Chemical Composition and Lipoxygenase Activity in Soybeans as Affected by Genotype and Environment

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ABSTRACT

Environmental and genetic influences on the chemical composition and lipoxygenase activity of 24 soybean genotypes (groups IV-S and V) were determined. The soybeans were grown at two climatically different locations within the state of Georgia. Oil and protein contents and fatty acid composition of the oil in soybeans were affected by the environment. Five genotypes from group IV-S had a fatty acid composition of the oil different from the other genotypes in the group. These differences appeared to be genetically controlled. The level of lipoxygenase activity in soybeans also appeared to be genetically controlled and not influenced by the environment. The study indicated that soybean genotypes could be selected for a specific climatic region based on oil fatty acid composition and lipoxygenase activity.

INTRODUCTION

Recent studies have shown that soybean oil and soy products are adversely affected by certain constituents in the bean (1,2). High levels of linolenic acid are undesirable in soybean oil because oxidative breakdown products reduce flavor stability of the oil and produce "off flavors" in foods cooked with soybean oil (2). Also, low quality soybean products are thought to result from certain enzymatic actions. High levels of lipoxygenase activity are thought to cause beany flavors in most soy food products (1,3).

Several studies have indicated that the levels of unsaturated fatty acids and oil in soybeans are strongly influenced by the environment but that lipoxygenase activity appears to be genetically controlled (1,2,4).

Thus, the environmental and genetic influence on these components during soybean development would contribute considerably to bean quality. Criteria for soybean selection could be based on oil fatty acid composition and lipoxygenase activity as well as oil and protein contents in soybeans. The intent of this study was to determine the effect of environment and genotype on these chemical properties of soybeans grown in two climatic regions in Georgia.

EXPERIMENTAL PROCEDURES

Soybeans, groups IV-S and V, were grown in 1973 at the Mountain Experiment Station, Blairsville, GA, and the Coastal Plain Experiment Station, Tifton, GA. Groups IV-S and V were planted on May 11 at Tifton and on May 15 at Blairsville. The average maturity date for Group IV-S was August 21 at Tifton and September 23 at Blairsville. Maturity dates for Group V were September 3 at Tifton and October 9 at Blairsville.

The climates of these two locations differ appreciably although only 203 nautical miles apart. Tifton (latitude 31° 21′N) is 360 ft above mean sea level and has an average daily summer temperature of 78.3 F (May-September). Blairsville (34° 51′ N) is 1917 ft above mean sea level and has an average summer daily temperature of 64.5 F. Summer rainfall is normally about the same for the two loca-

TABLE I

Chemical Composition and Lipoxygenase Activities of 24 Soybean Genotypes Grown at Tifton, Georgia

Soybean genotype	Moisture (%)	Protein[a] (%)	Oil[a] (%)	Free fatty acid as oleic (%)	Fatty acid composition of oil as % total fatty acids					Lipoxygenase activity[b]
					16:0	18:0	18:1	18:2	18:3	
Group IV-S										
Kent	8.9	40.0	23.5	0.50	9.7	4.6	18.4	58.9	8.0	1.02
Columbus	8.8	40.2	22.1	0.50	10.5	4.9	19.8	57.5	7.4	0.91
Oksoy	8.1	37.6	24.2	0.43	11.0	4.2	36.4	42.5	5.4	0.60
Clark 63	8.5	41.9	25.1	1.19	10.6	4.7	20.1	57.2	6.8	0.40
D66-5566	8.9	43.0	23.2	0.53	10.6	3.1	36.0	44.8	5.0	0.45
D67-3297	8.8	39.2	22.1	0.43	11.8	2.9	24.8	53.6	6.7	0.73
S63-5328S	8.9	39.8	24.0	0.37	10.8	4.2	40.3	40.5	4.1	0.46
V68-1242	9.0	42.1	23.4	0.47	10.9	3.0	37.0	45.6	3.4	0.55
K1002	8.9	41.4	24.9	0.58	10.0	3.4	36.5	46.4	3.7	0.28
K1007	8.8	39.8	23.2	0.41	11.9	4.2	21.5	57.2	5.3	0.74
L69L-208	9.6	44.0	20.9	0.91	12.2	3.5	24.5	55.0	4.5	0.46
SL 13	10.0	44.4	22.5	1.36	11.1	5.0	21.1	57.4	5.3	0.85
Group V										
Essex	9.6	41.1	21.7	0.38	11.5	3.2	19.5	58.5	7.2	0.80
Forrest	7.7	41.8	21.4	0.41	10.8	3.9	28.9	51.1	5.2	0.60
Mack	8.8	42.7	23.3	0.43	11.1	3.6	31.0	49.4	4.8	0.55
S67-80	9.9	42.6	23.9	0.42	11.2	3.6	31.8	48.9	4.4	0.58
V68-920	9.8	38.6	22.5	0.42	12.7	2.7	21.9	56.9	5.7	0.74
D70-2650	9.5	42.5	22.9	0.37	12.1	3.0	18.9	58.6	7.3	0.34
D70-5107	9.9	42.0	23.4	0.47	12.1	2.9	25.9	53.9	5.2	0.54
D70-5154	9.7	40.5	23.6	0.39	11.6	3.1	29.6	50.4	5.2	0.39
R70-176	10.0	39.4	23.2	0.39	12.8	3.1	20.8	57.4	5.9	0.53
R70-306	10.0	41.2	22.4	0.48	11.3	3.1	21.3	57.1	6.8	0.69
V68-1171	10.1	40.4	24.0	0.40	10.9	3.5	25.8	53.3	6.1	0.60
V69-156	9.9	41.4	22.4	0.43	11.5	3.4	25.1	54.0	6.0	0.68

[a]Dry wt basis.

[b]Specific activity (units of activity per mg protein).

TABLE II

Chemical Composition and Lipoxygenase Activities of 24 Soybean Genotypes Grown at Blairsville, Georgia

Soybean genotype	Moisture (%)	Protein[a] (%)	Oil[a] (%)	Free fatty acid as oleic (%)	Fatty acid composition of oil as % total fatty acids					Lipoxygenase activity[b]
					16:0	18:0	18:1	18:2	18:3	
Group IV-S										
Kent	10.1	39.7	22.1	0.41	9.6	4.9	20.7	57.3	7.8	0.91
Columbus	10.6	40.4	21.9	0.42	10.3	4.8	21.8	56.5	6.5	0.85
Oksoy	10.1	36.7	22.1	0.43	11.1	5.0	30.4	47.7	5.7	0.66
Clark 63	10.5	41.4	22.3	0.44	10.6	5.0	21.4	56.3	6.7	0.66
D66-5566	10.2	39.6	23.0	0.41	11.3	4.3	24.7	53.5	6.1	0.46
D67-3297	10.0	39.5	21.1	0.40	11.7	3.8	24.3	52.8	7.3	0.73
S63-5328S	10.3	37.5	22.4	0.38	11.0	5.1	33.2	45.3	5.4	0.59
V68-1242	10.1	40.2	22.1	0.40	9.7	3.6	36.8	45.3	4.6	0.49
K1002	10.2	40.6	22.7	0.42	9.6	4.2	30.5	50.4	5.1	0.58
K1007	9.9	40.6	20.9	0.40	11.3	4.3	22.8	55.1	6.5	0.76
L69L-208	10.6	40.8	19.0	0.49	11.4	3.5	20.9	57.0	7.1	0.74
SL 13	10.4	40.1	20.2	0.39	10.6	5.0	21.3	56.4	6.7	1.03
Group V										
Essex	10.7	40.6	21.0	0.41	10.9	3.4	21.1	56.6	8.0	0.85
Forrest	10.0	36.2	21.7	0.42	10.8	3.5	21.1	56.7	7.9	0.62
Mack	10.4	39.4	22.3	0.43	11.2	3.8	23.3	54.3	7.4	0.47
S67-80	10.5	40.4	21.5	0.44	10.6	3.7	27.4	51.1	7.2	0.66
V68-920	11.2	37.7	20.4	0.42	12.4	3.1	18.0	58.3	8.1	0.66
D70-2650	10.7	39.7	21.5	0.40	11.7	3.3	20.1	56.7	8.2	0.54
D70-5107	10.6	38.0	21.8	0.43	12.1	3.3	21.2	55.7	7.7	0.56
D70-5154	10.8	37.5	22.2	0.45	12.0	3.4	20.7	55.6	8.3	0.34
R70-176	10.8	37.6	22.5	0.45	12.7	2.9	20.9	56.3	7.2	0.54
R70-306	9.75	38.4	22.0	0.43	10.7	3.9	23.6	53.4	8.2	0.61
V68-1171	9.64	37.5	21.5	0.36	10.5	4.1	24.3	52.8	8.1	0.59
V69-156	9.5	38.3	31.4	0.44	11.1	3.1	20.7	56.5	8.5	0.63

[a]Dry wt basis.

[b]Specific activity (units of activity per mg protein).

tions; however, in 1973, Tifton received 16.9 in. and Blairsville received 27.2 in.

Sample Preparation and Analysis

Soybean samples were ground to 16 mesh in a Wiley mill; and moisture, protein, oil, and free fatty acids were determined by AOCS methods (5-8). Fatty acid composition of the oil was determined by gas chromatography with a modified procedure of Metcalfe et al. (9). The chromatograph was equipped with flame ionization detectors and a stainless steel column (2.44 m x 2.0 mm, inside diameter) packed with 10% ethylene glycol succinate methyl silicone polymer on 100/120 mesh Gaschrom P. The oven was operated at 200 C, with the injector and detector at 210 and 260 C, respectively. Nitrogen, air, and hydrogen flow rates were 22, 245, and 35 ml/min. An Infotronics (CRS-100) digital integrator was used to quantitate peak areas. Fatty acids were quantitated from standard curves prepared with standard methyl esters (Nu-Chek-Prep, Elysian, MN).

Lipoxygenase Assay

Milled soybeans (2 g) were liquefied with 150 ml of cold (2 C) 0.1M phosphate buffer, pH 6.0, in a Virtis "45" homogenizer at 35% of maximum speed for 2 min. Crushed ice was packed around the homogenizing flask to prevent heat buildup. The resulting mixture was squeezed through four layers of cheese cloth, centrifuged at 39,000 x g for 10 min at 0 C, and the supernatant filtered through Whatman #4 paper. The filtered supernatant was used as the enzyme source and stored at 2 C. Lipoxygenase activity was assyed according to Surrey (10), but with 0.1M phosphate buffer, pH 6.0, instead of borate buffer. Protein was determined spectrophotometrically with the biuret method (11). One unit of enzyme activity (U) is defined as the production of 1 μmole of hydroperoxyoctadecadienoic acid per min at 234 nm for the conjugated diene (ϵ = 25,000) and the assay conditions used (12). Specific activity is expressed as one unit of activity per mg protein (U/mg prot). The enzyme solution was assyed within 3 hr after preparation because a 30-50% loss in activity occurred when stored overnight at 2 C. Substrate solutions (linoleic acid) were also freshly prepared as needed. All chemical and enzymatic determinations were conducted in duplicate.

RESULTS AND DISCUSSION

The protein and oil content and fatty acid composition of the oil varied very little among the 24 soybean genotypes at either location. However, five genotypes in Group IV-S (Oksoy, D66-5566, S63-5328S, V68-1242, and K1002) from Tifton and Blairsville showed a variation in fatty acid composition of the oil which was different from the other genotypes in the group (Table I and II).

The oil from three genotypes in Group IV-S (Clark 63, L69L-208, and SL 13) grown at Tifton (Table I) had high levels of free fatty acids and exhibited high absorption at 234 nm. These samples had noticeable field damage, which is directly related to these properties (13).

Although moisture was slightly higher, the mean protein and oil contents were significantly lower ($P<0.01$) in Groups IV-S and V genotypes from Blairsville than from Tifton (Table III). The average lipoxygenase activity was essentially the same at both locations. The average percentages of oleic and linolenic acids in oil composition of the 24 genotypes varied significantly ($P<0.01$) between locations. Genotypes of groups IV-S and V showed higher levels of oleic acid and lower levels of linolenic acid when grown at Tifton than at Blairsville. Linoleic acid levels were about the same at both locations.

The genotypes in Group IV-S were divided into two subgroups because the oils of five genotypes from both locations were different in fatty acid composition. These genotypes were designated Subgroup A and the remaining seven genotypes were designated Subgroup B (Table IV). For each location, the fatty acid composition of Subgroup A was significantly ($P<0.01$) different from that of Subgroup B. Subgroup A contained 58.3% higher levels of oleic

TABLE III

Average Compositional and Lipoxygenase Activity Values of Groups IV-S and V Soybean Genotypes at Two Locations[a]

Location	Group	Moisture (%)	Protein (%)	Oil[b] (%)	Fatty acid composition[c]			Lipoxygenase[b] activity (U/mg Prot)
					Oleic	Linoleic	Linolenic	
Tifton	IV-S	8.9 ± 0.14	41.1 ± 0.59	23.2 ± 0.32	28.0 ± 2.4	51.4 ± 1.9	5.5 ± 0.42	0.62 ± 0.06
	V	9.6 ± 0.19	41.2 ± 0.37	22.9 ± 0.24	25.0 ± 1.3	54.1 ± 1.0	5.8 ± 0.27	0.58 ± 0.04
Blairsville	IV-S	10.3 ± 0.07	39.7 ± 0.39	21.6 ± 0.33	25.7 ± 1.6	52.8 ± 1.3	6.3 ± 0.28	0.70 ± 0.05
	V	10.4 ± 0.16	38.4 ± 0.38	21.6 ± 0.16	21.9 ± 0.7	55.3 ± 0.59	7.9 ± 0.12	0.59 ± 0.03

[a]Mean values with standard error of the mean.

[b]Protein and oil contents were significantly ($P<0.01$) different between locations. Lipoxygenase activities were not significantly different.

[c]As % of total fatty acids. Palmitic and stearic acids did not vary significantly between locations and are not included.

TABLE IV

Fatty Acid Composition of Oil from Soybean Genotypes within Group IV-S[a]

Location	Subgroups	Fatty acid composition as % of total fatty acids		
		Oleic	Linoleic	Linolenic
Tifton	A[b]	37.5 ± 0.66	44.2 ± 0.92	4.0 ± 0.27
Blairsville		30.9 ± 1.28	48.5 ± 1.0	5.5 ± 0.19
Tifton	B[c]	21.4 ± 0.90	56.7 ± 0.67	6.3 ± 0.48
Blairsville		21.9 ± 0.48	55.9 ± 0.58	6.9 ± 0.18

[a]Mean values with standard error of the mean.

[b]Subgroup A includes only the genotypes Oksoy, D66-5566, S63-53285, V68-1242, and K1002.

[c]Subgroup B includes all genotypes in Group IV-S except those in subgroup A.

and 17.7% and 28.0% lower levels of linoleic and linolenic acids, respectively, than Subgroup B. Within Subgroup A, the Tifton-grown genotypes contained higher levels of oleic acid and lower levels of linoleic and linolenic acids than the Blairsville-grown genotypes; but within Subgroup B, the fatty acid compositions were essentially the same at both locations. These data suggest a genetic influence in oil composition inasmuch as fatty acid contents were atypical at both locations.

The data in Table III indicate an environmental influence on protein and oil contents and fatty acid composition of the oil. Earlier findings have shown that oil content and quality are directly related to environmental influences. Soybeans grown under warmer temperatures produced beans with higher oil content, whereas those grown under cooler temperatures yielded oils with higher levels of linoleic and linolenic acids (2). Investigations with developing seed and leaf tissue in several plant species have shown that the levels of these two acids are directly related to O_2 solubility in cell sap, which is inversely related to temperature. At constant temperature, plants grown under higher O_2 tensions produced higher levels of unsaturated fatty acids in leaves and seeds than those grown under lower O_2 tensions (4). Unsaturated fatty acid levels also increased directly with photosynthetic rates (14). As compared with those grown at Tifton, then, the 24 soybean genotypes grown at Blairsville would be expected to grow at cooler temperatures and thus produce higher levels of unsaturated fatty acids and lower levels of oil. Our data appear to support this suggestion. The later maturity for groups IV-S and V genotypes at Blairsville may also be related to the lower levels of oleic and higher levels of linoleic and linolenic acids found in these soybeans. The data also indicate that soybeans grown under warmer conditions produced more protein in Groups IV-S and V (Table III).

Lipoxygenase specific activity ranged from 0.28 U/mg prot to 1.02 U/mg prot for Tifton genotypes and 0.34 U/mg prot to 1.03 U/mg prot for the Blairsville genotypes (Tables I and II). Most duplicate samples varied 10% or less, and only a few varied ca. 20% in enzyme activity. The specific activity was essentially the same at either location for each genotype. This suggests that environment had little effect on the level of lipoxygenase in these samples. However, a threefold variation in specific activity among the 24 genotypes at either location possibly indicates genetic control of this enzyme.

These results suggest that selection of soybeans could be based on fatty acid composition of the oil as affected by the environment during plant growth, as well as protein and oil content. During commercial processing of soybeans, lipoxygenase activity must be eliminated to produce an acceptable product. These processes require either high temperature or ethanol extraction to effectively denature the enzyme (15). If, however, the initial lipoxygenase levels are low in soybeans, then inactivation should be easier to accomplish commercially. For this reason, low lipoxygenase activity could also be used as a criterion for selection.

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