Fatty Acid Composition of Oil from Soybean Leaves Grown at Extreme Temperatures

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Leaves from soybean (*Glycine max* (L.) Merr.) plants were assayed to determine if the relationship between temperature and relative fatty acid composition observed in the seed oil also existed for the triglycerides in the leaf oil. Leaf samples were harvested from eight soybean lines (A5, A6, C1640, Century, Maple Arrow, N78-2245, PI 123440 and PI 361088B) grown at 40/30, 28/22 and 15/12°C day/night. At 40/30 and 28/22°C, seven fatty acids were observed at a level greater than 1.0%. These included the five major fatty acids found in the seed oil: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acid; plus two fatty acids that had retention times the same as palmitoleic (16:1) and γ-linolenic (18:3 g) acid. In addition, an eighth fatty acid that had a retention time the same as behenic (22:0) acid was found in the leaves of all lines at 15/12°C. Palmitic, palmitoleic and stearic acid content did not differ significantly over temperatures. The oleic and linoleic acid content were each highest at 15/12°C, while the γ-linolenic and the linolenic acid content were each highest at 40/30°C. The fatty acid composition of the triglyceride portion of the leaf oil did not display the same pattern over temperatures as that observed for seed oil.

KEY WORDS: Fatty acids, leaves, soybean, temperature.

The relative content of each fatty acid in soybean seed oil has been shown to be related to temperatures during seed development. The trend was toward a higher content of saturated (palmitic acid and stearic acid) and monounsaturated (oleic acid) fatty acids under high temperatures and toward a higher content of polyunsaturated (linoleic acid and linolenic acid) fatty acids under low temperature conditions (1,2).

Martin and Rinne (3) reported that the relative fatty acid content of soybean leaf oil was very different from that of seed oil. Leaf oil was isolated in two forms, triglycerides (TG) such as those found in the seed, and total polar lipids (TPL). Among the triglycerides, linolenic acid was the largest single component of leaf oil, followed by linoleic acid and palmitic acid. The relative fatty acid composition of leaf oil was studied at a single temperature, 28/24°C.

The current study was conducted to determine the relative response to extreme temperatures of the fatty acid components of the triglyceride portion of the leaf oil. For this study, soybean lines with high stearic acid, high oleic acid, low linolenic acid or standard fatty acid content in the seed oil were assayed. The materials used in this study were derived from the same plants as those reported by Rennie and Tanner (1).

EXPERIMENTAL PROCEDURES

Leaf tissue from five low linolenic acid soybean lines: A5 (4), C1640 (5), N78-2245 (6), PI 123440 (7) and PI 361088B (8), with N78-2245 also having a high oleic acid content; one line with a very high stearic acid content, A6 (9); and two commercial cultivars, Century and Maple Arrow, were used in this study. Plants were grown at three temperature regimes: 40/30°C, 28/22°C and 15/12°C day/night under controlled indoor conditions. The experiment was in a randomized complete block design in a split-plot format with temperature regimes as main plots, and lines as subplots. The method of plant growth, the temperature regimes and the experimental set-up have been described elsewhere (1).

Two trifoliate leaf samples were harvested from nodes 5 to 7 at the R4 and R5 growth stage (10). Each leaflet was taken as a sample and five samples were assayed for each plant, with the mean of these samples being used as the value for that plant. Each sample was dried at 65°C for 48 hr and then was ground to a fine powder with a mortar and pestle. The oil was extracted, and the fatty acid methyl esters prepared as described elsewhere (1). The identity of each fatty acid was determined by use of standard samples (from Serdary Research Laboratories, London, Ontario) of palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, γ-linolenic acid, linolenic acid and behenic acid. When a known and an unknown fatty acid peak had the same retention times on the gas chromatograph (GC), it was assumed that they were the same fatty acid.

RESULTS AND DISCUSSION

There were seven fatty acids detected, at greater than 1.0%, in the triglyceride portion of the leaf oil from plants grown at 40/30°C or 28/22°C (Table 1). These included the five major fatty acids found in seed oil: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3). In addition, there was one fatty acid peak that had a retention time the same as samples of palmitoleic acid (16:1), and one fatty acid peak that had a retention time the same as samples of γ-linolenic acid (18:3 g). Plants grown at 15/12°C also displayed a peak that had a retention time the same as that of behenic acid (22:0), at a level greater than 1.0%.

The only report of comparable levels of palmitoleic acid was from soybean cell cultures (11) and of behenic acid was from wild *Glycine* seeds (12). There have been no reports of γ-linolenic acid in soybean tissues.

Martin and Rinne (3) assayed leaf triglycerides at 28/24°C, for six lines including A5, A6 and PI 123440. Their results for these three lines were similar to those reported here at 28/22°C for palmitic acid, stearic acid and oleic acid content. Their values for linoleic acid and linolenic acid content were higher than those reported here. However, the γ-linolenic acid component reported here does account for much of this difference. Those authors also reported values for leaf triglycerides from the line N78-2245, but their values were very different from those reported here. There is no obvious reason why the results should be similar for three lines and yet differ for N78-2245. Martin and Rinne (2) did not report that

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TABLE 1

Fatty Acid Composition by Relative Percent of the Leaves of Eight Soybean Lines at 40/30, 28/22 and 15/12°C

Temperature	Line	16:0	16:1	18:0	18:1	18:2	18:3 g	18:3	22:0[a]
				Fatty acid composition by GC %					
40/30°C	A5	21.3	2.2	6.9	6.8	8.9	12.8	39.4	0.4
	C1640	20.9	2.2	6.0	7.0	7.2	12.1	43.3	0.0
	N78-2245	17.5	2.0	5.3	10.5	11.9	8.6	41.1	0.0
	PI 123440	17.1	2.2	5.7	6.0	10.4	9.2	48.5	0.0
	PI 361088B	20.1	2.2	5.6	6.0	11.2	11.5	38.5	0.0
	Century	19.1	2.7	5.4	4.7	9.5	11.1	47.0	0.0
	Maple Arrow	22.1	2.4	6.0	6.0	8.8	12.0	41.3	0.0
	A6	24.3	1.9	7.2	7.1	9.0	13.7	36.1	0.0
	mean	20.3	2.2	6.0	6.8	9.6	11.4	41.9	0.0
28/22°C	A5	21.7	2.7	7.3	10.2	17.4	8.6	30.3	0.5
	C1640	19.2	3.4	6.5	10.6	13.8	6.8	35.0	0.2
	N78-2245	18.6	3.3	6.0	11.7	16.9	9.8	29.2	0.4
	PI 123440	17.8	1.3	6.4	12.9	17.0	6.8	29.6	0.4
	PI 361088B	20.9	2.3	6.8	9.0	15.4	9.4	34.9	0.3
	Century	20.5	3.6	6.8	13.6	12.1	12.8	28.4	0.0
	Maple Arrow	25.3	4.0	7.7	11.5	13.9	8.7	27.7	0.0
	A6	20.0	2.9	7.3	9.0	17.9	11.3	32.4	0.3
	mean	20.5	2.9	6.9	11.1	15.6	9.3	30.9	0.3
15/12°C	A5	22.0	3.3	7.1	15.0	17.7	10.3	21.4	1.6
	C1640	21.7	3.9	6.9	15.8	15.8	11.8	23.1	1.5
	N78-2245	18.9	2.5	5.3	14.1	20.6	7.7	21.8	2.1
	PI 123440	20.0	2.8	5.8	15.4	18.4	8.7	18.5	1.6
	PI 361088B	20.0	3.1	5.6	12.5	16.4	10.4	26.6	1.6
	Century	18.5	3.0	5.7	15.9	17.5	8.6	27.9	1.6
	Maple Arrow	19.8	3.1	6.1	12.4	16.3	8.4	23.1	1.8
	A6	22.3	3.1	7.3	12.0	18.0	7.4	22.3	2.1
	mean	20.4	3.1	6.2	14.1	17.6	9.2	23.1	1.7
Temperature	L.S.D.	1.2	0.9	1.0	3.1	3.4	2.1	4.7	0.5
Line	L.S.D.	1.9	0.5	0.8	2.0	2.1	1.6	2.8	0.4

[a]Palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), gamma-linolenic acid (18:3g), linolenic acid (18:3) and behenic (22:0) acid.

they had observed any evidence of detectable levels of palmitoleic acid, γ-linolenic acid or behenic acid in leaf tissue.

The fatty acid profile in the leaves of each line differed greatly between the two extreme temperature treatments (Table 1). There was variation among lines for each fatty acid, but these were not consistent across temperatures.

There were no significant differences in palmitic acid content, palmitoleic acid content or stearic acid content across temperatures (Table 1). The oleic acid content and linoleic acid content were two-fold higher at 15/12°C than at 40/30°C. The γ-linolenic acid content was greatest at 40/30°C. The linolenic acid content at 15/12°C was half the level observed at 40/30°C. The combined content of γ-linolenic acid and linolenic acid displayed the same response as the linolenic acid content alone. The behenic acid content for all lines grown at 40/30°C or 28/22°C was below 0.6%, but was above 1.4% for all lines grown at 15/12°C.

The trend observed for relative fatty acid percent over temperatures for the triglyceride portion of the leaf oil was very different from that observed for seed oil (1). The total polyunsaturated fatty acid content in leaf oil was highest at high temperatures but was lowest at high temperatures in seed oil. In seed oil, linoleic and linolenic acid displayed a similar pattern, but in leaf oil the relative content of linoleic acid was lowest at 40/30°C while that of linolenic acid was greatest at this temperature. It was not known what factors were influencing the relative content of the various fatty acid components in the triglyceride portion of the leaf oil. Researchers may be able to exploit the unique responses of seed oil and leaf oil to different temperatures, in the future.

The presence of palmitoleic acid, γ-linolenic acid or behenic acid in the leaves may not be of any direct use to the soybean oil industry. However, these fatty acids may be of interest if the plant could be modified such that these fatty acids could be produced in the seeds at detectable levels.

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