 18:1 apparently is inversely related to the combined level of linoleic acid (18:2) and 18:3. In TG from N70-3436, levels of 18:1 (40.1 mol %) represent an increase of 122% over 18:1 in TG from Dare, while 18:2 (43.3 mol %) and 18:3 (5.1 mol %) show a decrease of 29 and 37%, respectively. Compared to Dare, the levels of the saturated fatty acids palmitic (16:0) and stearic (18:0) are unchanged in the high 18:1 selections.

Changes in proportions of fatty acids by means of genetic shifts inevitably must lead to changes in proportions of TG molecular species. The combined level of the TG structures D_2T, D_3, SD_2, and SMD found in the oil extracts from the var. Dare and N70-3001, N70-3432, and

TABLE I

Oil Content of Soybeans

Line	Triglyceride (μm)[a]	Triglyceride (%)[b]	Diglyceride (μm)	Diglyceride (%)	Total polar lipids (μm)	Total polar lipids (%)	Grams dry wt per 100 seed	Percent oil (NMR)	Grams oil per 0.5 g dry wt
N70-3436	86.3	91.1	2.5	2.7	5.9	6.2	17.9	21.3	0.106
N70-3432	67.8	90.3	2.0	2.7	5.2	7.0	16.3	21.1	0.105
N70-3001	75.0	89.6	2.9	3.5	5.8	6.9	17.9	21.9	0.109
Dare	74.1	90.7	2.5	3.1	5.1	6.2	13.5	24.2	0.121

[a]μmol/0.5 g dry weight, calculated from fatty acid methyl esters.

[b]Mol % oil (excluding free fatty acids, unsaponifiable hydrocarbons, terpenes, sterols, and tocopherols).

TABLE II

Fatty Acid Distribution of Triglyceride

Soybean line	Mol %				
	16:0	18:0	18:1	18:2	18:3
N70-3436	9.5	2.0	40.1	43.3	5.1
N70-3432	10.5	1.5	38.1	44.2	5.5
N70-3001	11.6	1.9	34.7	45.8	6.0
Dare	10.6	1.9	18.1	61.2	8.1

DISCUSSION

Rizek et al. (10) estimated that the average consumption of fat in the United States (1972) was 156 g/person/day. This represents an increase of 20% over estimates from the early 1900s, and can be directly attributed to increased use of vegetable oil. Although animal fat still provides > 60% of the daily nutrient fat consumed per person, dietary trends indicate greater future demand for edible vegetable oils. Most edible vegetable oils contain high levels of polyunsaturated fatty acids. Although there is little supportive evidence, the popular view that substitution of dietary polyunsaturates for saturated fatty acids may help reduce the incidence of cardiovascular accidents has stimulated the demand for vegetable oils (11).

TABLE III

Mol Percent Triglyceride Molecular Species in Seed Oil

TG[a] species	Soybean				Safflower[b]	Sunflower[b]	Cotton[b]	Peanut[b]	Corn[c]
	Dare	N70-3001	N70-3432	N70-3436					
D_2T[d]	13.5	12.9	12.1	15.6	--[e]	--	--	--	0.2
D_3	26.7	19.7	22.0	15.2	47	14	11	--	22.3
MD_2	13.3	18.9	21.5	20.7	19	39	9	5	25.2
SD_2	23.4	12.7	11.3	13.3	18	14	27	--	16.2
M_2D	9.2	12.0	9.5	11.6	5	19	5	23	11.8
SMD	7.9	8.2	3.7	4.1	7	11	20	14	12.9
M_3	3.1	11.8	14.3	17.8	1	--	2	26	3.6
S_2D	--	--	--	--	2	1	17	4	3.1
SM_2	2.2	3.3	5.3	1.5	1	1	3	23	3.5
S_2M	0.6	0.4	0.3	0.2	--	1	6	5	1.2

[a]S = 16:0 + 18:0, M = 18:1, D = 18:2, T = 18:3.
[b]Ref. 8.
[c]Ref. 9.
[d]Also contains other unresolved species.
[e]Not detected.

TABLE IV

Fatty Acid Distribution at *sn*-Position 2 of Triglyceride in Various Vegetable Oils

Seed oil	Mol %				
	16:0	18:0	18:1	18:2	18:3
N70-3436	0.8	0.2	51.5	42.6	4.9
N70-3432	0.9	0.2	48.6	45.3	5.0
N70-3001	0.9	0.2	42.3	50.4	6.1
Dare	1.0	0.2	21.2	68.9	8.6
Olive[a]	1.4	Trace	82.9	14.0	0.8
Corn[a]	2.3	0.2	26.5	70.3	0.7
Peanut[a]	1.6	0.3	58.5	38.6	Trace

[a]Ref. 8.

N70-3436, respectively, is 71.5, 53.5, 49.1, and 48.2% of the total TG; and the composite amount of M_3, MD_2, and M_2D accounts for 25.6, 42.7, 45.3, and 50.1% of the TG structures in the respective lines; where S = 16:0 + 18:0, M = 18:1, D = 18:2, and T = 18:3 (Table III). The positioning of 18:1 in TG from high 18:1 lines apparently is not a random process. In fact, there must be selective utilization of DG in the formation of these molecules. Therefore, selection for high 18:1 soybean oil has resulted in simultaneous selection for specific TG combinations. For comparative purposes, we show the TG distribution reported in safflower, sunflower, cotton, peanut, and corn in Table III (8,9).

The fatty acid distribution of *sn*-position 2 monoglycerides (MG) from lipase hydrolysis of total TG fractions in Dare and the experimental lines is given in Table IV. The level of the essential dietary fatty acid, 18:2, at *sn*-position 2 in the oil from Dare is high and compares to that reported for corn oil (8). However, as the TG structures in high 18:1 lines are modified, 18:2 decreases at the *sn*-position 2. In N70-3436, the concentration of 18:2 at this position is similar to that reported in peanut oil (8).

The fatty acid composition reported for several edible vegetable oils is given in Table V (12-14). Soybean oil, which accounts for 73% of all edible vegetable oils used in the United States (12), also has high polyunsaturated levels. Raw soybean oil, however, has a greater proportion of 18:3 than most other edible vegetable oils. Since oxidation of 18:3 is primarily responsible for soybean oil unstability (15), reduction of the concentration of 18:3 in extracted oil by selective hydrogenation is necessary for improved oil flavor and stability (16). The fatty acid distribution reported for soybean oil hydrogenated to various degrees is included in Table V. A significant proportion of *trans* fatty acid isomers are generated in hydrogenated oil by rearrangement of *cis* bonds during saturation. The effect of dietary levels of *trans* fatty acid isomers on human health is not clearly defined; however, recent evidence suggests these isomers may disrupt certain metabolic processes (11).

It seems desirable, therefore, to search for soybean types having lower levels of unsaturated oil. Four thousand varieties in the World Soybean Collection have been surveyed (4). The range of 18:2 and 18:3 is 37-60% and 6-13%, respectively. Breeders have attempted to modify soy oil composition by selection (4), and by induction of mutants followed by appropriate genetic manipulation (5). Progress may be hindered by genotype x environment interaction (17); however, our results show that selection can result in substantial alteration of soybean oil composition.

Synthesis of polyunsaturated fatty acids in soybean seed, as well as in several other crops, is reported to occur by consecutive desaturation of 18:1 (18-20). Therefore, selection for high 18:1 content should result in reduced synthesis of 18:2 and 18:3. In the strain N70-3436, the relative level of 18:1 is ca. 22 mol % greater than in the control variety, while the relative concentration of 18:2

TABLE V

Fatty Acid Distribution of Major Edible Vegetable Oil Sources (%)

Fatty acid	Olive[a]	Peanut[a]	Safflower[a]	Cotton[a]	Corn[b]	Sunflower[c]	Palm[a]	Partially and fully hydrogenated soybean[a]		
16:0	13.6	11.0	7.8	25.0	11.1	5.8	46.8	11.0	11.0	11.0
18:0	3.2	2.3	2.7	2.8	2.0	5.3	3.8	4.0	7.0	83.0
cis										
18:1	74.0	51.0	13.9	17.1	24.1	19.8	37.6	27.0	24.0	--[d]
trans										
18:1	--	--	--	--	--	--	--	21.0	52.0	6.0
18:2	6.7	30.9	73.0	52.7	61.9	68.6	10.0	34.0	6.0	--
18:3	0.8	--	0.8	--	0.7	--	--	3.0	--	--
Iodine value	88	100	143	57	124	133	56	107	76	5

[a]Ref. 12.
[b]Ref. 13.
[c]Ref. 14.
[d]Not detected.

and 18:3, respectively, is ca. 18 and 3 mol % lower. The distribution of TG species in these new lines also has been altered. Lipase hydrolysis of total TG reveals increases in 18:1 and reduction of 18:2 and 18:3 at the *sn*-position 2 (Table IV).

Although raw soybean oil probably should have 18:3 concentrations of 3 mol % or less to remain stable without hydrogenation (2), the trend shown here for lowering 18:3 in soybean oil is evidence that the problem can be approached by selection for high 18:1. Further selection now underway for lines with higher 18:1 may achieve this goal.

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