JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 10163–10174 10163
DOI:10.1021/jf902344h

Effect of Genotype, Environment, and Their Interaction on Chemical Composition and Antioxidant Properties of Low-Linolenic Soybeans Grown in Maryland

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Eight soybean genotypes grown in three environments in Maryland were analyzed for total phenolic content (TPC), antioxidant capacity, isoflavone composition, lutein, tocopherols, fatty acid composition, and oil content. Fatty acid composition, isoflavones, lutein, tocopherols, and specific antioxidant assays had significant variation by genotype (G) ($P < 0.05$). Environment (E) had a significant effect on fatty acids, lutein, individual tocopherols, oxygen radical absorbance capacity (ORAC), and the isoflavone glycitein ($P < 0.05$). In addition, the interaction between genotype and environment (G × E) showed a significant effect on antioxidant capacity, isoflavones, lutein, tocopherols, and fatty acids ($P < 0.05$). Factorial designed analysis of variance of all data indicated that G had a larger effect than E on the majority of fatty acids, total isoflavones, lutein, and total tocopherols. E had a larger effect than G on stearic acid (18:0), glycitein, δ-tocopherol, and ORAC. The results of this study show that the genotype, growing environment, and their interactions in Maryland-grown soybeans may alter the levels of specific health-enhancing properties.

KEYWORDS: Isoflavones; soybeans; antioxidants; tocopherols; lutein

INTRODUCTION

Soybeans contain numerous compounds that are beneficial to human health. In recent years, soy compounds have been evaluated for their role in the prevention of cardiovascular disease, cancer, osteoporosis, and other diseases (*1*). Although soy protein is gaining popularity among consumers, soybean oil is the major soybean component found in the American diet, due to its presence in processed foods (*2*). Soy oil contains 7% α-linolenic acid (18:3n-3), an unstable fatty acid that can be easily oxidized (*3*). With this level of 18:3n-3, soy oil is hydrogenated during processing to prevent the off-flavors associated with auto-oxidation (*3*). Hydrogenation of oil may produce harmful *trans*-fatty acids that are associated with increased risk of cardiovascular diseases (*4*). Through cross-breeding and genetic modification, soybeans have been developed with reduced levels of 18:3n-3. The oil of these soybeans can be used in processed foods without the need for hydrogenation (*5*). Because the FDA has mandated labeling of *trans*-fats in foods, demand for soybeans low in 18:3n-3 has increased (*5*). Our previous research has demonstrated that low-18:3n-3 soybean genotypes grown in Maryland may possess antioxidant capacity and isoflavone, tocopherol, and carotenoid composition similar to those of the nonmodified genotypes (*6*).

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It has previously been shown that the nutrient composition in food crops is affected by genotype (G), environment (E), or interaction between G and E (G × E) (*7*−*9*). For example, Moore et al. (*8*) reported an effect of G, E, and G × E on phenolic acid composition and antioxidant capacity in hard winter wheat grain. They also observed an effect of elevated temperature on total phenolic content in wheat varieties grown in Colorado. G, E, and interaction between G and E are known to cause variation in soybean components (*7*). In 1994, Wang and Murphy (*7*) found that the ratio of isoflavones in soybeans varied due to differences in genotype, location, and growing season. Britz et al. (*10*) reported variation in soybean tocopherol levels related to growing season, genotype, and location. Additionally, Lee et al. (*11*) reported the effect of environmental conditions on lutein content in soybeans. However, no previous study has examined the effect of G, E, and their interaction on antioxidant properties and other health-enhancing components of soybeans bred for low α-linolenic acid.

Individual environmental factors such as temperature and precipitation/irrigation have also been shown to affect the isoflavone composition of soybeans (*9*). The study of Ohio soybeans by Riedl et al. (*9*) found that precipitation rather than temperature was correlated with isoflavone levels. Soybean fatty acid composition may also vary by exposure to environmental conditions. It was found that warmer growing temperatures might increase α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), but might decrease the levels of oleic acid (18:1n-9) (*3*). Ray et al. (*12*)

Published on Web 10/09/2009

found that soybeans with an earlier planting date had reduced 18:3n-3 compared to those with a later planting date. These previous research data indicated possible effects of G, E, and G × E on chemical compositions in soybeans with reduced α-linolenic acid content. Therefore, the present study was conducted to determine whether and how G, E, and G × E may alter the selected health components and antioxidant properties of Maryland-grown low α-linolenic soybeans. This research is part of our continuous effort to enhance the value-added production of Maryland-grown soybeans.

MATERIALS AND METHODS

Materials and Chemicals. Whole soybeans from the 2007 growing season were collected by Dr. William Kenworthy of the Department of Plant Sciences and Landscape Architecture, University of Maryland, College Park. The soybeans used in this study were grown in three environments in Maryland: the Wye Research Center near Queenstown (full-season crop) and two environments at the Poplar Hills field near Quantico, MD (both full-season and double-crop soybeans were analyzed and considered to be different environments). Seven genotypes were modified for reduced 18:3n-3, and one was a nonmodified cultivar commonly grown in Maryland. The soybeans were products of a traditional breeding program.

Thirty percent ACS-grade hydrogen peroxide was purchased from Fisher Scientific (Fair Lawn, NJ). 2,2′-Azobis(2-aminodopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Fluorescein (FL), iron(III) chloride, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Oil Extraction. Whole soybeans were ground to particle size 20-mesh using a hand-held coffee bean grinder. Five grams of ground soybeans were combined in a tube with 10 mL of petroleum ether. Tubes were vortexed for 15 s and held for 20 h at ambient temperature in the dark. The supernatant was removed and stored. The extraction was repeated twice. The petroleum ether was evaporated overnight under nitrogen, and the remaining oil was weighed. The oil samples were stored in the dark until further testing.

Antioxidant Extraction. The defatted soy flour that remained following oil extraction was air-dried overnight at ambient temperature. One gram of each soy flour sample was combined in a test tube with 10 mL of 50% acetone. The tubes were vortexed 3 times for 15 s each and kept in the dark at ambient temperature overnight. The supernatant was removed, filtered, and stored in the dark until further testing.

Fatty Acid Composition. The soybean oil was prepared for gas chromatography (GC) analysis according to a previously described procedure (*13*). The soybean oil was saponified and methylated to form fatty acid methyl esters (FAME) and dissolved in iso-octane. GC analysis was performed with a Shimadzu GC-2010 with FID. Helium was the carrier gas at a flow rate of 2.2 mL/min. The stationary phase was a fused silica capillary column SP-2380 (30 m × 0.25 mm with a 0.25 μm film thickness) from (Supelco, Bellefonte, PA). Injection volume was 1 μL at a split ratio of 10:1. Oven temperature started at 136 °C, was increased by 6 °C/min to 184 °C, was held for 3 min, and was then increased by 6 °C/min to a final temperature of 226 °C. Fatty acids were identified by comparing FAME retention time with that of known external standards. The FAMEs were quantified by the area under the curve of each identified peak. The ratio of individual FAME area to total area of all FAMEs was calculated to determine the percentage of each fatty acid in the oil.

Total Phenolic Contents (TPC). The total phenolic content of each soy flour extract was determined according to a previously described laboratory procedure (*14*). The final reagent mixture contained 50 μL of soy flour extract or standard, 250 μL of Folin−Ciocalteu reagent, 1.5 mL of 20% sodium carbonate, and 1.5 mL of ultrapure water, using gallic acid as a standard. After 2 h of reaction time at ambient temperature, absorbance was read at 765 nm. The reactions were conducted in duplicate, and results are reported in milligrams of gallic acid equivalents (GAE) per gram of whole soybean.

Isoflavone Composition. Three milliliters of soy flour extracts in 50% acetone was combined with 0.75 mL of 36% hydrochloric acid and heated for 2 h in a water bath at 55 °C. This step was performed to hydrolyze isoflavones to the aglycone form. The acetone was then evaporated under nitrogen. The remaining solution was extracted three times with 4 mL of ethyl ether/ethyl acetate (1:1, v/v) and washed with 3 mL of distilled water. The ethyl ether/ethyl acetate was removed using a nitrogen evaporator. The remaining soy extract was quantitatively redissolved in 0.5 mL of methanol and filtered through a 0.45 μm filter prior to HPLC analysis. HPLC was performed according to a previously described method (*15*), using a Shimadzu LC-20AD with autosampler. The column was a Phenomenex C18 (150 × 4.6 mm, 5 μm). The eluent consisted of 99.9% distilled deionized water with 0.1% acetic acid (v/v) (solvent A) and 99.9% acetonitrile with 0.1% acetic acid (v/v) (solvent B). The gradient progressed from 25 to 32% solvent B over 20 min. The detection wavelength was set at 254 nm. Oven temperature was 30 °C. Peak area of samples was compared to that of known standards to quantify isoflavone content.

DPPH Radical Scavenging Capacity Estimation. DPPH scavenging capacity was determined according to a previously described laboratory procedure (*16*), using a Victor^3 multilabel plate reader (Perkin-Elmer, Turku, Finland). DPPH radical solution (0.2 mM) was prepared in 50% acetone and filtered through a P5 grade filter paper (Fisher Scientific). Trolox standards were prepared in 50% acetone at concentrations of 6.25, 12.5, 25, 37.5, and 50 μM. Each final reaction mixture contained 100 μL of soybean extract, Trolox standard, or 50% acetone (control), and 100 μL of 0.2 mM DPPH solution. The absorbance was read at 515 nm. The radical scavenging capacity (RDSC) was calculated from the area under the curve and reported in micromoles of Trolox equivalents (TE) per gram of whole soybean.

Oxygen Radical Absorbance Capacity (ORAC). The ORAC values were determined using a previously reported laboratory procedure with fluorescein (FL) as a fluorescent probe (*14*). Trolox standards were prepared in 50% acetone at concentrations of 20, 40, 60, 80, and 100 μM. The other reagents were prepared in 75 mM phosphate buffer. In the initial reaction, 225 μL of 8.16×10^{-8} M FL was combined with 30 μL of sample, standard, or blank in a 96-well plate. The plate was heated at 37 °C for 20 min in a Victor^3 multilabel plate reader (Perkin-Elmer). Twenty-five microliters of 0.36 M AAPH was added to each well, and the fluorescence of the mixture was recorded every 2 min over a 40 min period at 37 °C. Excitation and emission wavelengths were 485 and 535 nm, respectively. The results were reported as micromoles of Trolox equivalents (TE) per gram of whole soybean, based on area under the curve calculations (*17*).

Hydroxyl Radical Scavenging Capacity (HOSC). The HOSC assay was conducted using a previously reported laboratory procedure (*18*). Trolox prepared in 50% acetone was used as the standard at concentrations of 20, 40, 60, 80, and 100 μM. Fluorescein was used as a fluorescent probe, and the assay was performed using a Victor^3 multilabel plate reader (Perkin-Elmer). Iron(III) chloride (3.43 M) and 0.1999 M hydrogen peroxide were prepared in ultrapure water, and 9.28×10^{-8} M FL was prepared in 75 mM sodium phosphate buffer (pH 7.4). The reaction mixture consisted of 170 μL of 9.28×10^{-8} M FL, 30 μL of sample, standard, or blank, 40 μL of 0.1999 M hydrogen peroxide, and 60 μL of 3.43 M iron(III) chloride. The fluorescence was recorded every 4 min for 4 h. Antioxidant capacity was calculated by area under the curve (AUC) described by Moore and others (*18*). Results were reported as micromoles of Trolox equivalent per gram of whole soybean.

Lutein Content. The soybean oil samples and standards were diluted in methanol/acetonitrile/chloroform (7:7:6, v/v/v) and filtered through a 0.45 μm filter. Soybean oil was diluted 1:5 to fall within the standard curve with a lutein concentration range of 1−10 μg/mL. HPLC analysis was performed according to a previously described method (*19*) using a Phenomenex C-18 column (250 × 4.6 mm, 5 μm) with a Phenomenex security guard cartridge. The mobile phase was isocratic, using methanol/acetonitrile/chloroform (45:45:10, v/v/v) with 0.05% ammonium acetate (w/v) in the methanol and 0.1% triethylamine (v/v) in the acetonitrile. Fifty microliters of each standard or sample was injected, and run time was 10 min, with each sample conducted in duplicate. A standard curve was developed from the known standards, and peak area of unknown samples was compared to this for quantification.

Tocopherol Content. Soybean oil and tocopherol standards were diluted 1:10 in methyl-*tert*-butyl ether and filtered through a 0.45 μm filter.

Table 1. Environmental Conditions during Soybean Growth from Planting to Harvest[a]

	abs high temp (°C)	abs low temp (°C)	av high temp (°C)	av low temp (°C)	overall av temp (°C)	precipitation (in.)
AG2091 V/PF	36.7	6.1	29.4	15.0	22.3	9.39
AG2091 V/PD	36.7	6.1	29.6	15.2	22.5	8.68
AG2091 V/W	37.8	11.7	28.9	20.2	24.5	9.14
AG3521 V/PF	36.7	6.1	29.4	15.0	22.3	9.39
AG3521 V/PD	36.7	6.1	29.6	15.2	22.5	8.68
AG3521 V/W	37.8	11.7	28.9	20.1	24.5	9.14
MD 04-6006/PF	36.7	6.1	29.4	15.0	22.3	9.39
MD 04-6006/PD	36.7	6.1	29.6	15.2	22.5	8.68
MD 04-6006/W	37.8	11.7	28.9	20.1	24.5	9.14
MD 05-5656/PF	36.7	6.1	29.4	15.0	22.3	9.39
MD 05-5656/PD	36.7	6.1	29.6	15.2	22.5	8.68
MD 05-5656/W	37.8	11.7	28.9	20.2	24.5	9.14
MD 05-6377/PF	36.7	6.1	29.4	15.0	22.3	9.39
MD 05-6377/PD	36.7	6.1	29.6	15.2	22.5	8.68
MD 05-6377/W	37.8	11.7	28.9	20.2	24.5	9.14
MD 05-6381/PF	36.7	6.1	29.4	15.0	22.3	9.39
MD 05-6381/PD	36.7	1.7	29.1	14.6	22.0	9.95
MD 05-6381/W	37.8	11.7	28.7	19.9	24.3	9.14
MD 04-5217/PF	36.7	6.1	29.4	15.0	22.3	9.39
MD 04-5217/PD	36.7	6.1	29.6	15.2	22.5	8.68
MD 04-5217/W	37.8	11.7	28.9	20.1	24.5	9.14
Manokin/PF	36.7	6.1	29.4	15.0	22.3	9.39
Manokin/PD	36.7	1.7	29.1	14.6	22.0	9.95
Manokin/W	37.8	11.7	28.7	19.9	24.3	9.14

[a] Temperatures reported for each location and genotype represent absolute high, absolute low, average high, average low, and overall average in °C during the 2007 season from planting to harvest. Precipitation is reported in inches. Differences among genotypes at the same location are due to differing number of days to maturity. PF, Poplar Hills full seed crop (Salisbury, MD); PD, Poplar Hills double cropped (Salisbury, MD); W, Wye Research Center (Queenstown, MD).

Reversed-phase HPLC with UV detection was performed according to a previously described procedure (*20*) with modifications. The stationary phase was a Waters C-30 column (250 × 4.6 mm, 5 μm). The mobile phase consisted of methanol/MTBE/water (81:15:4, v/v/v) (solvent A), and MTBE/methanol (91:9, v/v) (solvent B). The mobile phase was run from 0 to 16% solvent B in 13 min, 100% solvent B was maintained from 13 to 23 min, and the column was re-equilibrated with 100% solvent A from 23 to 32 min. Flow rate was 1.0 mL/min., and injection volume was 30 μL. The UV detector wavelength was 295 nm. Each standard and sample were run in duplicate.

Data on Environmental Conditions. The precipitation at each location during the growing season was reported by Kenworthy and others of the Maryland Cooperative Extension in "Agronomy Facts 32" (*21*). Daily temperature highs, lows, and averages were obtained from records kept by National Oceanic and Atmospheric Administration (NOAA) weather stations in the vicinity of the soybean fields. The environmental conditions including the precipitation and temperature highs, lows, and averages at each location during the growing season are summarized in **Table 1**. The Wye Research Center is a coastal location on the Chesapeake Bay. The Poplar Hills location is 60 miles southeast of the Wye Research Center and has more extreme low temperatures than the first location.

Statistical Analysis. Data were analyzed using SPSS (SPSS for Windows, version rel. 10.0.5., 1999, SPSS Inc., Chicago, IL). Factorial design analysis of variance (ANOVA) was performed on the data using a general linear model (GLM) with three replicates, using genotype and environment as fixed effects. Replicates were randomly selected samples from each test plot at each location. Tukey's post hoc test was used to determine differences between means after ANOVA analysis. Correlation was analyzed using a two-tailed Pearson's correlation test. Statistical significance was noted for values of $P < 0.05$ ($\alpha > 0.95$).

RESULTS AND DISCUSSION

The present study evaluated the chemical compositions and antioxidant properties of eight soybean cultivars grown in the three different environmental conditions. The effects of environmental conditions (E), genotype (G), and the interaction between G and E (G × E) on chemical compositions and antioxidant properties of soybeans were also investigated. In addition, the correlation between each examined chemical component and antioxidant property was calculated.

Chemical Compositions of the Eight Soybean Cultivars Grown in the Three Maryland Environments. *Oil Content and Fatty Acid Composition*. Oil content of the soybeans ranged from 14.0 to 18.2 g/100 g among all genotypes from the three growing locations under the experimental conditions (**Table 2**). The fatty acid profiles of the soybeans under the different growing conditions might differ significantly (**Table 2**). MD 05-6377 had the lowest 18:3n-3 concentration, ranging from 1.04 to 1.20%, which was significantly lower than all other soybean cultivars from all growing locations. AG2091 V, AG3521 V, and MD 05-6381 soybeans had 18:3n-3 content between 2.1 and 2.5%, which was significantly higher than that in MD 05-6377 cultivar at all three locations. This range was significantly lower than that in MD 04-6006, MD 05-5656, and MD 04-5217 soybeans grown at Poplar Hills (double cropped) in Salisbury (PD), and that in MD 04-6006 and MD 05-5656 at the Wye Research Center location in Queenstown, MD (**Table 2**). MD 05-6377 soybean from all three locations also had significantly lower palmitic acid (16:0) level, with a range of 4.2−4.7 g/100 g fatty acids, than the other soybeans grown at all tested locations. Interestingly, all seven low-linolenate soybeans grown at Wye Research Center had higher or the same concentration of oleic acid (18:1n-9) compared to the same genotype grown at the other two locations (**Table 2**). These data suggested that both genotype and growing environment could alter oil content and fatty acid composition in soybeans.

Total Phenolic Content. Phenolics are potential antioxidative components (*22*). TPC of the soybeans was between 1.2 and 2.1 mg of GAE/g of whole soybean (**Figure 1**). These values are consistent with previously reported levels of soybean TPC values of 1.5−5.4 mg of GAE/g (*6*, *9*). AG3521 V and Manokin grown at the Wye Research Center location significantly differed in their TPC values, suggesting the possible effect of genotype on TPC.

Table 2. Oil Content and Fatty Acid Composition of Soybeans[a]

	oil	16:0	18:0	18:1n-9	18:2n-6	18:3n-3
AG2091 V/PF	18.2e ± 0.1	10.7j–l ± 0.1	4.6b–d ± 0.2	31.0ef ± 2.1	51.4d ± 2.12	2.3bc ± 0.0
AG2091 V/PD	17.3de ± 1.3	11.1 lm ± 0.4	6.1f–h ± 0.3	26.9cd ± 1.4	55.0gh ± 1.4	2.5cd ± 0.1
AG2091 V/W	16.6b–e ± 0.5	10.8j–l ± 0.1	4.2ab ± 0.1	32.3fg ± 0.5	50.6d ± 0.5	2.2b ± 0.0
AG3521 V/PF	16.9c–e ± 0.2	10.5jk ± 0.2	5.0d ± 0.6	26.0c ± 1.4	56.5hi ± 2.2	2.5cd ± 0.1
AG3521 V/PD	17.5de ± 2.0	10.6jk ± 0.1	4.8d ± 0.0	22.0ab ± 0.3	59.7h ± 0.2	2.5de ± 0.1
AG3521 V/W	16.7b–e ± 0.5	10.5j ± 0.1	3.8a ± 0.0	26.5c ± 0.6	56.7hi ± 0.6	2.5cd ± 0.1
MD 04-6006/PF	14.6a–c ± 1.3	6.8f ± 0.1	6.3g–i ± 0.1	36.5i ± 0.7	47.2c ± 0.7	3.1ef ± 0.1
MD 04-6006/PD	14.6a–c ± 0.4	7.6g ± 0.2	5.5e ± 0.1	27.9cd ± 1.2	55.1gh ± 1.1	3.5fg ± 0.1
MD 04-6006/W	16.1a–e ± 3.7	6.8f ± 0.1	4.6b–d ± 0.1	41.4l ± 1.2	43.9a ± 0.9	3.6hi ± 0.2
MD 05-5656/PF	14.0a ± 1.0	6.0e ± 0.1	5.9e–h ± 0.3	37.4jk ± 1.5	47.6c ± 1.6	3.1ef ± 0.1
MD 05-5656/PD	15.7a–e ± 2.3	5.8c–e ± 0.2	6.7l ± 0.1	39.9l ± 0.3	44.1ab ± 0.2	3.5fg ± 0.1
MD 05-5656/W	14.3ab ± 0.2	6.0e ± 0.1	4.6b–d ± 0.1	39.3kl ± 0.8	46.5c ± 0.9	3.6hi ± 0.2
MD 05-6377/PF	16.3a–e ± 0.2	4. 3a ± 0.1	5.0ef ± 0.3	34.3gh ± 0.1	54.7f–h ± 0.3	1.0a ± 0.04
MD 05-6377/PD	17.2de ± 0.4	4.7b ± 0.2	5.0d ± 0.0	27.0c ± 0.2	62.2k ± 0.2	1.0a ± 0.05
MD 05-6377/W	15.2a–d ± 0.5	4.2a ± 0.2	4.6b–d ± 0.1	37.7jk ± 0.5	52.3de ± 0.5	1.2a ± 0.17
MD 05-6381/PF	16.3a–e ± 0.2	5.5c ± 0.3	5.7ef ± 0.3	30.2ef ± 0.5	56.6hi ± 0.6	2.1b ± 0.2
MD 05-6381/PD	17.2de ± 0.4	5.9de ± 0.3	4.8d ± 0.1	26.5c ± 0.5	60.6jk ± 0.8	2.2bc ± 0.2
MD 05-6381/W	15.2a–d ± 0.5	5.6cd ± 0.1	4.2ab ± 0.1	29.2de ± 0.6	58.7ij ± 0.3	2.3bc ± 0.3
MD 04-5217/PF	17.2de ± 0.7	9.5l ± 0.2	6.3hi ± 0.5	34.0hi ± 1.2	46.2bc ± 1.5	3.0de ± 0.1
MD 04-5217/PD	17.0c–e ± 0.4	9.8 ± 0.2	5.8e–g ± 0.5	26.8c ± 2.0	54.0efg ± 2.1	3.4f–h ± 0.2
MD 04-5217/W	16.2a–e ± 0.5	9.2h ± 0.2	4.3a–c ± 0.1	39.3kl ± 1.6	44.2ab ± 1.4	3.3ef ± 0.2
Manokin/PF	16.6b–e ± 0.6	10.9k–m ± 0.1	4.8cd ± 0.1	23.2ab ± 0.2	54.1e–g ± 0.1	7.0j ± 0.1
Manokin/PD	15.3a–d ± 0.4	10.7jk ± 0.2	5.5e ± 0.1	23.7b ± 0.3	52.6d–f ± 0.1	7.5k ± 0.1
Manokin/W	15.4a–d ± 0.5	11.2m ± 0.1	4.5b–d ± 0.1	21.1a ± 1.0	55.2gh ± 0.5	8.1l ± 0.4

[a] Data are expressed as the mean of three replicate plots, each tested in duplicate, ± SD ($N = 6$). Oil is expressed as g/100 g of whole soybean. Fatty acids are expressed as g/100 g of oil. All genotypes are low 18:3n-3, except Manokin, which is a nonmodified genotype. Values marked by the same letter within each fatty acid group are not statistically different. PF, Poplar Hills full seed (Salisbury, MD); PD, Poplar Hills double cropped (Salisbury, MD); W, Wye Research Center (Queenstown, MD).

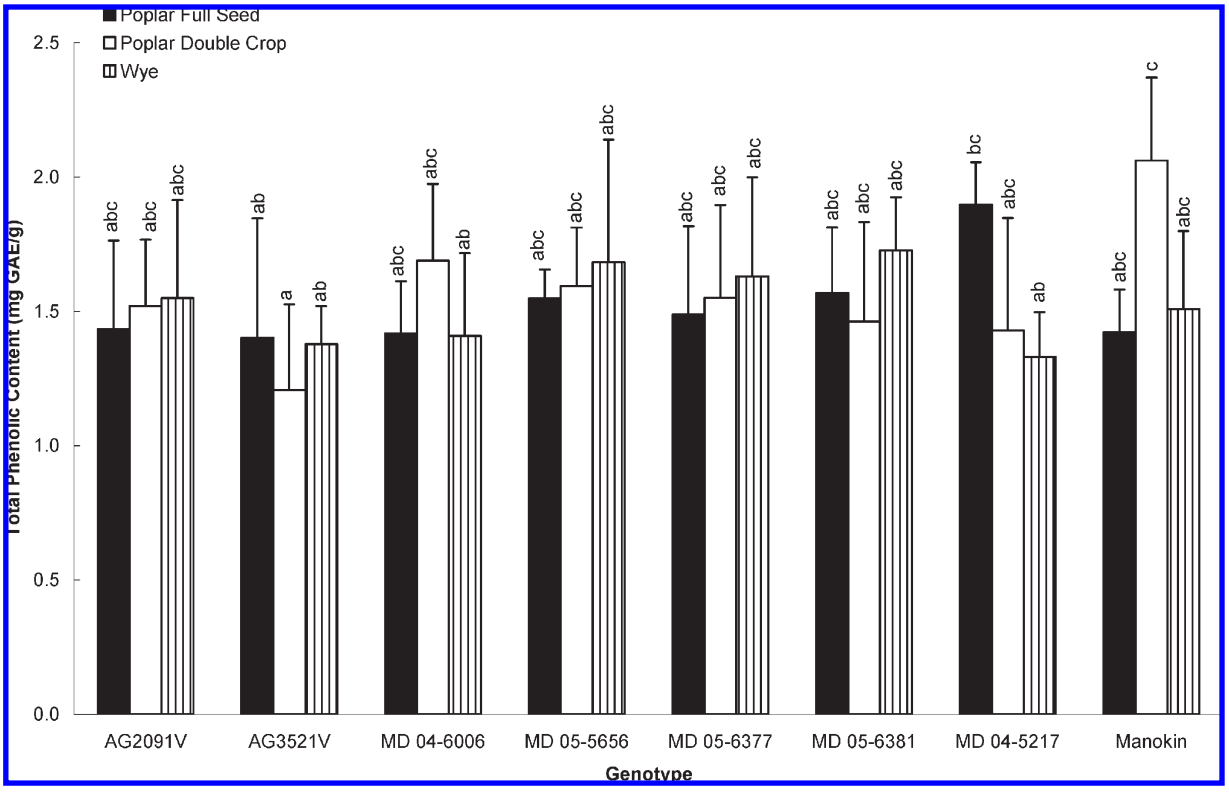

Figure 1. Total phenolic content of soybeans. Data are expressed as milligrams of gallic acid equivalent (GAE) per gram of soybean. Values represent the mean of three replicate plots ± SD ($n = 6$). Values marked by the same letter are not statistically different. Poplar Full Seed, Poplar Double Crop, and Wye indicate growing environment.

No difference in TPC was observed in any tested soybean genotype grown at the different locations.

Isoflavones. The total and individual isoflavones were estimated and reported in their aglycone levels. Total isoflavones in the soybean samples ranged from 0.37 to 0.90 μmol/g of soybean among all genotypes grown at different environments (**Table 3**). This total isoflavone content is lower than that reported previously. Riedl et al. (*9*) found total isoflavones in Ohio soybeans

Table 3. Isoflavone Composition of Soybeans[a]

	daidzein	glycitein	genistein	total isoflavones
AG2091 V/PF	31.2ab ± 9.6	68.4d−g ± 16.2	21.6ab ± 6.4	0.45a ± 0.01
AG2091 V/PD	38.5b−d ± 9.4	58.2b−g ± 17.6	39.8a−d ± 9.8	0.50ab ± 0.12
AG2091 V/W	34.2ab ± 3.2	95.8g ± 7.6	30.9a−d ± 4.4	0.59a−c ± 0.05
AG3521 V/PF	34.1ab ± 7.6	84.6 ± 7.8	31.6a−d ± 10.0	0.55a−c ± 0.08
AG3521 V/PD	31.2ab ± 1.3	44.6a−f ± 3.9	33.1a−d ± 2.1	0.40a ± 0.01
AG3521 V/W	33.2ab ± 3.2	61.0c−g ± 3.1	34.4a−d ± 1.4	0.47a ± 0.02
MD 04-6006/PF	41.0a−d ± 1.6	75.1fg ± 11.0	25.3ab ± 2.7	0.52ab ± 0.03
MD 04-6006/PD	63.8a−e ± 4.4	33.7a−c ± 1.12	46.5a−d ± 5.3	0.54ab ± 0.04
MD 04-6006/W	53.8a−e ± 11.8	74.3e−g ± 32.9	38.3a−d ± 13.0	0.62a−c ± 0.21
MD 05-5656/PF	78.2d−f ± 7.5	49.9a−g ± 5.4	52.9b−e ± 6.7	0.68a−c ± 0.07
MD 05-5656/PD	58.3a−e ± 18.6	41.7a−d ± 6.3	43.0a−d ± 14.7	0.54ab ± 0.14
MD 05-5656/W	84.2ef ± 22.9	78.2g ± 10.5	61.9a−d ± 18.9	0.83bc ± 0.18
MD 05-6377/PF	29.3a ± 8.4	61.2c−g ± 25.9	15.3a ± 5.3	0.39a ± 0.14
MD 05-6377/PD	38.8a−d ± 3.5	35.3a−c ± 4.5	36.0a−d ± 3.0	0.41a ± 0.04
MD 05-6377/W	32.4ab ± 3.7	46.3a−f ± 10.5	22.2ab ± 2.2	0.37a ± 0.04
MD 05-6381/PF	44.2a−d ± 6.4	55.6a−g ± 12.2	18.9a ± 3.8	0.44a ± 0.06
MD 05-6381/PD	33.7ab ± 5.1	35.3ab ± 4.5	30.0a−d ± 2.6	0.34a ± 0.03
MD 05-6381/W	39.2a−c ± 5.4	46.3a−c ± 7.5	26.7a−d ± 2.2	0.36a ± 0.03
MD 04-5217/PF	39.0a−c ± 16.0	88.4 g ± 10.3	28.8a−d ± 11.4	0.57a−c ± 0.09
MD 04-5217/PD	40.0a−c ± 9.9	43.9a−e ± 9.8	36.1a−d ± 7.7	0.45a ± 0.10
MD 04-5217/W	45.1a−d ± 1.8	61.6c−g ± 12.4	39.3a−d ± 3.1	0.54ab ± 0.05
Manokin/PF	107.7e ± 76.0	47.8a−g ± 36.2	83.0e ± 61.1	0.90c ± 0.66
Manokin/PD	70.5b−e ± 12.2	25.8a ± 4.3	58.9c−e ± 6.2	0.56a−c ± 0.08
Manokin/W	69.5b−e ± 11.2	41.2a−d ± 23.4	51.4b−e ± 8.9	0.61a−c ± 0.10

[a] Data are expressed as the mean of three replicate plots, each tested in duplicate, ± SD ($N = 6$). Daidzein, genistein, and glycitein are expressed as μg/g of whole soybean. Total isoflavones are expressed as μmol/g of whole soybean. All genotypes are low 18:3n-3, except Manokin, which is a nonmodified genotype. Values marked by the same letter within each component are not statistically different. PF, Poplar Hills full seed (Salisbury, MD); PD, Poplar Hills double cropped (Salisbury, MD); W, Wye Research Center (Queenstown, MD).

in a range from 1.6 to 7.1 μmol/g when extracted from soy flour with acidic acetonitrile. Slavin et al. (*6*) found 0.9−2.4 μmol/g in Maryland-grown soybeans from the 2005 growing season. This difference might be partially due to the different extraction solvents, extraction procedures, growing seasons, and different soybean cultivars or lines (*7*). Also noted was that there were high standard deviations in isoflavone levels among replicates of the same location and genotype, suggesting that possible effects of other factors might have contributed to the variation.

Glycitein, daidzein, and genistein were detected in all tested soybean samples with a concentration of 25.8−95.8 μg/g for glycitein, 29.3−107.7 μg/g for daizein, and 15.3−83.0 μg/g for genistein (**Table 3**). On a per weight basis, glycitein was the primary isoflavone compound in AG2091 V, AG3521 V, and MD 04-5217 soybeans, but not in MD 05-5656 and Manokin genotypes grown at all three tested locations in Maryland (**Table 3**). Furthermore, glycitein was the primary isoflavone compound in MD 04-6006 and MD 05-6377 soybeans grown at Poplar Hills (full seed) and the Wye Research Center locations, but not necessarily the major one of these two soybean lines grown at Poplar Hills (double cropped). On the other hand, daidzein was the major isoflavone compound in MD 05-5656 and Manokin soybeans grown at all three locations. In the genotypes MD 04-6006 and MD 05-6377, daidzein was highest at Poplar Hills compared to other locations, although not at a statistically different level.

The ratio of isoflavones also varied by genotype and environment. In the AG2091 V soybean, the ratio of daidzein/glycitein/genistein was 1:2:1.5 in the Poplar Hills full seed environment, but was 1:2.8:0.9 in the Poplar Hills double cropped and 1:3:1 in the Wye Research Center environments, respectively. Also noted was that in the MD 05-6377 double-cropped soybeans, the ratio of daidzein/glycitein/genistein was approximately 1:1:1. Taken together, these results indicated the possible effects of genotype and growing environment on soybean isoflavones, providing background for further investigation into the effects of each and their potential interaction on soybean phytochemicals.

Lutein. Lutein has been identified previously as the predominant carotenoid in soybeans (*6*). In the present study, lutein levels ranged from 10.4 to 27.2 μg/g of oil (**Table 4**). Lutein was highest in the MD 05-6381 and MD 04-5217 genotypes and lowest in MD 05-5656 soybeans across all tested locations, suggesting the effect of genotype on soybean lutein concentration. A trend in environment was seen in five of the eight soybean lines, with the highest lutein levels in the Poplar Hills full seed environment followed by the double cropped, and with lowest levels in the Wye environment (**Table 4**). In addition, the highest lutein level in the double-cropped environment was in the MD 05-6381 genotype, whereas the highest level was found in the MD 04-5217 line at the Wye Research Center location, and in the full seed environment MD 05-6381 contained the most lutein. These results showed the possible effect of genotype and environment interaction on lutein content in soybeans.

Tocopherols. There have been several studies on the tocopherol levels of soybeans, including those with modified fatty acids (*10*, *24*−*26*). In the present study, α-, γ-, and δ-tocopherols were detected in all soybean samples (**Table 4**). Total tocopherols ranged from 2.3 to 3.1 μmol/g of oil, and α-tocopherol ranged from 259.5 and 317.7 μg/g of oil. The α-tocopherol levels and total tocopherols were generally lower than those reported for Maryland-grown soybeans by Slavin et al. (*6*), but are consistent with levels reported for Indian soybeans by Rani et al. (*27*). The soybeans evaluated by Slavin et al. were grown in Maryland during the 2005 season, and this may partially account for differences in tocopherol levels. Other studies have reported tocopherols on a per gram of soybean basis and so are not necessarily comparable due to different extraction procedures.

MD 04-6006 soybeans had higher or the same levels of total tocopherol, whereas MD 04-5217 genotype contained the highest α-tocopherol across all tested environments (**Table 4**). Four

Table 4. Lutein Content and Tocopherol Composition of Soybeans[a]

	lutein	α-tocopherol	γ-tocopherol	δ-tocopherol	total tocopherol
AG2091 V/PF	15.6de ± 2.4	286.9a−e ± 21.6	384.7a−d ± 55.9	403.4c−f ± 46.9	2.67a−e ± 0.17
AG2091 V/PD	17.0de ± 1.2	290.1a−e ± 14.2	368.2a−d ± 38.0	347.2a−e ± 38.2	2.49a−c ± 0.12
AG2091 V/W	17.5de ± 0.6	272.0ab ± 23.2	326.2a ± 45.4	394.6b−f ± 42.5	2.48a−c ± 0.21
AG3521 V/PF	18.2de ± 1.1	291.1b−e ± 32.6	400.5a-e ± 66.0	339.9a−e ± 32.2	2.55a−c ± 0.25
AG3521 V/PD	15.2c−e ± 1.0	306.0c−e ± 13.2	429.2a−f ± 66.3	301.3a ± 48.0	2.55a−c ± 0.28
AG3521 V/W	14.0a−d ± 0.6	268.9ab ± 5.4	371.1a−d ± 20.5	362.7a−f ± 35.8	2.49a−c ± 0.09
MD 04-6006/PF	18.6c−e ± 1.1	296.1b−e ± 11.0	524.6f ± 44.8	419.7ef ± 46.2	3.08e ± 0.22
MD 04-6006/PD	18.0c−e ± 1.0	276.9a−c ± 12.0	471.0c−f ± 6.9	360.0a−f ± 44.6	2.74b−e ± 0.07
MD 04-6006/W	14.9a−d ± 0.6	280.1a−d ± 8.3	414.1a−f ± 43.9	441.1f ± 63.9	2.83b−e ± 0.22
MD 05-5656/PF	12.3a−c ± 1.9	269.4ab ± 10.5	352.1ab ± 83.5	403.7d−f ± 51.8	2.56a−c ± 0.30
MD 05-5656/PD	10.6ab ± 0.6	281.7a−d ± 13.5	390.5a−e ± 47.1	445.6f ± 51.0	2.79b−e ± 0.15
MD 05-5656/W	10.4a ± 1.5	269.5ab ± 3.7	391.3a−e ± 27.6	427.5ef ± 42.4	2.71a−e ± 0.08
MD 05-6377/PF	17.5de ± 1.4	277.1a−c ± 6.8	498.0ef ± 26.2	357.8a−f ± 39.6	2.80b−e ± 0.12
MD 05-6377/PD	17.1de ± 2.2	273.9ab ± 12.1	478.9d−f ± 53.1	337.3a-e ± 36.3	2.69a−e ± 0.18
MD 05-6377/W	14.3a−d ± 1.6	289.1a−e ± 6.4	501.0ef ± 38.3	406.3d−f ± 43.2	2.97de ± 0.13
MD 05-6381/PF	25.0h−j ± 1.0	283.1a−d ± 13.0	442.5b−f ± 48.2	311.8a−c ± 35.9	2.56a−c ± 0.18
MD 05-6381/PD	27.2ij ± 0.9	274.7ab ± 13.0	401.9a−e ± 71.3	305.8ab ± 29.3	2.43ab ± 0.22
MD 05-6381/W	19.9c−e ± 0.8	279.1a−d ± 18.0	412.9a−e ± 25.3	347.4a−e ± 37.2	2.57a−d ± 0.11
MD 04-5217/PF	24.8ij ± 2.8	317.7e ± 18.4	472.3c−f ± 71.5	370.6a−f ± 53.3	2.87c−e ± 0.25
MD 04-5217/PD	23.0 g−j ± 5.8	308.4de ± 11.2	383.8a−d ± 93.9	336.9a−e ± 37.9	2.54a−c ± 0.27
MD 04-5217/W	22.8f−j ± 3.0	294.7a−d ± 10.5	374.8a−d ± 24.7	408.8d−f ± 43.2	2.68a−e ± 0.09
Manokin/PF	21.1e−i ± 6.6	270.4ab ± 17.0	346.0ab ± 55.2	325.9a−d ± 33.8	2.33a ± 0.20
Manokin/PD	17.0b−e ± 1.5	270.4ab ± 8.6	363.0a−c ± 23.5	397.1b−f ± 34.0	2.48a−c ± 0.08
Manokin/W	19.4d−g ± 1.2	259.6a ± 7.5	383.0a−d ± 43.3	390.2a−f ± 26.9	2.54a−c ± 0.17

[a] Data are expressed as the mean of three replicate plots, each tested in duplicate, ± SD ($N = 6$). Lutein and α-, γ-, and δ-tocopherol are expressed as μg/g of oil. Total tocopherol is expressed as μmol/g of oil. All genotypes are low 18:3n-3, except Manokin, which is a nonmodified genotype. Values marked by the same letter within each component are not statistically different. PF, Poplar Hills full seed (Salisbury, MD); PD, Poplar Hills double cropped (Salisbury, MD); W, Wye Research Center (Queenstown, MD).

soybean lines had the highest amount of total tocopherols in the Poplar Hills full seed environment, and another three soybeans produced greatest total tocopherols in the Wye Research Center environment. Furthermore, α-tocopherol was most abundant in four of the soybean lines at Poplar Hills full seed, whereas three others had higher levels in the double-cropped environment. MD 05-6377 was the only line that produced the highest level of α-tocopherol at the Wye Research Center location.

Antioxidant Properties. All genotypes under all growing conditions demonstrated scavenging capacity against DPPH (RDSC value), hydroxyl (HOSC), and peroxyl (ORAC) radicals (**Figures 2−4**). RDSC value ranged from 0.6 to 1.5 μmol of TE/g among the genotypes at all locations (**Figure 2**). A greater RDSC value is associated with a stronger DPPH radical scavenging capacity. The soybean line that had the greatest RDSC value at one growing location did not necessarily show the highest DPPH radical scavenging capacity in a different growing environment (**Figure 2**). Other groups have previously reported DPPH radical scavenging capacity of soybean extracts (*23*); however, it is difficult to compare the results from different laboratories because not all results were reported as relative DPPH radical scavenging capacity using a standard antioxidant such as Trolox in the present study.

HOSC values varied from 20.1 and 40.1 μmol of TE/g of whole soybeans under the experimental conditions (**Figure 3**). AG3521 V soybean in the Poplar Hills full seed environment showed an 11% stronger HOSC than its counterpart in the Poplar Hills double-cropped environment, whereas the MD 04-5217 line grown in the double-cropped environment had about a 90% higher HOSC value than that in the full seed environment (**Figure 3**).

ORAC values also varied by genotype and environment, as seen in **Figure 4**. ORAC values ranged from 22.4 to 58.4 μmol of TE/g. These ORAC values were within the range previously reported of 21.2−91.3 μmol of TE/g for yellow soybean by Xu and Chang (*23*). Interestingly, the soybean with the greatest ORAC value, which was Manokin in the Poplar Hills full seed environment, did not necessarily exhibit strongest DPPH and hydroxyl radical scavenging capacities in the same environment (**Figures 2−4**). These radical scavenging capacity results suggested that each soybean line or cultivar may respond to environment differently. These results also indicated that each antioxidant property may respond to individual environmental factors differently. Therefore, the contributions of genotype, environment, and their interaction were evaluated for their effect on chemical components and antioxidant properties.

Effects of Genotype (G), Environment (E), and the Interaction between G and E (G × E) on Soybean Composition and Antioxidant Property. *Oil Content and Fatty Acid Composition*. The percent of total mean square for each variable (G, E, and G × E) was determined to quantify the contribution of each variable to soybean components and antioxidant properties. G accounted for the most variations in soybean oil content (60%, $P < 0.001$). E accounted for 27% variation in soybean oil content ($P < 0.01$), whereas G × E contributed 13% of that ($P < 0.01$).

In the majority of fatty acids, G had a larger effect on variance than E (**Table 5**). Genotype showed the largest effects of 98.8 and 97.5% ($P < 0.001$), respectively, on 16:0 and 18:3n-3 contents. The line MD 05-6377 contained the lowest 18:3n-3 levels at all locations (1.0−1.2 g/100 g of oil). This line also contained the lowest level of 16:0 at all locations (4.20−4.72 g/100 g of oil). This line may be noted for future analysis, because soybean oil with low 18:3n-3 and low 16:0 is desirable for reduced-*trans* and saturated fat consumption (*5*). G also was the major contributor for total saturated fat (79%, $P < 0.001$).

E had a large effect (84.3%) on stearic acid (18:0) ($P < 0.001$), whereas G had more effect on the other fatty acids. When averages by environment were compared, 18:3n-3 and 16:0 were both lowest in the Poplar Hills full seed environment (earlier planting date) and highest in the Poplar Hills double-cropped

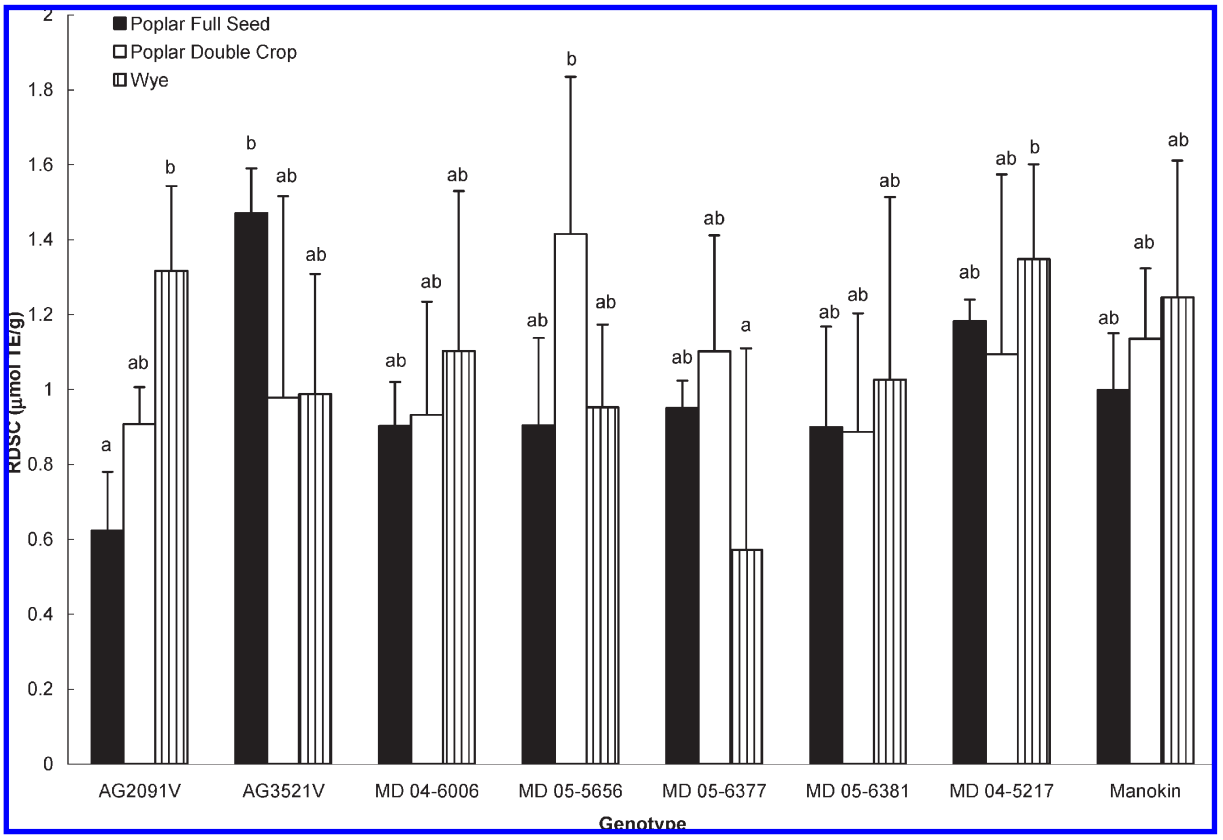

Figure 2. Relative DPPH scavenging capacity (RDSC) of soybeans. Data are expressed as micromoles of Trolox equivalent (TE) per gram of soybean. Values represent the mean of three replicate plots ± SD (n = 6). Values marked by the same letter are not statistically different. Poplar Full Seed, Poplar Double Crop, and Wye indicate growing environment.

environment (later planting date) at statistically significant levels. Ray et al. (*12*) also found lower 18:3n-3 levels in nonmodified soybeans with an earlier planting date. The same study found that 16:0 was lower at a later planting date, which was not observed in our results. The double-cropped soybeans contained the lowest level of 18:1n-9 and highest level of 18:2n-6 compared to other environments. The differences observed by planting date likely reflect changes in temperature or other environmental conditions (*28*). In addition, E had a significant effect on total saturated fat in soybeans (20%, $P < 0.001$) (**Table 5**).

Oleic acid (18:1n-9) is a desirable component of edible oil due to its benefits to cardiovascular health and stability in foods (*29*). The full seed soybeans in this study appeared to have a more desirable fatty acid profile compared to the double-cropped soybeans, due to lower 18:3n-3 and higher 18:1n-9.

The percent variation due to G × E was low in the fatty acids, ranging from 0.3 tp 5.8% (**Table 5**) ($P < 0.001$). In both Poplar Hills environments, 18:1n-9 was highest in the genotype MD 05-5656 (9.3−39.9 g/100 g of oil). However, at the Wye Research Center environment, 18:1n-9 was highest in the MD 04-6006 genotype (41.4 g/100 g of oil). The line AG2091 V produced the highest oil content in the Poplar Hills full seed environment, but AG3521 V contained the highest levels in the other two environments, although the differences are not statistically significant (**Table 2**).

Soybean TPC. There was not a significant effect of G or E individually on variation in soybean TPC; however, there was a significant effect of G × E interaction ($P < 0.05$). For example, the genotype MD 04-5217 demonstrated a high TPC level in the Poplar Hills double-cropped environment, whereas the AG2531 V genotype had a significantly lower level in the same environment. The effect of G × E interaction accounted for 53.6% of variation in TPC levels ($P < 0.01$) (**Table 5**). When analyzing wheat varieties from Colorado, Moore et al. (*8*) found that E accounted for most of the variation in TPC (79.5%). Riedl et al. (*9*) reported significant variation in soybean TPC by environment. However, the present study did not find a similar effect.

Isoflavone Levels. Others have reported significant differences in soy isoflavone level based on genotype (*7*). In the present study, there was significant variation by G in the isoflavone levels ($P < 0.01$). Overall, the Manokin soybean with regular 18:3n-3 concentration contained the highest levels of total isoflavones. Among the reduced 18:3n-3 genotypes, MD 05-5656 contained the highest average levels of total isoflavones, daidzein, and genistein across the different environments. Daidzein and genistein had the most variation attributed to G (88.5 and 78.6%, respectively, $P < 0.001$).

The variation in isoflavones due to environmental differences was also well documented in the literature (*7*, *9*, *28*). Our current study found that the total isoflavone levels and the isomer glycitein showed significant variation by environment ($P < 0.05$). This variation was demonstrated by reduced levels in the Poplar Hills double-cropped environment. Of the isoflavone isomers, only glycitein had the majority of percent variation attributed to E (64.8%, $P < 0.001$) (**Table 5**).

There was a small effect of G × E, ranging from 8.8 to 12.4% ($P < 0.05$) for total and individual isoflavones. The G × E combination with the highest total isoflavone level was MD 05-5656 at the Wye Research Center location (0.83 μmol/g of whole soybean), but G × E interaction was not statistically significant ($P = 0.069$).

Lutein Content. Previous research has reported that lutein content in soybeans might significantly vary across genotypes (*6*) and environments (*11*). Our results showed that G accounted for

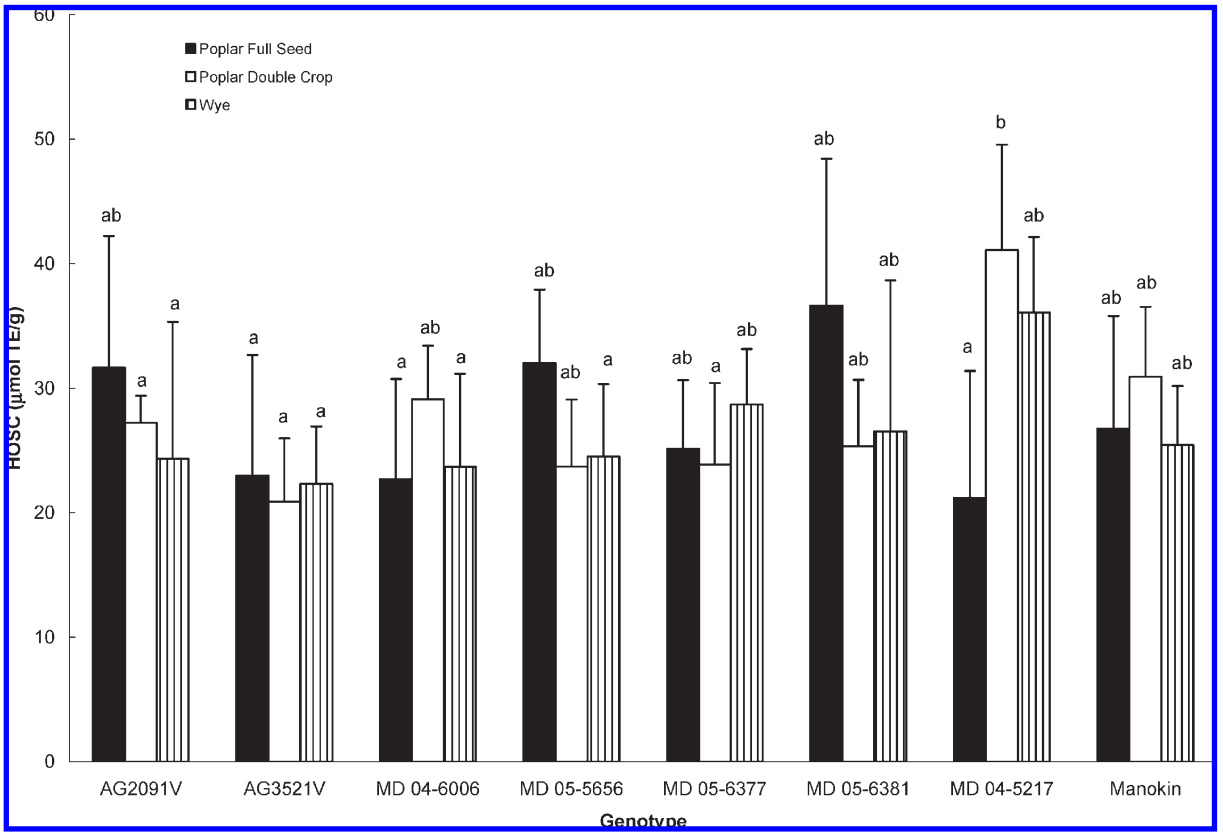

Figure 3. Hydroxyl radical scavenging capacity (HOSC) of soybeans. Data are expressed as micromoles of Trolox equivalent (TE) per gram of soybean. Values represent the mean of three replicate plots ± SD (n = 6). Values marked by the same letter are not statistically different. Poplar Full Seed, Poplar Double Crop, and Wye indicate growing environment.

78.6% of variation in lutein levels ($P < 0.001$) (**Table 5**). MD 05-6381 and MD 04-5217 were the lines with the highest overall lutein levels, ranging from 19.9 to 27.2 μg/g of oil. Environment also accounted for about 18% of the variation in lutein level ($P < 0.001$), with higher mean levels at the Poplar Hills location than at the Wye Research Center location. The combination of G and E that produced the highest lutein level was MD 05-6381 in the Poplar Hills double-cropped environment (27.2 μg/g of oil) (**Table 4**). On the basis of the proportion of total mean squares, the effect of G × E accounted for only 4.1% of the variation ($P < 0.01$).

Tocopherol Composition. α-, γ-, δ-, and total tocopherols showed significant variation by G ($P < 0.01$) (**Table 5**). The genotype MD 04-5217 contained the highest α-tocopherol levels at all locations. G contributed to the largest amount of variance in α-, γ-, and total tocopherols based on proportion of mean squares (57.1−70.3%, $P < 0.001$) (**Table 5**). Individual tocopherol isomers also showed significant variation by E. E contributed to the majority of variance in δ-tocopherol (51.2%, $P < 0.001$), about 30% of that in α-tocopherol ($P < 0.01$), and 20% of that in γ-tocopherol ($P < 0.05$). An environmental effect on soybean tocopherols was also noted by Britz et al. (*10*) and Dolde et al. (*25*), although in both studies G was responsible for more variation than E. In addition, G × E showed significant contribution to α-, γ- and total tocopherols ($P < 0.01$) and to δ-tocopherol ($P < 0.001$).

Antioxidant Capacity. There was a significant effect of G on RDSC ($P < 0.05$) and HOSC ($P < 0.01$). The largest proportion of variation in the HOSC assay was attributed to G (47.9%) (**Table 5**). G contributed 38.6% of the variation in soybean RDSC levels. Moore et al. (*8*) examined variation in antioxidant capacity of hard winter wheat and reported the variation of RDSC in winter wheat samples was attributed mainly to G (88.6%). Thus, food crops may have varying factors that influence antioxidant capacity, suggesting the possibility of improving the antioxidant properties in food crops such as soybeans and wheat through breeding effort or genetic modification.

There was significant variation by environment in the ORAC levels when averaged among all genotypes ($P < 0.05$). The double-crop soybeans had a higher ORAC level on average than the other environments. The largest variation in ORAC was attributed to E (55.8%, $P < 0.05$). The other antioxidant assays did not demonstrate significant variation by environment. This finding was in agreement with that for wheat by Moore and others (*8*). They found that the ORAC value of winter wheat was more affected by E (51.8%) than G, which is similar to the effect found on soybeans in the current study.

In addition, G × E might significantly alter RDSC and HOSC ($P < 0.001$). The effect of G × E interaction contributed the most variation to RDSC (49.3%, $P < 0.001$) (**Table 5**). This differs from the findings of Moore et al. (*8*), who reported that either G or E had a larger effect than G × E for most antioxidant properties in hard winter wheat varieties. No effect of G × E on ORAC was detected in the current study.

Effects of Individual Environmental Conditions on Soybean Composition and Antioxidant Property. The effects of environment (E) on chemical composition and antioxidant properties in soybean were observed in the present and previous studies (*7*). It is interesting to know whether and how individual environmental conditions may alter which chemical composition and antioxidant property in soybeans grown in Maryland. This information could be used to improve the agricultural practices to enhance the nutritional value of soybeans in Maryland and other locations worldwide.

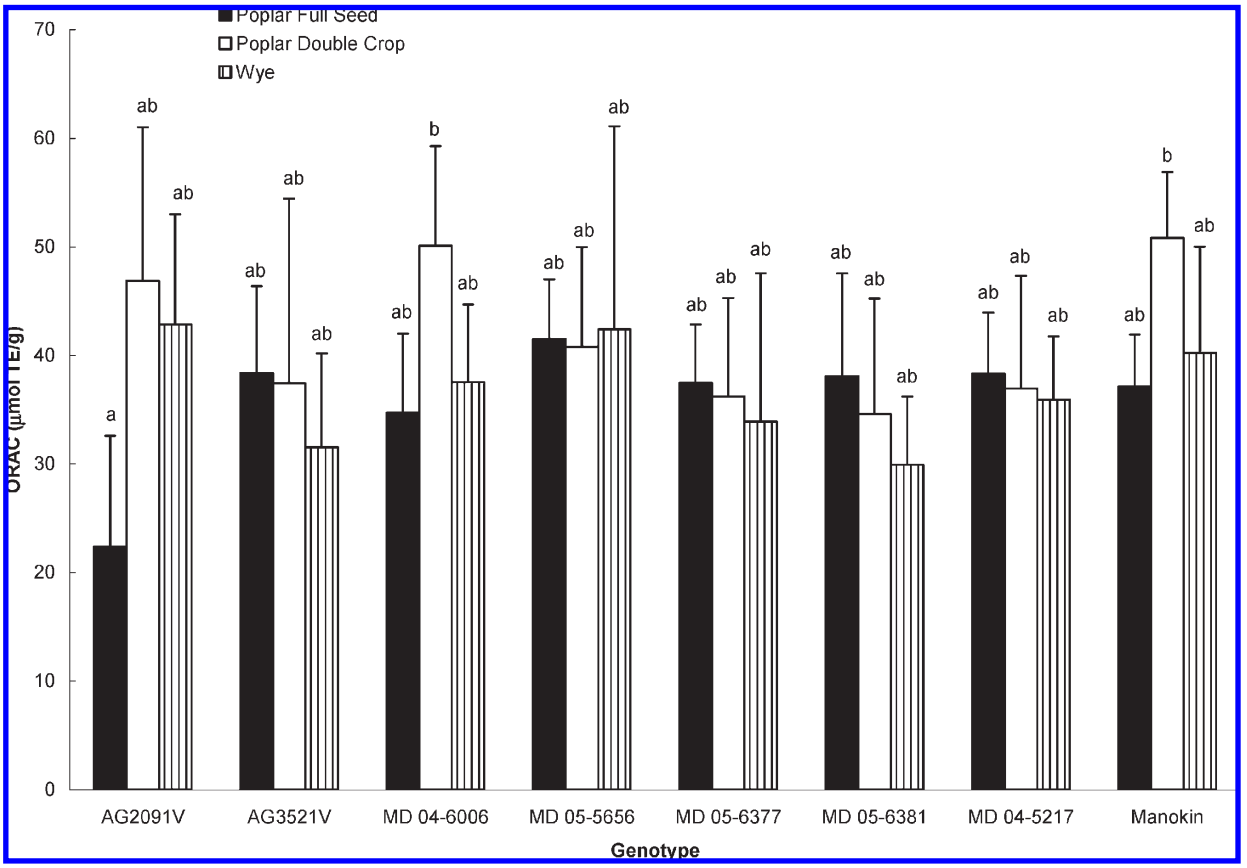

Figure 4. Oxygen radical absorbing capacity (ORAC) of soybeans. Data are expressed as micromoles of Trolox equivalent (TE) per gram of soybean. Values represent the mean of three replicate plots ± SD ($n = 6$). Values marked by the same letter are not statistically different. Poplar Full Seed, Poplar Double Crop, and Wye indicate growing environment.

Table 5. Effect of G, E, and G × E on Soybean Composition[a]

	% genotype (G)	% environment (E)	% G × E
oil content	60.15***	27.01**	12.84**
total saturated	78.99***	20.01***	1.00***
16:0	98.81***	0.93***	0.26***
18:0	10.24***	84.27***	5.49***
18:1n-9	53.34***	40.82***	5.84***
18:2n-6	55.85***	36.93***	7.23***
18:3n-3	97.45***	2.11***	0.44***
TPC	40.64	5.78	53.58**
daidzein	88.52***	1.67	9.81*
genistein	78.64***	8.99	12.37**
glycitein	26.38***	64.82***	8.80***
total ISF	67.19***	21.28*	11.52
lutein	78.26***	17.63***	4.11**
α-tocopherol	57.17***	30.81**	12.02**
γ- tocopherol	70.25***	19.08*	10.67**
δ-tocopherol	42.94***	49.04***	8.01*
total tocopherol	69.09***	16.70	14.21**
RDSC	38.56*	12.16	49.28***
HOSC	47.92**	5.96	46.12***
ORAC	21.37	55.77*	22.86

[a] Effect of genotype, environment, and genotype × environment on soybean composition and antioxidant properties expressed as percent of total mean square. ISF, total isoflavones; RDSC, relative DPPH• scavenging capacity; HOSC, hydroxyl radical scavenging capacity; ORAC, oxygen radical absorbance capacity. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Values without asterisks are not significant at $P < 0.05$.

Oil Content and Fatty Acid Composition. Oil content on soybeans was positively correlated with average high temperature with a Pearson correlation coefficient value of 0.199 ($P < 0.05$) and negatively correlated with overall average and average low temperatures, with r values of −0.182 ($P < 0.05$) and −0.190 ($P < 0.05$). No correlation between oil content and precipitation was detected.

In the present study, correlation analysis of air temperature and fatty acid levels showed a strong positive correlation between stearic acid (18:0) and average high air temperature ($r = 0.690$, $P < 0.01$) and the reverse for average low temperature ($r = -0.699$, $P < 0.01$) and overall average temperature ($r = -0.689$, $P < 0.01$) (**Table 6**). This finding may explain the large effect of E on 18:0 levels (**Table 5**). Small positive correlations were observed between overall average and average low air temperatures and 18:1n-9 level in soybean oil ($P < 0.01$). In contrast, the 18:2n-6 level was negatively correlated with overall average and average low air temperatures ($P < 0.01$), and the 18:3n-3 level was negatively correlated with average high air temperature ($P < 0.01$). Precipitation had a positive correlation with α-linolenic acid ($r = 0.22$, $P < 0.01$), but had no influence on other fatty acid concentrations.

TPC. TPC of soybeans had significant correlation with precipitation ($P < 0.05$). There were no significant correlations between TPC and individual environmental factors (**Table 6**).

Isoflavones. Previous research has shown negative correlation between isoflavones and air temperature during seed development (*28*). In the present study, total isoflavones, genistein, and daidzein did not have a significant correlation with air temperature (**Table 6**). Only glycitein was positively correlated with overall average air temperature ($r = 0.204$, $P < 0.05$) and average low temperature ($r = 0.204$, $P < 0.05$). Other reports have indicated that irrigation or precipitation during seed fill may influence soybean isoflavone levels (*9*). Overall precipitation levels did not have significant correlation with isoflavone levels

Table 6. Correlation between Soy Components, Antioxidant Capacity, and Weather Conditions[a]

	precipitation	av high temp	overall av temp	av low temp
oil content	−0.009	0.199*	−0.182*	−0.190*
16:0	−0.024	0.011	−0.015	−0.017
18:0	−0.024	0.690**	−0.686**	−0.699**
18:1n-9	−0.055	−0.148	0.309**	0.298**
18:2n-6	−0.016	0.144	−0.247**	−0.241**
18:3n-3	0.220**	−0.180**	−0.032	−0.009
TPC	0.167*	−0.068	−0.056	−0.04
daidzein	0.098	−0.045	−0.018	−0.009
glycitein	0.064	−0.119	0.204*	0.197**
genistein	0.01	−0.021	0.002	0.004
total isoflavones	0.074	−0.093	0.098	0.101
lutein	0.312**	0.024	−0.243**	−0.222**
total tocopherols	−0.113	0.036	0.078	0.067
α-tocopherol	−0.157	0.313**	−0.192*	−0.211*
γ-tocopherol	−0.054	0.182*	−0.14	−0.147
δ-tocopherol	−0.094	−0.258**	0.321**	0.320**
RDSC	−0.068	−0.031	0.052	0.048
HOSC	0.01	0.052	−0.057	−0.059
ORAC	−0.035	0.1	−0.091	−0.094

[a] Data are expressed as Pearson correlation coefficient (*r* value). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Values without asterisks are not significant at $P < 0.05$. Absolute high and low temperatures had similar correlations as the average highs and lows and so are not reported here. TPC, total phenolic content; RDSC, relative DPPH• scavenging capacity; HOSC, hydroxyl radical scavenging capacity; ORAC, oxygen radical absorbance capacity.

in the present study; however, precipitation is known only for the total growing season rather than seed fill dates.

Lutein. Lutein content was negatively correlated with overall average air temperature ($r = -0.243$, $P < 0.01$) and average low temperature ($r = -0.222$, $P < 0.01$) and was positively correlated with precipitation levels ($r = 0.312$, $P < 0.01$) (**Table 6**). In a review of the literature, we did not find previous studies examining correlation of individual environmental factors with lutein accumulation in soybean oil. On the basis of our current results, further investigation of this relationship may be warranted.

Tocopherols. α-Tocopherol had a positive correlation with average high temperature ($r = 0.313$, $P < 0.001$), whereas δ-tocopherol had a positive correlation with overall average temperature ($r = 0.321$, $P < 0.001$) and average low temperature ($r = 0.320$, $P < 0.001$) (**Table 6**). No effect of precipitation has been observed for tocopherol composition in soybeans in the present study. This suggests that increased air temperature may increase the level of α-tocopherol while reducing the δ-tocopherol concentration. Britz et al. (*10*) also found elevated α-tocopherol levels in warmer temperatures and with full seed planting dates. Low 18:3n-3 soybeans have been previously shown to have higher α-tocopherol content in warmer temperatures (*3*). As previously noted, the Poplar Hills locations had the highest α-tocopherol levels on average. This location did not have the highest average air temperatures, so there may be other factors involved in the production of α-tocopherol.

Antioxidant Capacity. Large effects of specific weather conditions on antioxidant capacity were not observed in the selected genotypes of soybeans. This may be due in part to the fact that selected growing locations were not exposed to extremely different weather conditions. The crops grown at Poplar Hills were exposed to lower temperatures than the crops at Wye Research Center, but high temperatures were similar throughout the growing season. The weather information used for analysis was collected from data available in records. Concurrent observation of specified weather conditions during crop growth may provide more accurate data for specific crop locations.

Additional environmental factors that were not measured in this study may have affected soybean composition. For example, statistical analysis showed that ORAC values had 55% variation due to E, but they did not have a significant correlation when compared with air temperature or precipitation. This may indicate that other environmental factors are responsible for ORAC variation. Solar radiation is one factor not measured by this study that may be responsible for crop variation (*8*).

Correlation between Individual Chemical Compositions and Antioxidant Properties. Table 7 shows the Pearson correlation coefficients between each chemical composition and antioxidant properties conducted in this research. Interestingly, the level of 16:0 was positively correlated with 18:3n-3 concentration ($r = 0.519$, $P < 0.01$) and negatively correlated with 18:1n-9 ($r = -0.538$, $P < 0.01$). This suggested the possibility of obtaining soybean lines low in α-linolenic and palmitic acids through breeding effort to enhance shelf stability of soybean oil without hydrogenation and to improve its nutritional value. The level of 18:3n-3 was positively correlated with daidzein, genistein, and total isoflavones ($P < 0.01$), but negatively correlated with glycitein content ($P < 0.01$), indicating the possible effect of reducing 18:3n-3 on isoflavones in soybeans. Also noted was a significant high correlation between oleic acid (18:1n-9) and δ-tocopherol. There was a negative correlation between 18:3n-3 and α-, γ-, and total tocopherols, indicating that reducing the 18:3n-3 level may be related to increased tocopherols in the selected genotypes. The results of Almonor et al. (*24*) support the finding that reduced 18:3 soybeans produce relatively higher amounts of α-tocopherol than nonmodified soybeans. However, later research by McCord et al. (*26*) demonstrated proportional changes in tocopherol isomers with changes in 18:3n-3. According to Dolde et al., the relationship between tocopherols and 18:3n-3 may be due primarily to similar environmental conditions that exert effects on both (*25*).

Lutein also had negative correlation with α- and δ-tocopherols. On the basis of the findings of Lee et al. (*11*) and Dolde et al. (*25*) these correlations may be primarily related to the effects of external conditions. These data suggest that in some cases, selection for one soybean component may occur under conditions that reduce levels of other desirable components. However, Wang et al. (*30*) demonstrated that α-tocopherol and lutein are highly heritable in soybean and that through genetic manipulation soybeans may be produced that contain elevated levels of each component. Lutein and α-tocopherol were negatively correlated with the majority of isoflavones in the present study.

Among antioxidant properties, ORAC was highly correlated with TPC, which is consistent with the results of previous studies on agricultural products (*8*). TPC was also positively correlated with HOSC ($r = 0.232$, $P < 0.01$). ORAC and TPC were also positively correlated with daidzein, genistein, and total isoflavones, as isoflavones are phenolic compounds with known antioxidant activity (*6*).

In conclusion, the health components and antioxidant properties of soybeans were affected by genotype, environment, and the interaction between genotype and environment. Each chemical component or antioxidant property may respond to genotype, environment, and their interaction at different levels. Furthermore, each soybean component and antioxidant property may respond to individual environmental factors differently. Among the soybeans studied, there was not one particular genotype or environment that produced outstanding levels of all health components. However, it may be possible to select the ideal genotype and environment for an enhanced level of a specific component. Continuation of this analysis over multiple growing

Table 7. Correlation between Soybean Composition and Antioxidant Assays[a]

	oil	16:0	18:0	18:1	18:2	18:3	TPC	RDSC	HOSC	ORAC	daid	glyc	geni	T ISF	lutein	α-toco	γ-toco	δ-toco
16:0	0.305**																	
18:0	−0.077	−0.164																
18:1n-9	−0.233**	−0.538**	0.196*															
18:2n-6	0.220**	0.037	−0.270**	−0.803**														
18:3n-3	−0.189*	0.519**	0.003	−0.388**	−0.133													
TPC	−0.129	−0.073	0.189*	0.032	−0.089	0.140												
RDSC	−0.070	0.195*	0.009	−0.018	−0.130	0.161	0.097											
HOSC	0.023	0.002	0.117	0.015	−0.039	0.037	0.232**	0.005										
ORAC	−0.123	0.089	0.225**	−0.061	−0.091	0.202*	0.673**	0.123	0.123									
daid	−0.288**	0.122	0.031	0.032	−0.219**	0.584**	0.266**	0.087	0.082	0.201*								
glyc	0.159	0.162	−0.023	0.381**	−0.411**	−0.221**	0.046	0.128	−0.090	−0.046	0.045							
geni	−0.220**	0.287**	−0.036	−0.163	−0.139	0.591**	0.269**	0.107	0.074	0.272**	0.936**	0.025						
T ISF	−0.164	0.225**	0.008	0.116	−0.370**	0.422**	0.292**	0.126	0.069	0.210*	0.853**	0.499**	−0.222**					
lutein	0.238**	0.106	0.028	−0.277	0.247**	0.021	−0.007	0.082	0.141	−0.041	−0.240**	−0.036	−0.237**	- −0.222**				
α-toco	0.206*	0.091	0.291**	0.129	−0.154	−0.245**	−0.014	0.028	−0.045	0.007	−0.387**	0.258**	−0.335**	−0.179*	−0.340**			
γ-toco	−0.159	−0.447**	0.185*	0.160	0.084	−0.318**	0.061	−0.050	−0.154	−0.097	−0.259**	0.068	−0.358**	−0.212*	0.137	0.487**		
δ-toco	−0.326**	−0.176*	0.031	0.710**	−0.755**	0.046	0.072	0.122	−0.144	−0.021	0.182*	0.289**	0.090	0.273**	−0.485**	−0.021	0.176*	
T toco	−0.264**	−0.380**	0.196	0.491**	−0.350**	−0.240**	0.076	0.032	−0.187*	−0.076	−0.157	0.239**	−0.262**	−0.040	−0.088	0.505**	0.866**	0.621**

[a] Data are expressed as Pearson correlation coefficients (*r* value). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Values without asterisks are not significant at $P < 0.05$. TPC, total phenolic content; RDSC, relative DPPH• scavenging capacity; HOSC, hydroxyl radical scavenging capacity; ORAC, oxygen radical scavenging capacity; daid, daidzein; glyc, glycitein; geni, genistein; T ISF, total isoflavone; toco, tocopherols.

seasons would provide a better indication of the best combination of genotype and environment for nutraceutical, chemical, and nutritional properties in these soybeans.

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Received July 7, 2009. Revised manuscript received September 14, 2009. Accepted September 21, 2009.