14. Steiman, I., J.J. Myher, A.G.D. Hoffman and A. Kuksis. Proc. Can. Fed. Biol. Soc. 24:182. Abstr. No. 328 (1981).
15. Curstedt, T. Biochim. Biophys. Acta 398:265 (1975).
16. Wadke, M., H. Brunengraber, J.M. Lowenstein, J.J. Dolhun and P. Arsenault. Biochemistry 12:2619 (1973).
17. Myher, J.J., A. Kuksis, L. Marai and S.K.F. Yeung. Anal. Chem. 50:557 (1978).
18. Bugaut, M., J.J. Myher, A. Kuksis and A.G.D. Hoffman. Biochim. Biophys. Acta 792:254 (1984).
19. Pind, S., A. Kuksis, J.J. Myher and L. Marai. Can. J. Biochem. Cell Biol. Submitted (1985).
20. Voyksher, R.D., J.R. Hass and M.M. Bursey. Anal. Chem. 54:2465 (1982).
21. Glaumann, H., A. Bergstand and J.L.E. Ericsen. J. Cell. Biol. 64:356 (1975).

[Received July 6, 1984]

Fatty Acid Development in a Soybean Mutant with High Stearic Acid[1]

G.L. GRAEF, Department of Agronomy; **L.A. MILLER**, Department of Food Technology; **W.R. FEHR**, Department of Agronomy, and **E.G. HAMMOND**, Department of Food Technology, Iowa State University, Ames, Iowa 50011

ABSTRACT

The fatty acid composition of developing soybean (*Glycine max* [L.] Merrill) seeds was evaluated in the mutant line, A6, and its parent, FA8077. Seeds of both lines were harvested at 2-day intervals from 15 to 39 days after flowering (DAF) and at 4-day intervals from 39 DAF until maturity. Significant differences between the two lines were observed for stearic and oleic acid percentages at 19 DAF. The maximum difference between the lines was at 25 DAF, when A6 had 45.4% and FA8077 had 4.1% stearic acid. The increase in stearic acid percentage in A6 was accompanied by a decrease in oleic acid to 16.8% at 25 DAF, compared with 53.7% oleic acid for FA8077. The two lines did not differ in development of palmitic, linoleic and linolenic acids. The protein and oil content of mature seeds were similar for the two lines.

INTRODUCTION

A soybean (*Glycine max* [L.] Merrill) mutant with high stearic acid was identified at Iowa State University in 1981. It was an M_2 plant selection from sodium azide (NaN_3) treatment of seeds from the line FA8077. The mutant, designated A6, showed a fatty acid composition never before reported for soybean (1). The average stearic acid content of soybean oil is 4.0%, with a range from 2.2% to 7.2% for genotypes available in the world collection (2). A6 contained about 28% stearic acid in its seed oil. Graef et al. (5) determined from the cross of A6 × FA8077 that the high stearic acid in A6 was controlled by a recessive allele at one locus.

In previous studies of developing soybean seeds, the stearic acid percentage of the oil seemed to remain relatively constant (3,4). Fehr et al. (3) reported that stearic acid percentages remained more or less constant during seed development, from 26 days after flowering (DAF) to maturity. Rubel et al. (4) observed a decrease in stearic acid percentage from 8% at 24 DAF to 3% at 32 DAF. The stearic acid percentage remained constant from 32 DAF until maturity. Palmitic and linolenic acid decreased in percentage during seed development, while oleic and linoleic acid percentages increased (3,4).

The objective of our study was to determine the stage in seed development at which the deviation in stearic acid synthesis for A6 is initiated and to examine the effect of high stearic acid percentage on the development of other fatty acids.

PROCEDURES

A6 and FA8077 were grown in 1.5-m rows spaced 1 m apart at the Agronomy Research Center near Ames, Iowa in May 1982. Two hundred and forty pods were tagged at the third, fourth or fifth node from the top of the main stem of plants of both lines (6). The age of seeds in each pod was expressed as DAF, which was the number of days between fertilization of the flower and harvest of the pod.

Seeds were harvested at 2-day intervals from 15 to 39 DAF and at 4-day intervals from 39 DAF to maturity (51 DAF). On each of the 16 sampling dates, 15 pods of each line were removed from the plants and taken to the laboratory. The seeds were removed from the pods and the weights of three 10-seed samples of each line were determined. Each 10-seed sample was placed in a test tube and frozen at -29 C until all samples from the 16 dates had been collected.

At the time of fatty acid analysis, each 10-seed sample was freeze-dried for 20 hr, and the dry weight of the samples was recorded. The beans were ground to a fine powder in a mortar with 20 ml/g dry weight of chloroform-methanol (2:1, v/v), then centrifuged for 15 min. A volume of water equal to 1/5 the volume of the chloroform-methanol solution was added to the supernatant. This caused the solvent to separate into two layers. The lower chloroform layer was collected, and the chloroform evaporated. The residual lipids were dissolved in hexane (10 mg/ml), and 1 ml of the hexane solution was transferred to a 2-ml reaction vial and evaporated to about 0.2 ml. Then 0.5-ml of 1.0 M sodium methoxide in methanol was added to the vials and allowed to react for 2 hr to convert the oil to methyl esters. Next, 1 ml of distilled water was added, and one hr later a few drops of distilled hexane were added to dissolve the fatty acids. The top layer of the solution (fatty acids in hexane) was removed and placed in a 2-ml glass vial. Approximately 1.5 µl of each sample were injected into a Beckman GC-5 gas chromatograph (Fullerton, California) fitted with hydrogen flame detectors. The column was 2 m long and 3.2 mm in diameter, packed with 15% EGSSX on Chromosorb W 100/120 mesh and maintained at 185 C. The N flow was 40 ml/min, H flow was 50 ml/min and air flow was 300 ml/min. Standard ester mixtures by Nucheck (Elysian, Minnesota) were run on a regular basis for calibration. A PET 2001 computer (Commodore Business Machines, Santa Clara, California) was used to control injection, integrate peak areas and calculate percentages of palmitic, stearic, oleic, linoleic and linolenic acids.

The oil samples were analyzed in three replications of a randomized complete-block design. A replication consisted of one 10-seed sample from each of the 16 harvesting periods for the two lines. Standard statistical procedures were used for data analysis. For the analysis of variance, lines and sampling periods were considered to be fixed effects.

[1]Journal Paper No. J-11444 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa, Projects 2475 and 2493.

TABLE I

Fresh Weight and Dry Weight of Seeds of A6 and FA8077 at Various Periods of Seed Development

DAF[a]	Fresh weight		Dry weight	
	FA8077	A6	FA8077	A6
	mg/seed			
15	40 ± 4	43 ± 3	8 ± 1	8 ± 0
21	143 ± 10	186 ± 3	36 ± 2	41 ± 1
27	332 ± 23	367 ± 1	94 ± 4	110 ± 2
33	431 ± 3	436 ± 5	152 ± 3	161 ± 2
39	441 ± 16	508 ± 5	186 ± 5	216 ± 8
47	421 ± 7	413 ± 11	208 ± 6	221 ± 5
51	241 ± 16	278 ± 1	183 ± 6	207 ± 7

[a]Days after flowering.

RESULTS AND DISCUSSION

The fresh weight and dry weight of seeds of FA8077 and A6 showed similar trends during seed development (Table I). The dry weight of seeds of A6 was greater than seeds of FA8077 for most of the dates sampled. Protein and oil composition were determined for seeds harvested from mature plants. Oil content on a dry basis was 18.7 ±0.3% for FA8077 and 18.3 ±0.1% for A6. Protein content on a dry basis was 43.9 ±0.1% for FA8077 and 43.3 ±0.1% for A6.

Significant differences between A6 and FA8077 were observed for stearic and oleic acid percentages beginning at 19 DAF (Figs. 1 and 2). The maximum difference between the lines for both fatty acids occurred at 25 DAF, when A6 had 45.4% stearic acid and 16.8% oleic acid compared with 4.1% stearic acid and 53.7% oleic acid for FA8077. At most of the sampling periods, the increase in stearic acid for A6 compared with FA8077 was associated with a proportionate decrease in oleic acid. For example, at 21 DAF the stearic acid content of A6 was 27.1 percentage units higher and oleic acid 26.6 percentage units lower than FA8077 (Table II). Ladd and Knowles (7) observed a similar relationship in safflower (*Carthamus tinctorius* L.). They reported that increases in stearic acid were accompanied by decreases in relative amounts of oleic acid, linoleic acid or both. A6 and FA8077 differed only in development of stearic and oleic acids. There were no significant differences between the lines for percentages of palmitic, linoleic or linolenic acids in mature seeds harvested at 51 DAF (Table II).

The development of stearic acid in FA8077 agreed with that reported for soybean genotypes previously studied (3,4). Stearic acid percentage was relatively high early in development (15 DAF) and decreased rapidly until about 23 DAF (Table II). The stearic acid percentage of FA8077 remained constant from 25 DAF to maturity.

The pattern of stearic acid development observed in A6 has not been reported previously for soybean. The stearic acid percentage of A6 increased rapidly from 17% at 15 DAF to 41% at 23 DAF, then remained more or less constant until maturity, when it decreased slightly. The development of palmitic, linoleic and linolenic acid in A6 and FA8077 was similar to that reported for other genotypes previously investigated (3,4). The results indicate that the genetic change that led to the altered fatty acid composition in A6 did not influence the pattern of development of palmitic, linoleic or linolenic acids.

Specific assays are required to determine the mechanism responsible for the change in stearic and oleic acid in A6. The mutation was shown to be a recessive allele at a single locus (5). Likely mechanisms for the effect of the mutant allele are an altered rate of stearate desaturation or an altered substrate specificity for acyl-ACP hydrolase. Acyl-ACP hydrolase has a high specificity for oleoyl-ACP, favoring oleoyl-ACP hydrolysis (8). If specificity of the enzyme was affected, stearic-ACP hydrolysis might be favored in A6, which would make stearic acid unavailable for desaturation. The stearic acid would be transferred out of the proplastid as free fatty acid and converted to stearoyl CoA for insertion into triacylglycerols.

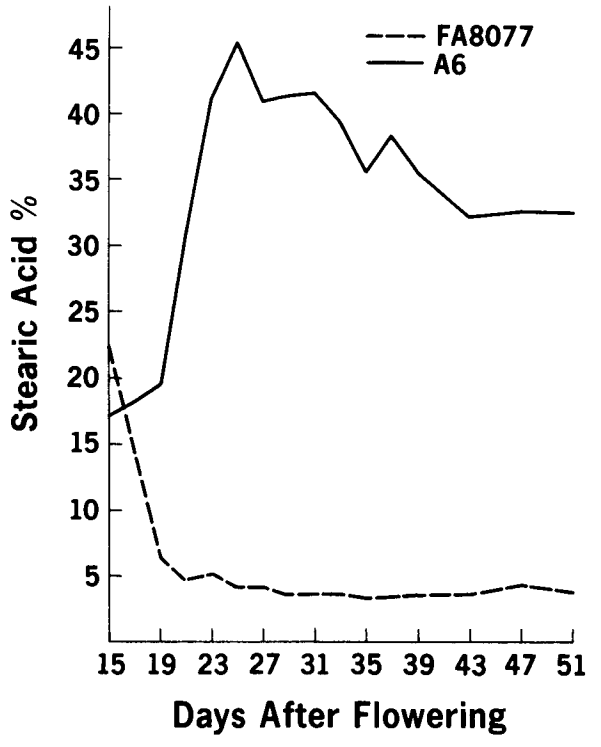

FIG. 1. Stearic acid percentage in oil of A6 and FA8077 during seed development. The least significant difference (p=0.05) for comparing the lines at each sampling period is 4%.

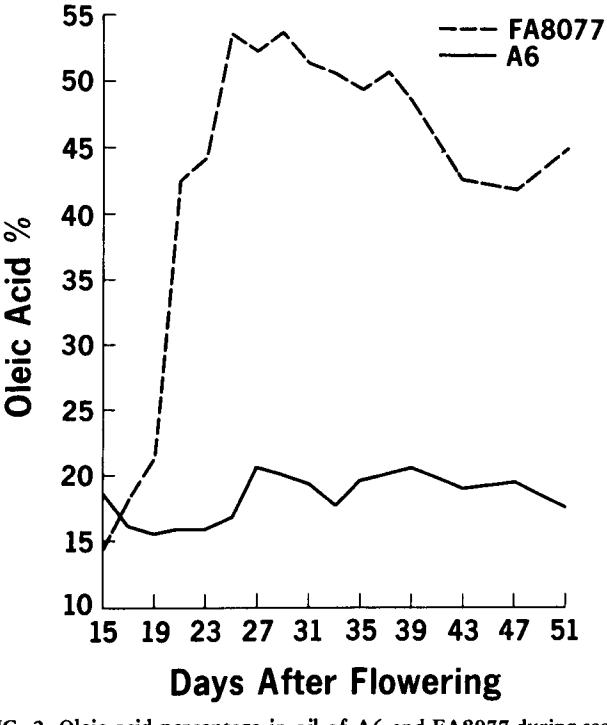

FIG. 2. Oleic acid percentage in oil of A6 and FA8077 during seed development. The least significant difference (p=0.05) for comparing the lines at each sampling period is 3%.

TABLE II

Mean Fatty Acid Percentage of A6 and FA8077 Based on Analysis of Three 10-Seed Samples Harvested at Various Periods of Seed Development

	Palmitic		Stearic		Oleic		Linoleic		Linolenic	
DAF[a]	FA8077	A6	FA8077	A6	FA8077	A6	FA8077	A6	FA8077	A6
15	31.7	25.6	22.6	17.1	14.4	18.7	21.9	26.4	12.9	12.1
17	16.7	23.7	14.9	18.2	18.2	16.1	33.4	27.7	17.2	14.2
19	21.5	16.7	6.5	19.6	21.3	15.6	31.8	32.6	15.4	12.2
21	14.1	10.6	4.9	31.0	42.5	15.9	24.7	28.1	13.7	14.3
23	12.3	9.2	5.1	41.1	44.1	15.9	26.1	22.9	11.4	10.1
25	12.0	8.9	4.1	45.4	53.7	16.8	21.7	21.4	8.3	7.5
27	9.8	7.8	4.1	41.0	52.2	20.8	25.9	22.8	8.0	6.8
29	9.5	7.6	3.8	41.5	53.9	20.0	25.3	23.6	7.5	6.5
31	9.4	7.6	3.7	41.7	51.5	19.2	28.4	24.8	7.0	5.9
33	9.1	8.1	3.7	39.6	50.7	17.8	29.9	28.6	6.4	5.9
35	9.4	8.2	3.3	35.7	49.5	19.6	31.2	30.5	6.6	6.0
37	9.1	8.2	3.6	38.6	50.9	20.0	29.8	27.5	6.6	5.6
39	8.8	8.5	3.8	35.7	48.5	20.6	32.7	29.4	6.3	5.8
43	8.9	8.4	3.8	32.1	42.6	19.0	38.2	34.2	6.4	6.2
47	8.7	8.2	4.3	32.7	41.9	19.5	36.8	33.6	6.3	6.1
51	8.6	8.3	3.9	32.6	45.0	17.8	36.7	35.2	5.8	6.1
LSD (0.05)	7.2		3.8		3.0		4.6		3.0	

[a]Days after flowering.

ACKNOWLEDGMENTS

The research was supported by grants from the American Soybean Association Development Foundation and the Iowa Soybean Promotion Board. P.K. Stumpf and J.B. Ohlrogge provided helpful comments.

REFERENCES

1. Hammond, E.G., and W.R. Fehr, Crop Sci. 23:192 (1983).
2. Downey, R.K. and D.I. McGregor, Curr. Adv. Plant Sci. 12:151 (1975).
3. Fehr, W.R., J.C. Thorne and E.G. Hammond, Crop Sci. 11:211 (1971).
4. Rubel, A., R.W. Rinne and D.T. Canvin, Ibid. 12:739 (1972).
5. Graef, G.L., W.R. Fehr and E.G. Hammond, Ibid. (In press) (1985).
6. Fehr, W.R., C.E. Caviness, D.T. Burmood and J.S. Pennington, Ibid. 11:929 (1971).
7. Ladd, S.L., and P.F. Knowles, Ibid. 10:525 (1970).
8. Ohlrogge, J.B., W.B. Shine and P.K. Stumpf, Arch. Biochem. Biophys. 189:382 (1978).

[Received May 10, 1984]

Lipids in Margarines and Margarine-like Foods

H.T. SLOVER, R.H. THOMPSON JR., C.S. DAVIS and **G.V. MEROLA,** U.S. Department of Agriculture, Beltsville Human Nutrition Research Center, Nutrient Composition Laboratory, Beltsville, Maryland

ABSTRACT

The lipid composition of margarines from stores in selected locations in the U.S. is reported. The lipids determined include the fatty acids, tocopherols and major plant sterols. Data are included for isomeric octadecenoic fatty acids (14 isomers or groups of isomers) and four isomeric octadecadienoic fatty acids common in partially hydrogenated vegetable fats, insofar as these are separable by capillary gas chromatography. Amounts of individual lipids found in vegetable oil margarines, spreads, imitation and diet margarines were: palmitate, 8.49 to 13.17% (normalized weight percent, calculated as triglyceride); stearate, 4.78 to 9.53%; linoleate, 6.06 to 46.39%; linolenate, 0.18 to 3.57%; α-tocopherol, 0.3 to 24.3 mg/100g; γ-tocopherol, 3.0 to 55.0 mg/100g; δ-tocopherol, 0.5 to 18.9 mg/100g; campesterol, 10.6 to 106.3 mg/100g; stigmasterol, 13.1 to 60.9 mg/100g; sitosterol, 42.3 to 412.9 mg/100g. Amounts of *trans*-unsaturated octadecenoic fatty acids in these margarines varied from 10.74 to 30.06%. Small amounts of the *trans,trans, trans,cis* and *cis,trans* isomers of linoleate also are reported.

INTRODUCTION

The USDA Nutrient Composition Laboratory has carried out a study of the lipids in consumer margarines to provide detailed information on fatty acids, tocopherols and sterols. Food fats may be described as either hidden, i.e. an integral part of the food and therefore not directly recognizable as fat, or visible, clearly recognizable as fat. Margarine is one of the major visible fats, although not the major contributor of fat to the U.S. diet. It was estimated in 1980 (1) that the per capita consumption of margarine in the U.S. was stabilized at 11 to 13 pounds per year, or about 11 to 13 grams of margarine fat per day. Margarine consumption at this level would supply 99 to 117 calories per day, or 5-6% of a 2000 calorie diet.

The vegetable oils from which most margarines are made are major sources for essential fatty acids and the vitamin E-active tocopherols, and variable amounts of these survive in the processed margarines. In addition, margarines contain plant sterols, principally sitosterol, campesterol and stigmasterol, that may be of significance in human diets; the few margarines made with animal fats will contain cholesterol. Processing of margarines includes partial hydrogenation, one of whose effects is the production of numerous isomeric unsaturated fatty acids differing in the position and geometric configuration of their double bonds. Data on the amounts of these lipids in foods, including margarine, are needed by those investigating the relationship of diet to health, selecting and recommending diets for the general public, or providing diets designed for specific therapeutic purposes. This work was undertaken to provide the maximum amount of such information that may be obtained using current analytical methods.