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ORIGINAL PAPER

Potential Relationships Between Fatty Acid Compositions and Phytochemicals of Selected Low Linolenic Soybeans Grown in Maryland

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Abstract Eight soybean genotypes grown in Maryland were analyzed for total phenolic content, antioxidant capacity, isoflavone composition, lutein content, tocopherol composition, and fatty acid profile. The soybean samples consisted of seven low α-linolenic (18:3n-3) genotypes and 1 standard genotype for comparison. 18:3n-3 levels were positively correlated with palmitic acid (16:0) content, and negatively correlated with oleic acid (18:1n-9) concentration. Daidzein and genistein concentrations were positively correlated with 18:3n-3 levels. α-Tocopherol, γ-tocopherol, and total tocopherol contents were negatively correlated with that for 18:3n-3. Two of the reduced 18:3n-3 genotypes contained significantly higher lutein levels than the non-modified genotype. All genotypes contained similar antioxidant capacity to the non-modified genotype. There were not significant differences among genotypes in relative 2,2-diphenyl-1-picrylhydrazyl (DPPH·) scavenging capacity or oxygen radical absorbing capacity. No correlation was observed between 18:3n-3 and antioxidant capacity. The results of this study show that the Maryland-grown low 18:3n-3 soybeans are sources of phytochemicals and antioxidants with potential health benefits. Specific genotypes may be selected for food production to obtain the most desirable combination of nutritional, nutraceutical, and chemical properties. The reduction in 18:3n-3 may also influence the levels of other fatty acids and antioxidant compounds in soybeans.

Keywords Radical scavenging · Antioxidant activity · Hydroxyl radical · ORAC · DPPH· · Fatty acid · TPC · Soybean · Isoflavone · Lutein, tocopherol

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Introduction

While traditionally served in some Asian cuisines, soy food products have enjoyed increasing popularity in Western cultures since the 1990s. In 2008, the United Soybean Board reported that 32% of consumers surveyed used soy foods or beverages at least once per month [1]. Soy provides a complete protein for humans with low saturated fat, and therefore is an alternative to many meat products [2]. Soy consumption has been associated with health benefits related to its bioactive compounds, including phenolics, isoflavones, and other potential nutraceutical components [2, 3]. According to the United Soybean Board, 35% of soy consumers cited a desire to improve health as a reason for use [1], indicating the importance of health beneficial properties in soybean based food ingredients and ready-to-eat food products.

Soybean oil is recognized as a healthier alternative to animal fats due to its high content of unsaturated fatty acids [4]. The significant α-linolenic acid (18:3n-3) content of regular soybean oil makes it a good source of omega-3 fats, which are associated with reduced cardiovascular disease risk [5]. However, α-linolenic acid can deteriorate easily during processing and storage, due to its highly unsaturated structure. It oxidizes twice as quickly as linoleic acid (18:2n-6) under stable conditions [6]. At high temperatures during cooking, it can degrade even more rapidly [6]. The

soybean oil is often chemically hydrogenated to improve stability before use in food products. Unfortunately, partially hydrogenated oils contain *trans* fats and are associated with an increased risk of cardiovascular diseases [7].

Several possible approaches to reduce the level of *trans* fat in food oils have been investigated, including modification of fatty acid composition in edible seed oils through breeding efforts [8]. Soybean genotypes have been cultivated with reduced levels of α-linolenic acid to improve the stability and avoid the need for hydrogenation [6]. Low α-linolenic soybeans may contain <3% α-linolenic acid versus the 7% in the conventional soybeans [9, 10]. Some low α-linolenic cultivars contain <1% linolenic acid. In commercial baked and fried food products, the desired α-linolenic acid portion is 2% for soybean oil to maintain stability without hydrogenation [6]. Due to rising public concerns over *trans* fats, low α-linolenic soybean oil is promising for use in food production.

The reduction of α-linolenic acid content naturally increases the content of other fatty acids in the soybean. The α-linolenic acid may be replaced with oleic (18:1n-9), linoleic (18:2n-6), stearic (18:0), or palmitic (16:0) acids. For health considerations, an increase in the monounsaturated oleic acid would be desirable. However, when α-linolenic acid is reduced, there also may be an increase in saturated fatty acids. This contributes to the stability of the oils, but a higher intake of palmitic acid may have deleterious health effects [11]. An increase in dietary stearic acid intake has been evaluated as a non-significant effect to cardiovascular risk, even though it is a saturated fatty acid [11, 12]. In addition, one clinical study demonstrated that a diet containing low α-linolenic soy oil improved human lipid profiles over diets containing high amounts of saturated or hydrogenated soy oils [13]. Thus the health benefits of reducing the *trans* fats help to justify modification of lipids in soy oil through soy breeding efforts.

Alteration in fatty acid composition may alter other properties of soybeans. For example, previous research indicated that tocopherol levels were decreased in some genotypes of soybeans with modified fatty acid profiles [14, 15]. Our recent study [9] evaluated a possible alteration of reducing linolenic concentration on phytochemical profiles and antioxidant properties of soybeans using three Maryland cultivars grown in 2005. The results of this preliminary research indicated that low α-linolenic soybean lines might have antioxidant capacity and nutraceutical compounds similar to the conventional soybean cultivars. The limitation of this research was that only three low α-linolenic acid soy lines from a single location in Maryland were included. To draw a general conclusion, more genotypes from multiple environments are required. As a continuation of our research on low α-linolenic soybeans, this study was conducted to examine the effects of the breeding effort to reduce α-linolenic acid concentration on desirable health beneficial properties such as phenolic, isoflavone, lutein, and tocopherol content, and antioxidant capacity. The study evaluated seven Maryland-grown low α-linolenic soybean lines and one control soybean cultivar with ordinary α-linolenic content grown under three different environments representative of Maryland soybean production systems.

Materials and Methods

Materials

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. Three soybean samples from each of eight lines or cultivar under each environment (8 soybean lines × 3 environments × triplicate plots under each condition × duplicate tests = total 144 samples) were selected. Soybeans were cultivated in traditional breeding programs. 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 handheld 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 (tubes held for 22 h in the subsequent extractions) and all supernatants were collected and combined. The petroleum ether was evaporated overnight, and the remaining oil was weighed. The oil samples were stored in the dark until further testing.

Antioxidant Extraction

The defatted soy flour was kept in a fume hood overnight at ambient temperature to evaporate all remaining petroleum ether. One gram of each soy flour sample was combined in a screw-capped tube with 10 mL of 50% acetone. The

tubes were vortexed three times for 15 s each, and kept in the dark at ambient temperature overnight. The tubes were centrifuged at 1,500 rpm for 5 min. The supernatant was collected in separate tubes 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 [16]. The soybean oil was saponified and methylated to form fatty acid methyl esters (FAME). After cleaning, FAME was quantitatively re-dissolved in isooctane. GC analysis was performed with a Shimadzu GC-2010 with a FID detector. Helium was the carrier gas with a flow rate of 2.2 m/min. The stationary phase was a fused silica capillary column SP^{TM}-2380 (30 m × 0.25 mm with a 0.25 μm film thickness) from Supelco Inc. (Bellefonte, PA). Injection volume was 1 μL at a split ratio of 10/1. The oven temperature started at 136 °C, increased by 6 °C/min until 184 °C was reached and held for 3 min, then increased by 6 °C/min to a final temperature of 226 °C. Each sample was tested in duplicate. Fatty acids were identified by comparing FAME retention time with that of known external standards. The FAMEs were quantified by calculating the area under the curve of each identified peak. Individual FAMEs were reported in g/100 g total fatty acids.

Total Phenolic Content (TPC)

The total phenolic content of each soy flour extract was determined according to a previously described laboratory procedure using gallic acid as the standard [17]. The final reaction mixture contained 50 μL of soybean extract, 250 μL of Folin–Ciocalteu reagent, 1.5 mL of 20% sodium carbonate, and 1.5 mL of ultra-pure water. After 2 h of reaction at ambient temperature, absorbance was read at 765 nm. The reactions were conducted in duplicate and results reported in gallic acid equivalents (GAE) per gram of whole soybean.

Isoflavone Composition

Three milliliters of the defatted soy flour extracts in 50% acetone were combined with 0.75 mL 36% hydrochloric acid and heated for 2 h in a water bath at 55 °C. This step hydrolyzed isoflavones to the aglycone form for HPLC quantification. The acetone was then evaporated under nitrogen. The remaining solution was extracted three times with 4 mL ethyl ether/ethyl acetate (1:1, v/v) each time, and the combined ethyl ether/ethyl acetate solution was washed with 3 mL distilled water. The ethyl ether/ethyl acetate was evaporated in a nitrogen evaporator. The remaining soy extract was re-dissolved in 0.5 mL methanol and filtered through a 0.45 μm filter prior to HPLC analysis. HPLC was conducted according to a previously described method (with modifications) [18]. A Phenomenex C-18 column (150 mm × 4.6 mm, 5 μm) was used. The mobile phase 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 linearly from 25 to 32% solvent B over 20 min. The detection wavelength was set at 254 nm. The column was kept at 30 °C. Samples were analyzed in duplicate.

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. HPLC was performed according to a previously described method [19] using a Phenomenex C-18 column (250 mm × 4.6 mm, 5 μm and Phenomenex C18 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. Then 50 μL of each standard and sample was injected and run time was 10 min per sample, each conducted in duplicate. A standard curve was developed from the known standards, and the 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. Reversed-phase HPLC with UV detection was performed according to a previously described procedure with slight modifications [20]. The stationary phase was a Waters C-30 column (250 mm × 4.6 mm, 5 μm). Solvent A of the mobile phase consisted of methanol/MTBE/water, (81:15:4, v/v/v), and Solvent B was MTBE/methanol (91:9, v/v). The mobile phase was run from 0 to 16% solvent B in 13 min, 100% Solvent B from 13 to 23 min, and re-equilibrated with 100% Solvent A from 23 to 32 min. The flow rate was 1.0 mL/min, and the injection volume was 30 μL. The UV detector wavelength was set at 295 nm. Each standard and sample was run in duplicate.

Relative DPPH· Scavenging Capacity (RDSC)

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH· scavenging capacity was determined according to a previously described laboratory procedure [21], using a Victor^3 multilabel plate reader (PerkinElmer, Turku, Finland). DPPH·

solution was prepared in 50% acetone and filtered through a medium porosity P5 paper filter (Fisher Scientific, Waltham, MA). Trolox was used as the standard. The final reaction mixture contained 100 μL soybean extract or Trolox standard or 50% acetone (the control), and 100 μL 0.2 mM DPPH solution. The absorbance was read at 515 nm every minute for 40 min. Each sample was tested in duplicate. The radical scavenging capacity (RDSC) was calculated from the area under the curve and reported in Trolox equivalents (TE) per gram of whole soybean.

Hydroxyl Radical Scavenging Capacity

The HOSC was examined using a previously reported laboratory procedure [22]. Fluorescein (FL) was used as the fluorescent probe and the assay was performed using a Victor^3 multilabel plate reader (PerkinElmer, Turku, Finland). Iron (III) chloride and hydrogen peroxide were prepared in ultra-pure water. 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 0.20 M hydrogen peroxide, and 60 μL of 3.43 M iron (III) chloride. The fluorescence was recorded every 4 min for 4 h. Trolox prepared in 50% acetone was used as the standard. The assay was conducted in duplicate for each sample. Antioxidant capacity was calculated by area under the curve (AUC) described by Moore and others [22]. Results were reported as μmol TE/g of whole soybean.

Oxygen Radical Absorbance Capacity

The oxygen radical absorbance capacity (ORAC) values were determined following a previously reported laboratory procedure [23], with fluorescein (FL) as the fluorescent probe. Trolox standards were prepared in 50% acetone and other reagents were prepared in 75 mM pH 7.4 phosphate buffer. Samples were analyzed in duplicate. In the initial reaction, FL was combined with 30 μL of sample, standard, or solvent in a 96-well plate. The plate was heated at 37 °C for 20 min in a Victor^3 multilabel plate reader (PerkinElmer, Turku, Finland). Twenty-five microliter 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 μmol TE/g whole soybean, based on AUC calculations.

Statistical Analysis

Data were analyzed using SPSS (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL). Data were reported as mean ± SD ($n = 18$). Differences between means were determined by analysis of variance (ANOVA) with Tukey's HSD post hoc test. A two-tailed Pearson Correlation Coefficient test was used to determine correlations among means. Significance was declared at $P < 0.05$.

Results and Discussion

Oil Content and Fatty Acid Composition

The tested soybean lines and cultivars significantly differ in their oil contents (Table 1). The oil content of the soybeans with lower α-linolenic acid ranged from 14.7 to 17.3 g/100 g (non-dried weight) under the experimental conditions,

Table 1 Oil content and fatty acid (FA) profiles of the soybeans*

	AG2921V	AG3521V	MD 04-6006	MD 05-5656	MD 05-6377 (1% 18:3n-3)	MD 05-6381 (1% 18:3n-3)	MD 04-5217	Manokin (non-modified)
Oil	17.3d ± 1.1	17.0c,d ± 1.2	15.1a,b ± 2.3	14.7a ± 1.6	16.0b–d ± 0.8	16.3b–d ± 0.9	16.8c,d ± 0.7	15.7a–c ± 0.8
16:0	10.9f ± 0.3	10.5e ± 0.1	7.1c ± 0.4	5.9b ± 0.2	4.4a ± 0.3	5.7b ± 0.3	9.5d ± 0.3	10.9f ± 0.3
18:0	5.00a–c ± 0.9	4.5a ± 0.6	5.5b,c ± 0.7	5.7c ± 0.9	5.1a–c ± 0.5	4.9a,b ± 0.6	5.5b,c ± 1.0	4.9a,b ± 0.5
18:1n-9	30.1b,c ± 2.5	24.8a ± 2.2	35.3d,e ± 5.8	38.9e ± 1.5	33.0c,d ± 4.6	28.6b ± 1.7	33.8d ± 5.5	22.7a ± 1.3
18:2n-6	52.5b ± 2.4	57.6d ± 2.0	48.7a ± 4.9	46.1a ± 1.8	56.4c,d ± 4.4	58.6d ± 1.8	48.1a ± 4.6	54.0b,c ± 1.1
18:3 n-3	2.4b ± 0.2	2.6c ± 0.2	3.5e ± 0.3	3.4e ± 0.2	1.1a ± 0.1	2.2b ± 0.2	3.1d ± 0.3	7.5f ± 0.5
SFA	15.9	15.1	12.5	11.6	9.5	10.6	15.0	15.9
MUFA	30.1	24.8	35.3	38.9	33.0	28.6	33.8	23.7
PUFA	54.8	60.3	52.2	49.5	57.5	60.8	51.2	61.5

All samples are low linolenic genotypes except Manokin, which has standard soybean fatty acid composition

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acid

* Soybeans were not dried prior to analysis. Data are expressed as means ± SD ($n = 18$). Values in the same row with different letters are significantly different ($P < 0.05$). Total oil is expressed as g/100 g soybean. Fatty acids are expressed as g/100 g oil

while the Manokin soybean, the control soybean cultivar with ordinary α-linolenic level, showed an oil content of 15.8/100 g (Table 1). Soybean cultivars on average contain 19–20% oil by weight [10]. The extraction procedure used in this research may not have yielded all oil present in the soybeans. Oil content can also vary due to environmental differences during soybean growth [6, 10]. A negative correlation between oil content and α-linolenic acid was detected ($r = -0.19$, $P = 0.024$). Therefore, there may be a relationship between α-linolenic content and total oil concentration of the soybeans.

Fatty acid compositions of these soybeans were also examined. The oil from Manokin soybeans had an average of 7.5% 18:3n-3, which is similar to many US cultivars. The other cultivars and experimental lines had a lower α-linolenic acid content as expected. MD 04-6006, MD-05-5656 and MD 04-5217 had about 3% α-linolenic acid, while MD 05-6377 had 1% α-linolenic acid (Table 1). In addition, AG2921V and AG3521V had 2.3–2.6% α-linolenic acid. MD 05-6377 contained a relatively high level of oleic acid (18:1n-9) (33%) compared to standard soybeans and very low α-linolenic acid (1%). MD 05-6377 also contained the lowest palmitic acid (4.4%) of these tested lines. High oleic and low palmitic acids are a desirable trait for cardiovascular health [11]. Interestingly, the two-tailed Pearson correlation test showed that the level of palmitic acid was correlated with α-linolenic content with a r value of 0.519 ($P < 0.001$), suggesting that the breeding effort to reduce α-linolenic acid may also decrease the palmitic acid (16:0) level in soybean oil. Also noted was that 18:1n-9 content was negatively correlated with 18:3n-3 level ($r = -0.388$, $P < 0.001$), suggesting that reduction of 18:3n-3 might increase 18:1n-9 content. In addition, the content of total saturated fat was correlated with the 18:3n-3 level, indicating the possibility of reducing total saturated fat of soybean oil while reducing 18:3n-3 content. These data suggested that reduction of α-linolenic acid through breeding efforts may result in soybean lines rich in oleic acid and low in palmitic and total saturated fatty acids. The resulting soybean oils may have improved oxidative stability.

Total Phenolic Content

The soybean cultivars and lines analyzed in this study contained total phenolic content (TPC) levels between 1.3 and 1.7 mg gallic acid equivalent (GAE)/g soybean (Table 2). These levels are comparable to that of 1.5–4.4 mg GAE/g soybean reported in previous studies, however fall at the lower end of the overall reported range [9, 24, 25]. No correlation between 18:3n-3 concentration and TPC value was observed, suggesting that a reduction of the 18:3n-3 level might not significantly alter total phenolic contents in the soybeans.

Table 2 TPC, isoflavone, lutein, and tocopherol content of soybeans*

	AG2091 V	AG3521 V	MD 04-6006	MD 05-5656	MD 05-6377 (1% 18:3n-3)	MD 05-6381 (1% 18:3n-3)	MD 04-5217	Manokin (non-modified)
TPC	1.5a,b ± 0.3	1.3a ± 0.3	1.5a,b ± 0.3	1.6a,b ± 0.3	1.6a,b ± 0.3	1.6a,b ± 0.3	1.6a,b ± 0.4	1.7b ± 0.4
Daidzein	35.3a,b ± 7.7	32.9a ± 4.7	52.9b ± 11.8	73.5c ± 20.1	33.5a,b ± 6.7	39.1a,b ± 6.7	41.4a,b ± 10.6	82.6c ± 46.5
Genistein	31.9a ± 10.0	33.0a ± 5.7	36.7a,b ± 11.9	52.6bc ± 15.7	24.5a ± 9.6	25.2a ± 5.6	34.8a ± 8.9	64.4c ± 36.4
Glycitein	74.9c ± 21.7	63.4b,c ± 17.7	61.1b,c ± 27.4	56.6a–c ± 17.6	47.6a,b ± 18.4	38.2a ± 14.5	64.7b,c ± 21.4	38.3a ± 22.6
Total ISF	0.52a–c ± 0.10	0.47a,b ± 0.08	0.56b–d ± 0.12	0.68c,d ± 0.18	0.39a,b ± 0.08	0.38a ± 0.06	0.52a–c ± 0.10	0.70c ± 0.39
Lutein	16.8b,c ± 1.5	15.8b ± 2.0	17.2b,c ± 2.1	11.2a ± 1.9	16.3b,c ± 1.7	24.1d ± 4.6	23.6d ± 3.1	19.2c ± 4.1
α-Tocopherol	282.5a,b ± 20.3	288.7b ± 24.9	284.4,b ± 13.1	273.6a,b ± 11.2	280.0a,b ± 10.7	279.0a,b ± 14.4	306.9c ± 16.3	266.8a ± 12.2
γ-Tocopherol	356.5a ± 49.2	400.3a,b ± 57.4	469.9c,d ± 57.7	378.0a,b ± 57.3	492.6d ± 39.6	419.1b,c ± 51.8	410.3a,b ± 79.5	364.0a,b ± 43.0
δ-Tocopherol	379.1b–d ± 46.8	334.6a,b ± 45.2	406.9c,d ± 60.5	425.6d ± 48.9	367.1a–c ± 47.8	321.7a ± 37.4	372.1b,c ± 52.1	371.0b,c ± 44.5
Total Toco.	2.5a,b ± 0.2	2.5a,b ± 0.2	2.9d ± 0.2	2.7cd ± 0.2	2.8d ± 0.2	2.5a,b ± 0.2	2.7c,d ± 0.2	2.4a ± 0.2

* Soybeans were not dried prior to analysis. Data represent the average by genotype ± SD ($n = 18$). Values in the same row with different letters are significantly different($P < 0.05$). All samples are low α-linolenic genotypes except Manokin, which has standard fatty acid composition. TPC is the total phenolic content in the respective soybean samples and is measured as milligram gallic acid equivalents/g (GAE mg/g) of whole soybean. Daidzein, glycitein, and genistein, are expressed as μg/g whole soybean, and lutein, α-, δ-, and γ- tocopherols are expressed as μg/g oil. Total isoflavones (ISF) are expressed as μmol/g whole soybean, and total tocopherols (Toco.) are expressed as μmol/g oil

Soybeans are known to contain multiple phenolic compounds, including isoflavones [26]. Phenolics contribute to total antioxidant capacity, and sub-classes of phenolics such as soy flavonoids are associated with the reduced risk of several aging-related chronic human diseases [27, 28]. Our previous research on modified-lipid soybeans found that there was no difference in the TPC content of modified-lipid soybeans and normal lipid soybeans [9]. The current study confirmed those previous findings.

Isoflavones

The 50% acetone extracts were hydrolyzed with concentrated HCl to cleave glycoside bonds and allow measurement of isoflavone aglycones. Each individual isoflavone, including daidzein, genistein, and glycitein, was measured in micrograms and total isoflavones were reported in μmol/g soybean since the different isomers vary in molecular weight. This study used 50% acetone as the extraction solvent since it has been previously used for total phenolic and antioxidant extraction from soybeans and our research was focused on multiple soy components [9], although acetonitrile with 0.1 N HCl has been used as a solvent for isoflavone extraction in several previous studies [24, 29–31]. Daidzein, genistein, and glycitein were detected in all the tested soybean cultivar and lines regardless of the 18:3n-3 content. The soybean lines and cultivar differed in their daidzein, genistein, and glycitein compositions (Table 2). Glycitein was the primary isoflavone compound in six of the seven tested low-linolenic acid soybean lines, whereas daidzein was the major isoflavone in MD 05-5656 and Manokin soybeans. While many studies have reported that glycitein is lower in soybean extracts than daidzein and genistein, at least one other group has reported glycitein (in aglycone equivalents) at higher levels than the other isomers [32].

The 18:3n-3 content was correlated to daidzein ($r = 0.571$, $P < 0.001$) and genistein ($r = 0.577$, $P < 0.001$) levels, and negatively correlated with glycitein content ($r = -0.210$, $P < 0.001$). Taken together, these data suggested that breeding efforts to reduce α-linolenic acid may significantly alter the isoflavone compositions and content in soybeans. It is widely accepted that changes in the composition of soybeans may occur over different growing seasons [31, 33]. Additional research is required to investigate the effects of selected growing conditions such as solar radiation and irrigation, genotype, and the interaction between genotype and individual growing conditions on soybean isoflavones.

The tested soybean lines and cultivar differed in their total isoflavone contents (Table 2). The Manokin genotype (standard fatty acid composition) and MD 05-5656 (18:3n-3) had the highest total isoflavone content at 0.70 μmol/g compared with the low 18:3n-3 lines (Table 2). The lowest total isoflavone level of about 0.4 μmol/g was detected in MD 05-6377 and MD 05-5656, each of which contained about 1% 18:3n-3. Riedl and others reported 1.5–7.1 μmol/g total isoflavones in Ohio-grown soybeans with acidic acetonitrile as the extraction solvent at ambient temperature [30]. The lower total isoflavone levels in the current study may be partially related to the extraction solvent used (50% acetone), which could not extract insoluble bound isoflavones from the soybean matrix [9]. Total isoflavone content was correlated with the level of 18:3n-3 ($r = 0.421$, $P < 0.001$), suggesting the possibility of a decrease in the total isoflavones due to the reduction of the 18:3n-3 level through breeding effort.

Lutein

Lutein has been previously identified as the major carotenoid in mature yellow soybeans [9, 34, 35]. Lutein has shown beneficial health effects for humans including prevention of aging-related macular degeneration and protection of skin from ultra-violet damage [36, 37]. All soybean samples tested in this study contained lutein ranging from 11.1 to 24.1 μg/100 g oil (Table 2). Two of the reduced 18:3n-3 lines had a significantly higher lutein level than the lines with a non-modified fatty acid profile, whereas two low 18:3n-3 lines had significantly lower lutein concentration than the non-modified soybean and the remaining three low 18:3n-3 lines contained the same lutein level as the ordinary soybean. This observation was in agreement with the findings from an earlier work from our group [9] that found variation in the level of lutein among both modified and non-modified oil soybeans. In addition, research by Wang and others demonstrated that lutein levels might be heritable in soybeans [38]. The reduced 18:3n-3 lines identified with high lutein levels may be further cultivated to maximize the health benefits of soy oil. The lutein level was positively correlated with total oil content ($r = 0.475$, $P < 0.01$) and the level of linoleic acid ($r = 0.272$, $P < 0.01$), but not correlated with α-linolenic acid level in the soybeans. The lutein content was negatively correlated with total isoflavones ($r = -0.233$, $P < 0.01$).

Tocopherols

Tocopherols confer antioxidant activity on soybean oil to prevent lipid oxidation, and also provide dietary vitamin E [6]. This study quantified α-, γ-, and δ- tocopherol isomers in the soybean oils. The soybean oils differed in their tocopherol compositions, with δ-tocopherol as the primary isomer in two of the low α-linolenic acid and ordinary soybean oils and γ-tocopherol as the major isomer in five low linolenic acid lines under the experimental conditions

(Table 2). Total tocopherols ranged from 2.5 to 2.9 μmol/g oil (Table 2). The levels of α-, γ-, and total tocopherols were all negatively correlated with 18:3n-3 ($P < 0.01$). The oil from the Manokin soybeans with the ordinary fatty acid profile contained the lowest total tocopherols compared to the other genotypes. These results suggest that lowering 18:3n-3 levels may result in similar or higher tocopherol levels in these particular genotypes. This conclusion was supported by our previous findings that some reduced 18:3n-3 genotypes had tocopherol levels comparable to the standard genotypes [9]. This conclusion is in contrast to the observation in another study by Dolde and others [39]. They reported that reduced 18:3n-3 soybean genotypes contain lower tocopherols than standard soybeans under the same growing conditions. In addition, there was no correlation between δ-tocopherol content and 18:3n-3 level, but δ-tocopherol content had a positive correlation with oleic acid (18:1n-9) ($r = 0.536$, $P < 0.001$).

Relative DPPH· Scavenging Capacity (RDSC)

There are multiple types of free radicals and different sources of antioxidants within a biological system. While there are several assays that can determine aspects of antioxidant capacity, there is not one single assay to determine total antioxidant capability [40]. Two or more antioxidant assays are required to determine the scope of antioxidant capacity of a single compound or antioxidant preparation. Assays for antioxidant capacity typically measure either single electron transfer reactions (SET) or hydrogen atom transfer reactions (HAT). Oxygen radical absorbing capacity (ORAC) and hydroxyl radical scavenging capacity (HOSC) are examples of HAT reaction assays, while the DPPH· scavenging capacity is considered a SET reaction assay [40, 41]. Three assays have been selected in this study to show the range of antioxidant property.

The DPPH· scavenging capacity ranged from 0.9 to1.2 μmol TE/g soybean and the tested soybean samples had no significant difference in their DPPH radical scavenging capacities (Fig. 1). Because of the varying laboratory methods for measuring and reporting DPPH· scavenging activity [21], it is difficult to compare the current results with many other analyses of soybeans. There was high variation in DPPH· scavenging capacity between different samples within the same soybean lines and cultivar. The data represents nine samples from each line taken from different plots. It was reported that growing conditions such as solar irradiation and temperature during selected growing period altered antioxidant properties in wheat grain [42]. The high variation of the DPPH· scavenging capacity data may have occurred from unknown differences in soil or other growing factors. No correlation between 18:3n-3 content and DPPH· scavenging capacity was detected under the experimental conditions, suggesting a lower possibility of altering DPPH· scavenging capacity in soybeans because of a breeding effort to reduce α-linolenic acid level.

Hydroxyl Radical Scavenging Capacity)

The hydroxyl radical (·OH) is a highly reactive molecule that may be generated under physiological conditions and

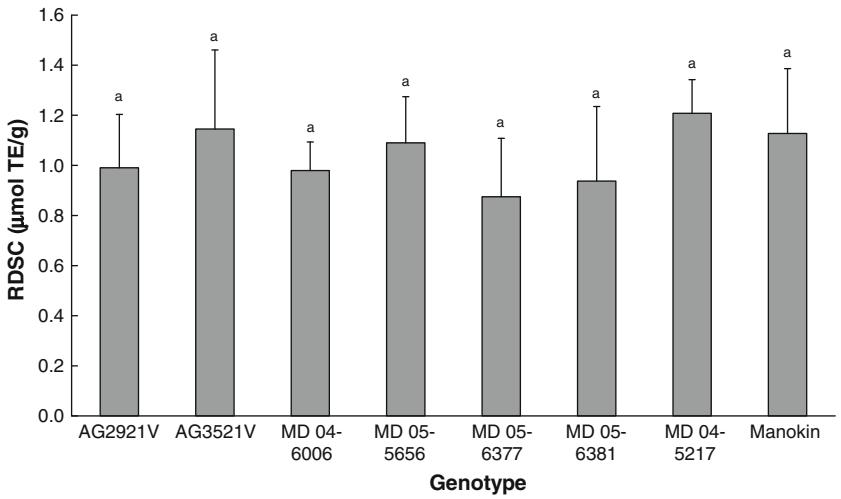

Fig. 1 DPPH radical scavenging capacity of soybeans. Data are expressed as Trolox equivalent (*TE*) in μmol/g whole soybean. The final reaction mixture contained 100 μL soybean extract or Trolox standard or 50% acetone (the control), and 100 μL 0.2 mM DPPH solution. The absorbance was read at 515 nm every minute for 40 min, and TE was calculated based on area under the curve (AUC) calculations compared to the standards. *Vertical bars* represent SD ($n = 18$). *Different letters* represent a significant difference ($P < 0.05$)

can damage lipids, proteins, and DNA [40]. The HOSC assay developed by Moore and others measures antioxidant capacity against OH radical generated by the Fenton reaction of Fe(III) and H_2O_2 [22]. All tested soybean extracts demonstrated a hydroxyl radical scavenging capacity (HOSC) (Fig. 2). The greatest HOSC value was observed in the MD 04-5217 soybeans which had about 3.1% 18:3n-3 in the total fatty acids, whereas the lowest hydroxyl radical scavenging capacity was detected in the AG3521V soybeans containing 2.6% 18:3n-3. The hydroxyl radical scavenging capacity of soybeans was not correlated with their 18:3n-3 content, suggesting that reduction of 18:3n-3 level may not alter HOSC in soybeans.

The HOSC values ranged from 22.04 to 32.78 μmol TE/g soybean (26.4–39.3 TE/g soy flour) under the present experimental conditions. This HOSC value range is lower than that of 68–104 μmol TE/g soy flour previously reported in Maryland soybeans by our group [9]. It needs to be pointed out that the focus of this study was to evaluate the effects of reducing 18:3n-3 content through breeding efforts on health beneficial properties in Maryland-grown soybeans, and many experimental conditions were selected to handle large numbers of samples but not to determine the maximum value of each property. Changes in extraction conditions might attribute to the lower HO· scavenging capacity range observed in this study. It was also possible that variation in growing season and environments might affect the antioxidant capacity between crop years. The growing season has been shown to have a significant impact on the composition of soybeans in previous research [31, 33]. Changes in the amount of phenolics, for example, would have an effect on the total antioxidant capacity of the soybeans.

Oxygen Radical Absorbing Capacity

The Oxygen Radical Absorbing Capacity (ORAC) assay measures scavenging activity against peroxyl radical, which may be formed under normal physiological conditions and may be involved in many harmful reactions in biological systems such as lipid peroxidation [23]. The ORAC values in the evaluated soybean samples was between 34.2 and 42.7 μmol TE/g using 50% acetone as the extraction solvent (Fig. 3). The ORAC levels are within the range reported by Xu and Chang in 50% acetone extract of yellow soybeans from North Dakota (22.1–91.2 μmol TE/g) [25]. A previous study by Xu and Chang found that extraction with 70% ethanol resulted in significantly higher ORAC values for yellow soybeans compared with 50% acetone extracts [43]. This was contradicted by the results of Slavin and others in which 50% acetone was preferred over 70% ethanol as an extraction solvent for soybean ORAC determination [9]. Furthermore, there was no significant difference among ORAC values of the soybean lines and cultivar at $P < 0.05$ in the present study. This might be due to the high standard deviation among soybean lines and cultivar, suggesting the possible effects of unknown factors related to growing conditions. In addition, no correlation was observed between the ORAC value and the 18:3n-3 acid content under the experimental conditions, indicating less concern over decreasing the ORAC in soybeans through breeding efforts to reduce 18:3n-3 content.

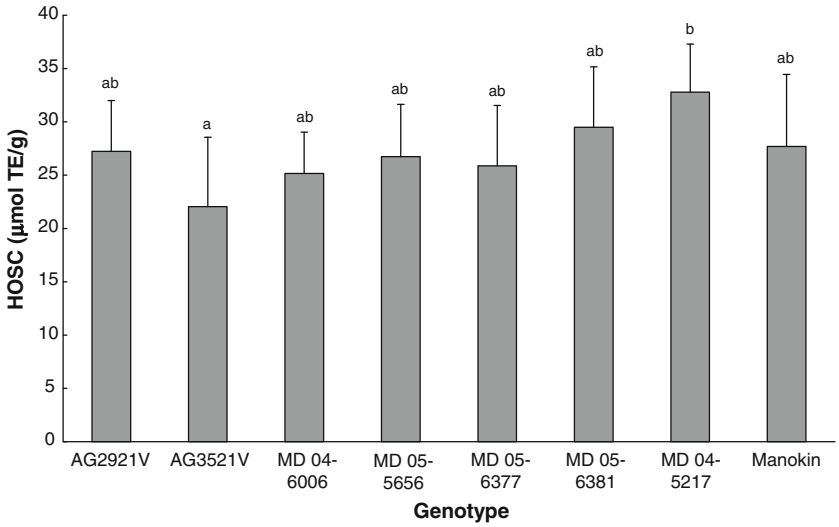

Fig. 2 Hydroxyl radical scavenging capacity (HOSC) of soybeans. Data are expressed as Trolox equivalent (*TE*) in μmol/g whole soybean. The reaction mixture consisted of 170 μL of 9.28×10^{-8} M FL, 30 μL of sample, standard, or blank, 40 μL of 0.20 M hydrogen peroxide, and 60 μL of 3.43 M iron (III) chloride. The fluorescence was recorded every 4 min for 4 h. Antioxidant capacity was calculated by area under the curve (AUC) of sample absorbance compared to absorbance of standards. *Vertical bars* represent SD ($n = 18$). *Different letters* represent significant difference ($P < 0.05$)

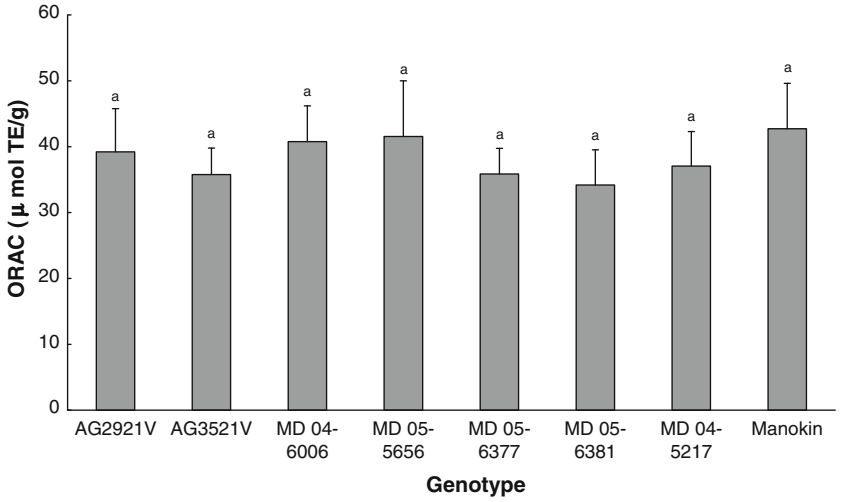

Fig. 3 Oxygen radical absorbance capacity (*ORAC*) of soybeans. Data are expressed as Trolox equivalent (*TE*) in μmol/g whole soybean. 225 μL of 9.28×10^{-8} M Fluorescein (FL) was combined with 30 μL of sample, standard, or solvent in a 96-well plate. The plate was heated at 37 °C for 20 min in a Victor^3 multilabel plate reader (PerkinElmer, Turku, Finland). 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-minute period at 37 °C. *Vertical bars* represent SD ($n = 18$). *Different letters* represent significant difference ($P < 0.05$)

In summary, this study indicates that breeding effort to reduce α-linolenic acid content in soybeans may result in soybean lines with comparable phytochemicals to non-modified soybeans. Additionally, experimental Maryland soybean lines have been identified that may be studied further due to their higher concentrations of specific components, such as elevated lutein and tocopherol levels. In a 50% acetone solvent extraction system, the low 18:3n-3 Maryland soybeans are rich in antioxidants and glycitein, although they may have lower levels of total isoflavone, daidzein, and genistein than non-modified soybeans. Additional research is needed to evaluate how genotype, individual growing conditions, and the interaction between the genotype and growing conditions on soybean oil content, fatty acid profile, antioxidant properties, and health beneficial phytochemicals.

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