Fatty Acid Composition within Each Structural Part and Section of a Soybean Seed

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Seed coat, axis, sections of cotyledons, and the whole seed of six soybean genotypes were analyzed by gas chromatography. Relative percentages of fatty acids were homogeneous within cotyledons but varied greatly among structural parts. The axis had highest percentage of polyunsaturated fatty acids, whereas the coat had highest percentage of saturated fatty acids. Due to the cotyledons representing the largest proportion, the fatty acid composition of cotyledons represented that of the whole seed, suggesting that slicing a portion of cotyledons for breeding screen of fatty acids is a reliable sampling technique. Furthermore, regardless of the great variation in fatty acid profile of the six genotypes selected, the ratio in relative percentage of an individual fatty acid between the axis and cotyledons was highly conserved, implying that (1) events of lipid metabolism in maturing seeds may be correlated between axis and cotyledons, and (2) given a fatty acid composition of one tissue, one can predict that of the other.

Keywords: *Fatty acid distribution; soybean cotyledons; axis; coat; breeding screen; fatty acid analysis*

INTRODUCTION

The nutritional value of soybeans is determined by not only quantity but also quality of oil and protein they contain. One quality factor, which has recently gained much attention, is the fatty acid composition of soybean oil. This is because there is increasing evidence of the relationship between consumption of saturated fat and elevated serum cholesterol level (Hegsted et al., 1965; Vessby, 1993) and of the relationship between linolenic acid content and the oxidative rancidity or loss of flavor stability in a food system (Frankel, 1984). As a result, there is a growing demand for speciality soybeans which have a modified fatty acid composition, such as soybeans with low saturated fat and soybeans with low linolenic acid content.

The fatty acid composition of soybeans is commonly determined by gas chromatography (GC) after first extracting oil from whole seed meals and then converting extracted oil into fatty acid methyl esters (FAMEs) (AOAC, 1984). This conventional sample preparation (derivatization) is unsuitable for breeding programs which requires fast and nondestructive evaluation of fatty acid profile in a single seed. Instead, an alternative method, such as one described by Dahmer et al. (1989), is often used. It involves slicing a portion of seed tissue from the opposite side of the embryo axis (about 5–25 mg) and then directly transesterifying lipids in the portion without prior oil extraction. The remaining seed thus remains viable. Obviously, the reliability of this nondestructive sampling technique depends totally on how homogeneously the fatty acids are distributed within the seed.

A cross section of a soybean seed shows the three structural parts: coat, cotyledons, and embryonic axis, with cotyledons as a major component. A literature search revealed that there is limited information on fatty acid distribution within a soybean seed (Singh et al., 1968; Stewart and Bewley, 1980). The present study was undertaken to provide additional information on this aspect as well as how fatty acid composition in one structural part is related to that of the other.

MATERIALS AND METHODS

Soybean Materials. Six Hartz soybean genotypes with varying fatty acid composition were selected. All were grown at the Research Farm of Jacob Hartz Seed Co., Inc., Stuttgart, AR, and harvested in 1992.

Seed Sectioning. A whole seed was first separated into three structural parts: coat, cotyledons, and axis, by using a razor blade. The two cotyledons were then halved, with cotyledon I section close to the axis area and cotyledon II section opposite the axis area. In other words, each cotyledon section contained two halves of each of two cotyledons. About 60 seeds were separated and sectioned for each genotype. Corresponding parts or sections from these 60 seeds were first combined and then ground into powders with a coffee mill. A portion of the sample powder (10 mg) was used for fatty acid analysis of the individual part or section. Another 60 intact seeds from each genotype were ground together, and a portion of the meal (10 mg) was used for fatty acid analysis of the whole seed.

Fatty Acid Analysis. The method involved preparing FAMEs by direct transmethylation and analyzing them with a GC instrument. For direct derivatization of lipids from seed tissues, 10 mg of each sample powder was weighed into 16 × 100 mm tissue culture tubes. Two milliliters of hexane was added next. And this was followed by adding 2 mL of 1.0% H_2SO_4 in MeOH solution. Sample tubes were screw-capped and then transferred to a heating block maintained at 85 °C and allowed to warm for 1 h. After cooling to room temperature and standing for 1 h, the reaction mixture was washed with anhydrous Na_2SO_4 (0.8–1.2 g). The top organic phase was finally transferred to Hewlett-Packard (HP) GC autosampling vials with Teflon-coated septa. For FAME analysis, a HP 5890 GC fitted with a HP autosampler 7673A, a FID detector, a computer with a HP ChemStation 3365, and a Supelco SUPELCOWAX 10 capillary column (Supelco Inc., Bellefonte, PA) was used. The detailed running condition was described elsewhere (Liu et al., 1995). Duplicate analyses were

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Table 1. Fatty Acid Composition of Structural Parts, Cotyledon Sections, and the Whole Seed of Six Hartz Soybean Genotypes[a]

Hartz genotype	tissue name	major fatty acids (relative %)				
		C16:0	C18:0	C18:1	C18:2	C18:3
A	whole	11.7	5.0	23.7	51.9	7.0
	cotyledon I	11.6	4.7	23.6	52.0	6.9
	cotyledon II	11.7	5.0	23.0	52.3	7.1
	axis	15.9	3.8	6.9	56.6	15.8
	coat	22.4	14.1	14.6	24.4	13.5
B	whole	10.0	22.3	17.2	43.6	5.4
	cotyledon I	10.3	22.7	16.8	42.8	5.6
	cotyledon II	9.6	21.8	18.3	44.1	5.1
	axis	12.4	17.5	4.3	54.4	11.1
	coat	28.9	17.8	15.6	15.6	6.2
C	whole	13.2	4.0	20.5	53.5	8.3
	cotyledon I	13.7	3.8	20.5	53.4	8.1
	cotyledon II	12.9	3.6	21.5	53.7	7.7
	axis	18.0	3.5	5.4	53.7	18.5
	coat	24.5	16.8	9.7	18.6	8.9
D	whole	13.1	3.9	18.7	59.7	3.9
	cotyledon I	13.1	3.8	18.9	59.6	3.9
	cotyledon II	12.4	3.6	20.3	58.9	4.0
	axis	16.8	3.0	6.1	63.3	10.0
	coat	23.3	13.1	13.2	19.0	7.7
E	whole	12.6	4.6	29.1	47.1	5.6
	cotyledon I	12.7	4.9	27.7	48.1	5.6
	cotyledon II	12.2	4.5	29.1	47.9	5.4
	axis	15.9	3.7	7.4	60.2	12.4
	coat	19.1	10.6	21.2	30.2	7.5
F	whole	5.7	3.2	36.2	48.6	6.0
	cotyledon I	6.0	3.3	35.6	48.3	5.5
	cotyledon II	5.7	3.2	36.2	48.6	5.8
	axis	9.9	3.5	9.3	65.3	10.4
	coat	21.2	16.5	15.2	28.6	7.4

[a] Means of duplicate measurements.

performed separately on each part or section as well as on the whole seed sample for each genotype.

RESULTS AND DISCUSSION

Table 1 shows the relative percentage of five major fatty acids in the whole seed, sections of cotyledons, seed coat, and embryonic axis of six Hartz soybean genotypes.

Fatty Acid Composition of the Whole Seed. Among the six selected genotypes, there was a great variation in major fatty acid composition determined from the whole seeds. Palmitic acid (C16:0) ranged from 5.7 to 13.2%; stearic acid (C18:0), 3.2 to 22.3%; oleic acid (C18:1), 27.2 to 36.2; linoleic acid (C18:2), 43.6 to 59.7%; and linolenic acid (C18:3), 3.9 to 8.3%. Apparently, genotype B was a high saturated fatty acid line, genotype F was a low saturated fatty acid line, and genotype D was a low linolenic line, whereas the other three genotypes were normal soybean lines.

Fatty Acid Distribution within Cotyledons. When each of two sections of soybean cotyledons, cotyledons I and II, was analyzed for fatty acid profile, no significant difference ($p < 0.01$) in relative percentages of the five major fatty acids existed. Furthermore, fatty acid composition determined from any section of the cotyledons did not significantly differ from that determined from the whole seed. These observations suggest that the nondestructive sampling technique for breeding screen of fatty acid profile in a single soybean seed is very reliable.

Fatty Acid Profile of Structural Parts. The relative percentage of the major fatty acids in three different structural parts: seed coat, cotyledons, and embryo axis varied considerably among themselves in all six genotypes selected (Table 1). Seed axis showed the lowest relative percentages of C18:0 and C18:1 and highest of C18:2 and C18:3. Cotyledons exhibited the lowest relative percentages of C16:0 and C18:3 and highest of C18:1. The seed coat had lowest C18:2 and highest C16:0 and C18:0. These features were generally followed in all six genotypes regardless of the great variation in their fatty acid profiles as determined from the whole seeds. Stewart and Bewley (1980) reported that the axis from unaged soybean seeds of Pride X005 cultivar contain 16.6% of C16:0, 3.1% of C18:0, 3.8% of C18:1, 57.3% of C18:2, and 19.3% of C18:3. Although they did not measure fatty acid composition of the whole seed or cotyledon tissues, their data also indicated that the axis contained lower C18:0 and C18:1 and higher polyunsaturated fatty acids when compared with fatty acid composition of a normal soybean cultivar (Synder and Kwon, 1986).

It is known that fatty acids in soybeans and some other plant species are synthesized in plastids up to oleoyl-CoA (Murphy, 1993). Oleoyl-CoA is then transported out of plastids, undergoing a variety of fates, such as further desaturation and elongation. Thus, oleic acid is a central metabolite in plant lipid metabolism. The lowest oleic acid content in axis is consistent with the fact that the axis is the most active tissue of soybean seeds. In terms of the food or feed values of soybean axes, relatively few studies have been done. However, according to Synder and Kwon (1986), there have been reports that the embryo axis is the source of beany off-flavors, and some processors of soymilk have tried to remove the germ to avoid off-flavors in soymilk. Our observation of highest polyunsaturated fatty acids in the soybean axis provides evidence to support this practice.

Data from Table 1 indicate that although there was a great variation in relative percentages of fatty acids among the structural parts, the fatty acid profile of cotyledons differed insignificantly from that of the whole seed. The explanation is as follows: By dry weight, the seed coat makes up about 8% of the whole bean, cotyledons make up 89–90%, and axis 2–3%, and by proximate analysis, cotyledons contain about 20% oil, the seed coat about 1% oil, and the axes contain about 10% oil (Wolf and Cowan, 1975). In other words, the cotyledon contributes some 98% of the oil, the axis some 1.5%, and the coat some 0.5%.

Relationships in Fatty Acid Profile among Structural Parts. On the basis of the data of Table 1, we calculated the ratio of the relative percentage of an individual fatty acid in one part vs that of the corresponding fatty acid in another part. These ratios were then pooled and statistically treated as shown in Table 2. We found that although the ratios in individual fatty acids between seed coat and cotyledons and between axis and seed coat varied greatly among the six Hartz genotypes selected, the ratios in five major fatty acids between axis and cotyledons exhibited little variation. The relative standard deviation for these ratios was between 8.9 and 12.6%. In other words, regardless of the great variation in the fatty acid profile of whole seeds among six selected genotypes (Table 1), the ratio between axis and cotyledons was highly conserved. The average ratio of six genotypes for C16:0 was 1.38 ± 0.15; C18:0, 0.86 ± 0.11; C18:1, 0.27 ± 0.02; C18:2, 1.17 ± 0.12; and C18:3, 2.22 ± 0.22.

This finding has two significant implications. First,

Table 2. Relationships in Relative Percentages of Major Fatty Acids between Different Structural Parts of Soybean Seeds[a]

relationship	Hartz genotype	ratio in relative % of major fatty acids C16:0	C18:0	C18:1	C18:2	C18:3
axis/cotyledon	A	1.37	0.79	0.30	1.08	2.26
	B	1.24	0.79	0.25	1.25	2.07
	C	1.36	0.94	0.25	1.00	2.35
	D	1.32	0.80	0.31	1.07	2.55
	E	1.28	0.78	0.26	1.25	2.25
	F	1.70	1.07	0.26	1.35	1.84
	av	1.38	0.86	0.27	1.17	2.22
	min	1.24	0.78	0.25	1.00	1.84
	max	1.70	1.07	0.31	1.35	2.55
	SD	0.15	0.11	0.02	0.12	0.22
	rel SD (%)	11.0	12.6	8.9	10.5	10.0
coat/cotyledon	A	1.93	2.89	0.63	0.47	1.92
	B	2.91	0.80	0.89	0.36	1.16
	C	1.85	4.54	0.46	0.35	1.13
	D	1.83	3.53	0.67	0.32	1.97
	E	1.53	2.26	0.75	0.63	1.36
	F	3.63	5.07	0.42	0.59	1.31
	av	2.28	3.18	0.64	0.45	1.48
	min	1.53	0.80	0.42	0.32	1.13
	max	3.63	5.07	0.89	0.63	1.97
	SD	0.74	1.42	0.16	0.12	0.34
	rel SD (%)	32.5	44.7	25.2	26.7	23.2
axis/coat	A	0.71	0.27	0.47	2.31	1.17
	B	0.43	0.98	0.28	3.48	1.79
	C	0.73	0.21	0.55	2.89	2.07
	D	0.72	0.23	0.46	3.34	1.30
	E	0.83	0.34	0.35	1.99	1.65
	F	0.47	0.21	0.61	2.28	1.41
	av	0.65	0.37	0.45	2.72	1.57
	min	0.43	0.21	0.28	1.99	1.17
	max	0.83	0.98	0.61	3.48	2.07
	SD	0.15	0.28	0.11	0.56	0.31
	rel SD (%)	22.8	74.0	25.1	20.6	19.6

[a] Refer to Table 1 for raw data. Values for cotyledons were the average values of the two cotyledon sections: cotyledon I and II.

it implies that events of lipid metabolism may be correlated in both axis and cotyledon tissues during seed development. This is consistent with the two tissues having the same embryonic origin. And second, by giving fatty acid composition of one tissue, we can predict that of the other. For example, if cotyledons are found to have 7.5% linolenic acid relative to total oil content from the tissue, the relative percentage of linolenic acid in the axis tissue would be around 16.7 (= 7.5 × 2.22).

Distribution of fatty acids in soybean seeds was studied previously by Singh et al. (1968). In their study, each seed was cut into five sections with the no. 1 section farthest from the axis and the no. 5 section containing the axis. Their finding is that palmitic, oleic, linoleic, and linolenic acids are homogeneously distributed throughout the seed but stearic acid is slightly lower in section 1. However, they did not analyze fatty acid profile within each structural part.

In conclusion, the ratio of individual fatty acids in soybean axis vs cotyledons was highly conserved regardless of genotypes, whereas fatty acid distribution within cotyledons was homogeneous. The fatty acid composition of cotyledons represented that of the whole seed even though the composition of the axis and seed coat significantly differed from that of cotyledons. This is because both axis and coat contribute only a small proportion (<2%) of the oil. The practical significance of this study lies in that fatty acid profile of a single seed can accurately be determined without destroying the viability of the seed, and that fatty acid composition of the axis can be predicted with good accuracy from the analysis of the cotyledon or the whole seed.

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