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Chemical Composition of 13 Commercial Soybean Samples and Their Antioxidant and Anti-inflammatory Properties

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ABSTRACT: A total of 13 commercial soybean samples were investigated and compared for their tocopherol and carotenoid compositions, fatty acid profile, total phenolic content (TPC), isoflavone and sterol compositions, and free radical scavenging properties. Free radical scavenging activities were estimated against peroxyl, hydroxyl, and $ABTS^{\bullet+}$ radicals. The commercial soybean samples differed in their chemical compositions and health properties. Among the tested samples, CN-DB soybean showed the highest TPC and antioxidant activities and the greatest amount of total isoflavones and lutein and tocopherol contents. In addition, four of the soybean samples were further tested for their potential anti-inflammatory activity. The four samples behaved differently in suppressing the IL-1β, IL-6, and COX-2 mRNA expressions, suggesting their different potential of anti-inflammatory activities. The results from this study suggested a need of improved quality control and measurement system to better reflect the health properties of commercial soybeans for general consumers and food manufacturers to improve the use of this important food ingredient.

KEYWORDS: *Soybean, carotenoid, isoflavone, tocopherol, free radical, antioxidant, anti-inflammation*

INTRODUCTION

Soybean is an important commercial crop in the world. In 2008, more than one-third of the world's soybeans was harvested in the United States, followed by Brazil, Argentina, and China.[1] In recent years, increasing evidence indicates that soybean components may reduce the risk of several chronic human diseases, including cancers, osteoporosis, cardiovascular diseases, and other aging-associated chronic health problems.[2]

It is believed that oxidative stress from free radicals may accelerate aging and the development of chronic disease.[3] Dietary antioxidants, including those found in soybeans, may reduce oxidative stress, and the antioxidant capacity is an important factor in assessing potential health benefits of a selected food. Chronic inflammation also plays a role in the development of aging-associated diseases,[4] and foods rich in anti-inflammatory components may reduce the risk of these health probems. To our knowledge, there are few reports on the anti-inflammatory properties of soybeans.

Soybean components that are reported to be beneficial to human health include α-linolenic acid, tocopherols, carotenoids, isoflavones, and other phenolics.[5−7] The growing environment and processing and storage conditions could alter the chemical profiles and, consequently, biological properties of soybean.[8−10] Studies of commercial soy foods have revealed a large variation in the quantity and composition of isoflavones. For example, Setchell and Cole reported that whole bean soymilk brands varied as much as 5-fold in isoflavone content.[11] They also reported that the isoflavone content could differ by 60% in the samples of the same brand soymilk, suggesting a huge variation in the chemical composition and health properties of commercial soybean products and ingredients.

When purchasing commercial soybeans or soy foods, consumers have little knowledge of the source of the soybeans or profiles of their health components. Therefore, the present study was conducted to provide an overview of available commercial soybeans and their variations. A total of 13 commercial soybean samples (5 from the United States, 2 from Brazil, 1 from Argentina, 1 from Canada, and the remaining 4 from China) were investigated for their chemical compositions and antioxidant and anti-inflammatory activities. The results may provide a broad picture of the health components in commercial soybeans.

MATERIALS AND METHODS

Materials and Chemicals. A total of 13 commercial whole soybean samples were obtained from Wilmar International, Ltd., Shanghai, China. Among the 13 samples, 5 soybean samples were grown in the United States (US-1−US-5), 2 from Brazil (BA-1 and BA-2), 1 from Argentina (AG-1), 1 from Canada (CA-1), and 4 from China (CN-HB, CN-HLJ, CN-DB, and CN-XJ). Information regarding the soybean cultivars or growing conditions was not available.

ABTS chromophore, diammonium salt, iron(III) chloride, fluorescein (FL), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), genistein, daidzein, lutein, β-carotene, β-cryptoxanthin,

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zeaxanthin, α-, δ-, and γ-tocopherols, dimethylsulfoxide (DMSO), and 2-propanol were purchased from Sigma-Aldrich (St. Louis, MO). AAPH was purchased from J&K Scientific, Ltd. (Beijing, China). Folin−Ciocalteu reagent was purchased from Ambrosia Pharmaceuticals (Shanghai, China). High-performance liquid chromatography (HPLC)-grade formic acid, acetonitrile, and methanol were obtained from Merck (Darmstadt, Germany). Analytical-grade methanol, ferulic acid, manganese dioxide, diethyl ether, ethyl acetate, sodium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from Sinopharm (Beijing, China). Fatty acid methyl ester (FAME) standard was purchased from UN-CHEK (Elysian, MN). RAW 264.7 mouse macrophage was acquired from Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and 1× phosphate-buffered saline (PBS) were purchased from Gibco (Life Technologies, Carlsbad, CA). TRIzol reagent was obtained from Invitrogen (Life Technologies, Carlsbad, CA). Lipopolysaccharide (LPS) from *Escherichia coli 0111:B4* was obtained from Millipore (Millipore, Billerica, MA). IScript Advanced cDNA synthesis kit was purchased from Bio-Rad (Bio-Rad Laboratories, Hercules, CA), while AB Power SYBR Green PCR Master Mix was purchase from ABI (Applied Biosystems, Carlsbad, CA). Amphotericin B/streptomycin/penicillin was purchased from Invitrogen (Life Technologies, Carlsbad, CA). Ultrapure water was prepared by a Millipore ultra-genetic polishing system with <5 ppb total organic carbon (TOC) and resistivity of 18.2 mΩ (Millipore, Billerica, MA) and was used for all experiments.

Oil Extraction. Whole soybeans were ground in a standard household coffee grinder to a 20-mesh particle size. A total of 5 g of soybean powder was extracted with 10 mL of petroleum ether (boiling point of 35−60 °C) at room temperature 4 times. Petroleum ether was removed using a nitrogen evaporator to a constant weight. The oils were stored at ambient temperature under nitrogen in the dark until testing.

Extraction of Antioxidant and Anti-inflammation Components. The soybean flour remaining after oil extraction was then extracted with 50% aqueous acetone at a ratio of 1 g/10 mL overnight in the dark, according to the previous work by Xu and Chang[12] and our group.[5] Extraction was performed in three replicates for each soybean flour. The extracts were filtered through 0.45 μm syringe filters and kept under nitrogen in the dark until testing.

Chemical Component Analysis. *Fatty Acid Composition.* FAMEs were prepared from the oils by the saponification and methylation method according to a laboratory protocol and subjected to gas chromatography (GC) analysis.[13] An Agilent 7890A GC with a flame ionization detector (FID) and a G4513 injector (Agilent, Loveland, CO) was used for fatty acid analysis. Carrier gas was helium at a flow rate of 2.2 mL/min. A fused silica capillary column DB-23 (60 m × 0.25 mm with a 0.25 μm film thickness) from Agilent (Loveland, CO) was used. The injection volume was 1 μL at a split ratio of 50:1. The initial oven temperature was 100 °C, increased 10.5 °C/min to 184 °C, held for 3 min, and increased 6 °C/min to a final temperature of 240 °C. Individual fatty acids were identified through comparison of GC retention times with the standard FAMEs. Quantification was based on the area under each fatty acid peak, as compared to the total area of all fatty acid peaks.

Carotenoid, Sterol, and Tocopherol Contents. The content (μg/g of soybean) of lutein, stigmasterol, campesterol, β-sitosterol, and α-, δ-, and γ-tocopherols were determined simultaneously using a Shimadzu LC-30AD series system equipped with a Shimadzu ultrahigh-pressure liquid chromatography (UHPLC) series diode array detector and an evaporative light scattering detector (ELSD) and an auto-sampler (Shimadzu Technologies, Japan).[14] Triplicate UHPLC analysis was performed for each of the 13 soy meal samples.

A XTerra phenyl column, 3.9 mm inner diameter × 150 mm, 3.5 μm particle size (Waters, Milford, MA), was used with a 3.9 mm inner diameter × 20 mm, 3.5 μm particle size guard column (Waters, Milford, MA). Methanol was used as solvent A, and acetonitrile/water (48:29.5, v/v) was used as solvent B. The elution was carried out at 77.5% of solvent B for 40 min, followed by a 4 min linear gradient from 77.5 to 0% B. The column was re-equilibrated at the initial condition for 10 min before the next injection. The injection volume was 20 μL, and the oven temperature was 30 °C. ELSD settings were as follows: drift tube temperature, 40 °C; gain setting, 8; frequency of data acquisition, 10 Hz; and nitrogen pressure, 350 kPa. Quantification was achieved using ultraviolet (UV) and ELSD spectra with authentic compounds.

Isoflavone Composition. The 50% acetone extractions were hydrolyzed with acid and analyzed using the Shimadzu UHPLC instrument.[5] Briefly, 2 mL of soybean extraction was mixed with 0.5 mL of 12 M HCl, kept in a 55 °C water bath for 2 h, and then cooled to room temperature, and acetone was evaporated under nitrogen. The water suspension was extracted with 4 mL of ether/ethyl acetate (1:1, v/v) 3 times. The three ether/ethyl acetate extractions were combined, and the organic phase was removed using a nitrogen evaporator. The residue was redissolved in 1 mL of HPLC-grade methanol. After filteration through a 0.22 μm GHP syringe filter, the methanol solution was injected to a BEH C18 column (2.1 mm inner diameter × 150 mm, 1.7 μm; Waters, Milford, MA) and kept in a 40 °C oven. Solvent A consisted of 0.1% formic acid in water, and solvent B consisted of 0.1% formic acid in acetonitrile. The gradient changed linearly from 90:10 (A/B, v/v) to 70:30 (A/B, v/v) in 10 min and to 10/90 (A:B, v/v) in 3 min, kept at this ratio for 2 min, and then returned to initial conditions for 2 min to re-equilibrate the column for the next injection. The flow rate was 0.5 mL/min; the injection volume was 5 μL; and the detection wavelength was 260 nm. The isoflavones were identified and quantified using external standards.

Total Phenolic Content (TPC). The TPC of the soybean samples was measured according to a laboratory procedure described previously.[15] Briefly, 100 μL of each 50% acetone extract was mixed with 500 μL of the Folin−Ciocalteu reagent, 1.5 mL of 20% sodium carbonate, and 1.5 mL ultrapure water. Absorbance was read at 765 nm on a UV1800 spectrophotometer (Shimadzu, Japan) after 2 h of reaction at ambient temperature in the dark. Reactions were conducted in triplicate, and results were reported as milligrams of gallic acid equivalent (GAE) per gram of the defatted soybean flour.

Antioxidant Activity Assays. *$ABTS^{\bullet+}$ Scavenging Ability.* The scavenging capacity against $ABTS^{\bullet+}$ was measured following a previously reported method.[16] Briefly, an $ABTS^{\bullet+}$ working solution was prepared by oxidizing ABTS to $ABTS^{\bullet+}$ using MnO_2. The reaction mixture was filtered through a Whatman No. 1 filter paper and diluted to an absorbance of 0.7 at 734 nm to obtain the $ABTS^{\bullet+}$ working solution. A total of 1 mL of $ABTS^{\bullet+}$ working solution was combined with 80 μL of standard or diluted sample extract, and the absorbance at 734 nm was read after sitting for 30 s and vortexing for 90 s. The $ABTS^{\bullet+}$ scavenging capacity was reported in Trolox equivalents per gram of the defatted flour.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay was conducted according to the previously reported laboratory protocol.[17] Trolox standards were prepared in 50% aqueous acetone, and all other reagents were prepared in 75 mM sodium phosphate buffer (pH 7.4). The initial assay mixture contained 225 μL of freshly made 8.16×10^{-8} M fluorescein and 30 μL of sample, standard, or blank solution. Reaction mixtures were preheated at 37 °C for 20 min. Then, 25 μL of freshly made 0.36 mM AAPH was added to initiate the reactions. The fluorescence of the assay mixture was recorded on a Synergy 2 multilabel plate reader (Biotek, Winooski, VT) once every minute for 2 h at 37 °C, with λ_{Ex} = 485 nm and λ_{Em} = 535 nm. Results were expressed as micromoles of Trolox equivalent (TE) per gram of the defatted soybean flour.[18]

Hydroxyl Radical Scavenging Capacity (HOSC) Estimation. The HOSC assay was conducted following a previously reported laboratory protocol[19] using fluorescein as the fluorescent probe and a Synergy 2 multilabel plate reader (Biotek). Briefly, 170 μL of freshly made 9.28×10^{-8} M fluorescein, 30 μL of sample, 40 μL of 0.1990 M H_2O_2, and 60 μL of 3.43 mM $FeCl_3$ were mixed. The fluorescence of the reaction mixture was recorded approximately every minute for 6 h at ambient temperature, with λ_{Ex} = 485 nm and λ_{Em} = 535 nm. Standards were prepared in 50% aqueous acetone, and the 9.28×10^{-8} M fluorescein was prepared freshly for each assay from stock solution in 75 mM sodium phosphate buffer (pH 7.4).[19] Results were expressed as

Table 1. Fatty Acid Composition of Soybean Oil (g/100 g of Oil)[a]

sample ID	16:0	18:0	total SFA	18:1	18:2	18:3	total PUFA
US-1	10.89 ± 0.24 a	3.98 ± 0.23 c	14.87 ± 0.47 bc	20.99 ± 0.39 e	55.20 ± 0.40 bc	8.94 ± 0.18 b	64.14 ± 0.58 ab
US-2	10.95 ± 0.15 a	3.98 ± 0.26 c	14.93 ± 0.41 bc	23.68 ± 0.22 c	55.74 ± 0.31 b	5.65 ± 0.03 e	61.39 ± 0.33 bc
US-3	11.69 ± 1.64 a	3.60 ± 0.05 d	15.30 ± 1.69 abc	18.38 ± 0.43 g	56.76 ± 0.84 ab	9.56 ± 0.55 b	66.32 ± 1.39 ab
US-4	11.10 ± 0.18 a	4.36 ± 0.11 abc	15.45 ± 0.29 abc	21.72 ± 0.30 d	55.14 ± 0.11 bc	7.69 ± 0.29 c	62.83 ± 0.40 b
US-5	11.04 ± 0.15 a	4.10 ± 0.12 bcd	15.14 ± 0.27 bc	22.32 ± 0.15 d	55.47 ± 0.49 b	7.08 ± 0.35 cd	62.54 ± 0.83 b
BA-1	11.20 ± 0.16 a	3.86 ± 0.13 c	15.06 ± 0.29 bc	25.16 ± 0.20 b	53.29 ± 0.34 c	6.49 ± 0.47 d	59.78 ± 0.81 c
BA-2	11.18 ± 0.07 a	3.76 ± 0.14 d	14.94 ± 0.21 bc	23.82 ± 0.03 c	54.22 ± 0.12 c	7.02 ± 0.21 cd	61.24 ± 0.32 bc
AG-1	11.02 ± 0.28 a	4.19 ± 0.14 abc	15.21 ± 0.42 abc	20.28 ± 0.14 f	56.96 ± 0.13 ab	7.55 ± 0.39 c	64.51 ± 0.51 ab
CA-1	10.69 ± 0.12 a	3.88 ± 0.34 c	14.57 ± 0.46 c	22.81 ± 0.29 d	53.82 ± 0.25 c	8.80 ± 0.14 b	62.62 ± 0.39 b
CN-HB	11.63 ± 0.11 a	2.72 ± 0.08 e	14.35 ± 0.19 c	30.62 ± 0.05 a	49.43 ± 0.09 d	5.60 ± 0.13 e	55.03 ± 0.21 d
CN-HLJ	11.87 ± 0.11 a	2.93 ± 0.10 e	14.81 ± 0.21 bc	20.46 ± 0.09 ef	56.11 ± 0.40 ab	8.63 ± 0.21 b	64.73 ± 0.61 ab
CN-DB	11.60 ± 0.13 a	4.57 ± 0.41 ab	16.17 ± 0.54 ab	18.71 ± 0.21 g	54.18 ± 0.12 c	10.94 ± 0.23 a	65.12 ± 0.35 ab
CN-XJ	11.88 ± 0.06 a	4.77 ± 0.08 a	16.64 ± 0.14 a	20.66 ± 0.24 e	53.68 ± 0.47 c	9.02 ± 0.17 b	62.70 ± 0.64 b

[a]Values are based on triplicate measurements. Mean ± SD values are shown. Individual sample values in the same column marked by the same letter are not significantly different ($p < 0.05$). SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid.

micromoles of Trolox equivalent (TE) per gram of defatted soybean flour.

Anti-inflammation Effects of Soybean Extracts in RAW 264.7 Mouse Macrophage Cells. RAW 264.7 mouse macrophage cells were cultured in the DMEM containing 10% FBS and 1% amphotericin B/streptomycin/penicillin at 37 °C with 5% CO_2 in air. To determine the anti-inflammation activities of the soybean extracts, RAW 264.7 mouse macrophages were cultured in 6-well plates overnight to reach a 80% confluence. Each extract was added into cell cultures at 10 and 100 μg/mL at 24 h prior to induction. After 24 h of pretreatment, LPS was added to the media at an initial concentration of 10 ng/mL. After induction, culture media were discarded and cells were collected to perform total RNA isolation and real-time polymerase chain reaction (PCR).

RNA isolation and real-time PCR were performed according to the previously published protocols.[20] After 4 h of induction with LPS, cells were washed with 1× PBS, and TRIzol reagent was added for total RNA isolation. IScript Advanced cDNA synthesis kit was used to reverse transcribe cDNA. Real-time PCR was performed on an ABI 7900HT Fast Real-Time PCR System using AB Power SYBR Green PCR Master Mix. Primers were as follows: IL-1β (forward, 5′-GTTGACGGACCCCAAAAGAT-3′; reverse, 5′-CCTCATCCTG-GAAGGTCCAC-3′); IL-6 (forward, 5′-CACGGCCTTCCCTACTT-CAC-3′; reverse, 5′-TGCAAGTGCATCATCGTTGT-3′); COX-2 (forward, 5′-GGGAGTCTGGAACATTGTGAA-3′; reverse, 5′-GCACGTTGATTGTAGGTGGACTGT-3′). The mRNA amounts were normalized to an internal control, GAPDH mRNA (forward, 5′-AGGTGGTCTCCTCTGACTTC-3′; reverse, 5′-TACCAGGAAAT-GAGCTTGAC-3′). The following amplification parameters were used for PCR: 50 °C for 2 min, 95 °C for 10 min, and 46 cycles of amplification at 95 °C for 15 s and 60 °C for 1 min.

Statistical Analysis. Data were reported as the mean ± standard deviation (SD) for triplicate determinations. One-way analysis of variation (ANOVA) and Tukey's post-hoc test were employed to identify differences in means. The *t* test was used to identify different values on both the TPC and antioxidant activity assays. The Pearson correlation was used to identify the correlations. Statistics were analyzed using SPSS for Windows (version release 10.0.5, SPSS, Inc., Chicago, IL). Statistical significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical Component Analysis. *Fatty Acid Composition.* As shown in Table 1, the 13 soybean oils significantly differed in their total saturated fatty acid (SFA), polyunsaturated fatty acid (PUFA), and monounsaturated fatty acid (MUFA) contents. The fatty acid composition of soybean oils may affect the overall nutritional quality of soybeans, because linoleic (18:2ω-6) and α-linolenic (18:3ω-3) are essential fatty acids. The level of 18:3ω-3 is also important for the dietary ω-3 PUFA level and ω-3/ω-6 fatty acid ratio, which are critical for human health. While higher levels of ω-3 fatty acids may contribute to a better nutritional value, the reduction of shelf stability resulting from the high levels of unsaturated fatty acids is undesirable. Thus, soy oils with different levels of ω-3 fatty acids are needed for desirable nutritional benefits and shelf stability for different food uses.

In all 13 soybean samples, there was no significant difference in the palmitic acid (16:0) level of about 11 g/100 g of oil, comparable to the previously reported 16:0 range of 3−12 g/100 g for soybean oils.[5−7] The stearic acid (18:0) contents ranged from 2.72 to 4.77 g/100 g. The CN-XJ oil contained the highest 18:0 concentration of 4.77 g/100 g, followed by CN-DB oil (4.57 g/100 g). CN-XJ and CN-DB also contained the highest total SFA among the 13 soybean samples. Although saturated fats are chemically stable, a lower SFA content is generally desirable because of the well-known connection between SFA consumption and elevated cardiovascular disease (CVD) risk. However, recent studies with stearic acid have indicated that it may be less detrimental to CVD-predictive blood lipid levels than other, shorter chain SFAs.[21] The higher level of stearic acid in several soybeans may therefore provide increased oxidative stability without the negative health impact.

The content of oleic acid (18:1ω-9) varied most in the tested samples, from 18.38 to 30.62 g/100 g of oil. CN-HB from China contained the highest amount of 18:1 with the lowest total PUFA, whereas US-3 from the United States contained the lowest amount of 18:1 with the highest total PUFA. In addition, all of the samples contained about 60−65 g of PUFA/100 g of oil, except CN-HB at 55.03 g/100 g of oil. US-3 oil contained the highest amount of linoleic acid (18:2ω-6) (56.76 g/100 g of oil), and the lowest level of 18:2ω-6 was found in CN-HB soybeans (49.43 g/100 g of oil). The highest amount of α-linolenic acid (18:3ω-3) was observed in CN-DB oil (10.94 g/100 g of oil), while the lowest amount of 18:3ω-3 was found in CN-HB (5.60 g/100 g of oil), indicating the significant variations in fatty acid compositions among these soybean samples from China. The US-2 and CN-HB soybeans from the United States and China, respectively, contained the lowest levels of 18:3ω-3 and, therefore, may be of interest for further breeding for a low-linolenic-acid soybean line that does not require hydrogenation for desirable oxidative stability. It needs to be pointed out that the level of 18:3ω-3 in the US-2 and

Table 2. Lutein, Tocopherol, and Steroid Contents in the Soybeans[a]

	μg/g of soybean flour					
	lutein	δ-tocopherol	γ-tocopherol	β-sitosterol	campesterol	stigmasterol
US-1	13.05 ± 0.23 a	12.21 ± 0.03 de	31.51 ± 0.79 ef	409.9 ± 7.6 b	136.2 ± 3.3 a	112.8 ± 2.2 ef
US-2	10.95 ± 0.02 b	16.03 ± 0.00 c	34.29 ± 0.93 cde	456.7 ± 1.5 a	114.3 ± 3.5 b	163.6 ± 2.8 a
US-3	11.09 ± 0.08 b	8.53 ± 1.03 f	32.65 ± 0.48 def	316.1 ± 1.8 f	105.5 ± 2.4 bc	100.5 ± 0.7 g
US-4	10.93 ± 0.28 b	8.09 ± 0.26 f	35.40 ± 0.60 bcd	354.9 ± 1.0 d	111.9 ± 1.2 b	126.8 ± 0.1 d
US-5	10.56 ± 0.02 b	3.62 ± 0.15 h	33.83 ± 0.66 cde	328.7 ± 4.7 ef	93.9 ± 5.5 cde	119.2 ± 0.9 de
BA-1	10.47 ± 0.19 b	6.80 ± 0.19 fg	35.59 ± 0.35 bcd	406.2 ± 1.4 b	102.5 ± 2.7 bc	147.6 ± 3.9 b
BA-2	8.76 ± 0.06 c	4.19 ± 0.20 h	32.68 ± 2.07 def	378.7 ± 0.7 c	96.3 ± 1.4 cd	124.9 ± 2.5 d
AG-1	7.14 ± 0.13 de	4.97 ± 0.20 gh	30.21 ± 1.33 f	259.5 ± 2.5 h	79.9 ± 0.4 e	111.2 ± 2.1 ef
CA-1	7.00 ± 0.17 de	14.37 ± 0.68 cd	35.09 ± 0.56 bcd	296.5 ± 6.6 g	106.0 ± 0.6 bc	137.1 ± 2.7 c
CN-HB	2.29 ± 0.08 g	11.63 ± 0.65 e	32.97 ± 0.38 def	333.9 ± 4.2 e	83.0 ± 2.8 de	123.4 ± 1.3 d
CN-HLJ	5.41 ± 0.08 f	20.64 ± 1.07 b	36.87 ± 0.31 bc	172.9 ± 0.4 k	91.3 ± 3.6 cde	124.8 ± 1.4 d
CN-DB	8.22 ± 0.15 cd	23.97 ± 1.16 a	42.58 ± 0.28 a	247.1 h ± 0.7	81.1 ± 3.6 e	95.3 ± 1.7 g
CN-XJ	6.56 ± 0.01 ef	22.02 ± 0.09 ab	38.36 ± 0.14 b	218.7 ± 1.5 j	83.4 ± 8.7 de	103.5 ± 4.3 fg

[a]Values are based on triplicate measurements. Mean ± SD values are shown. Values in the same column marked by the same letter are not significantly different ($p < 0.05$).

Table 3. Isoflavone Compositions in the Soybeans[a]

	μmol/g of soybean flour			
	daidzein	genistein	glycitein	total isoflavones
US-1	0.30 ± 0.00 a	0.04 ± 0.00 f	0.11 ± 0.00 e	0.45 ± 0.00 c
US-2	0.04 ± 0.00 g	0.04 ± 0.00 f	0.08 ± 0.00 h	0.16 ± 0.00 g
US-3	0.28 ± 0.00 b	0.05 ± 0.00 e	0.10 ± 0.00 f	0.44 ± 0.01 c
US-4	0.18 ± 0.00 e	0.04 ± 0.00 e	0.09 ± 0.00 g	0.31 ± 0.00 f
US-5	0.20 ± 0.01 c	0.05 ± 0.01 e	0.10 ± 0.00 f	0.34 ± 0.02 e
BA-1	0.19 ± 0.00 de	0.04 ± 0.00 ef	0.14 ± 0.00 c	0.37 ± 0.00 d
BA-2	0.14 ± 0.00 f	0.04 ± 0.00 f	0.12 ± 0.00 d	0.31 ± 0.00 f
AG-1	0.20 ± 0.00 cd	0.15 ± 0.00 a	0.17 ± 0.00 a	0.51 ± 0.01 b
CA-1	0.18 ± 0.01 e	0.11 ± 0.00 bc	0.07 ± 0.00 h	0.37 ± 0.01 d
CN-HB	0.04 ± 0.00 g	0.06 ± 0.00 d	0.07 ± 0.00 h	0.16 ± 0.01 g
CN-HLJ	0.20 ± 0.00 c	0.04 ± 0.00 f	0.08 ± 0.00 h	0.31 ± 0.00 f
CN-DB	0.27 ± 0.00 b	0.12 ± 0.00 b	0.15 ± 0.00 b	0.54 ± 0.00 a
CN-XJ	0.14 ± 0.00 f	0.10 ± 0.00 c	0.08 ± 0.00 g	0.32 ± 0.00 f

[a]Values are based on triplicate measurements. Mean ± SD values are shown. Values in the same column marked by the same letter are not significantly different ($p < 0.05$).

CN-HB soybeans was greater than that of 1−3 g/100 g of oil, typically considered to be "low-linolenic" soybeans.[5]

Carotenoid, Tocopherol, and Sterol Contents. Carotenoids and tocopherols are natural antioxidants and may protect the oil from auto-oxidation. They may also prevent oxidative damage to human cellular components. Lutein is also recognized for its role in eye health and may prevent age-related macular degeneration.[22] Lutein was detected in the defatted soybean flours with a concentration ranging from 2.29 to 13.05 μg/g of soybean flour (Table 2). The lowest lutein level was observed in the CN-HB soybean from China, and the highest lutein content was detected in the US-1 soybean. This lutein concentration range was comparable to the previously reported level of 0.8−10.8 μg/g in yellow soybeans.[23−25] Oil processing often removes carotenoids during the bleaching process that removes chlorophyll to enhance oil stability, but the presence of carotenoids (within limits) exerts a positive effect on oil stability and is welcomed by processors. Also, soybeans with high levels of carotenoids would be desirable for whole bean consumption or soy food applications to improve human health. The lutein content of the tested soybeans was positively correlated with the 18:2ω-6 fatty acid level (r = 0.567; $p < 0.05$), and this relationship agrees with the previous report by Whent et al. for Maryland-grown soybeans.[7]

The 13 soybean flours significantly differed in their δ- and γ-tocopherol contents, and no α-tocopherol was detected in any tested soybean samples (Table 2). α-Tocopherol is typically found in soybeans but is often in lower quantities than the other tocopherols. Seguin et al. reported an α-tocopherol concentration at 8.7−33.2 μg/g of soybean in 20 genotypes grown in eastern Canada.[26] Several others have reported α-tocopherol in soybean oil; however, it is known that genotype and environmental conditions may alter soybean tocopherol content and composition.[27−29] The results from this study showed that δ-tocopherol ranged from 3.62 μg/g (US-5) to 23.97 μg/g (CN-DB) of soybean, while γ-tocopherol ranged from 30.21 μg/g (AG-1) to 42.58 μg/g (CN-DB) of soybean (Table 2). In comparison, Seguin et al. reported a δ-tocopherol level at 79−121 μg/g and a γ-tocopherol content of 153−193 μg/g in Canadian soybeans.[26]

Campesterol, stigmasterol, and β-sitosterol were detected in all 13 soybeans. The campesterol content ranged from 79.9 μg/g (AG-1) to 136.2 μg/g (US-1) for the defatted soybean flours. The β-sitosterol content ranged from 172.9 μg/g (CN-HLJ) to 456.7 μg/g (US-2). The stigmasterol content ranged from 95.3

μg/g (CN-DB) to 163.6 μg/g (US-2) in the defatted soybean flour samples. Sterols recovered from soybean oil deodorizer distillate (SODD) are also of interest as a functional food ingredient and dietary supplement because their consumption may be associated with a significantly lower blood cholesterol level without toxic effects[30] and a decreased risk of certain cancers.[31]

In this study, β-sitosterol was negatively correlated with 16:0 and 18:3ω-3 fatty acid concentrations ($r = -0.615$; $p < 0.05$ and $r = -0.572$; $p < 0.05$, respectively). Sigmasterol was negatively correlated with 18:3ω-3 ($r = -0.727$; $p < 0.01$). These data may indicate a relationship between fatty acid and sterol levels in soybeans.

Isoflavone Composition. The total isoflavone content may vary depending upon soy genotype, growing environment, postharvest treatments, storage period of time and conditions, and the analytical method. Isoflavone composition of the 13 soybean samples was investigated using the hydrolyzed 50% acetone extracts (Table 3). The CN-DB soybean from China exhibited the greatest content of total isoflavones (0.54 μmol/g of soybean flour), followed by that of AG-1 at 0.51 μmol/g of soybean flour. The total isoflavone content in the 13 soybean samples was 0.16−0.54 μmol/g of soybean flour, which was similar to that of 0.39−0.90 and 0.14−1.62 μmol/g of soybean flour using 50% acetone as the extraction solvent in the previous studies.[6,7] A similar study of 20 soybean cultivars and lines from Serbian, United States, Russian, and Chinese origin showed that the greatest level was 315% of the lowest value, suggesting a huge variation, although direct comparison of values was difficult because of different extraction and analysis protocols.[32] Taken together, commercial soybeans are not equal in their ability to provide dietary isoflavones.

The 13 commercial soybean samples significantly differed in their isoflavone profiles (Table 3). Daidzein, genistein, and glycitein were present in all 13 tested samples, with daidzein being the primary isoflavone in most of them. The US-1 soybean possessed the greatest daidzein content of 0.30 μmol/g of the defatted soybean flour, which was about 7 times that in the US-2 and CN-HB flours (Table 3). The genistein content was 0.04−0.15 μmol/g, whereas the glycitein level ranged from 0.07 to 0.17 μmol/g in the flours. The soybean sample with the greater daidzein level did not necessarily contain the higher level of genistein or glycitein. For instance, the US-1 soybean with the highest daidzein level had the lowest genistein content, while the AG-1 flour with a moderate concentration of daidzein contained the highest levels of both glycitein and genistein (Table 3). In addition, both daidzein and total isoflavone content had a significant positive correlation with the 18:3ω-3 level ($r = 0.778$; $p < 0.01$ and $r = 0.746$; $p < 0.01$, respectively). These correlations supported the findings by Whent et al.[7] and suggested a potential relationship between isoflavones and 18:3ω-3 in soybeans. In summary, these data suggest that commercial soybeans may significantly differ in their total isoflavone content and isoflavone compositions, which may be of interest to consumers and producers of soy-based functional foods.

TPC and Antioxidant Activities. The 13 soybean samples differed in their total phenolic contents, with a TPC value of 0.85−2.38 mg of GAE/g of defatted soybean flour (Figure 1). This range was consistent with the previously reported TPC values of 0.8−2.2 mg of GAE/g of soybean flour.[5] The CN-DB soybean from China had the greatest TPC of 2.38 mg of GAE/g, followed by the US-3 soybean from the United States at 2.04

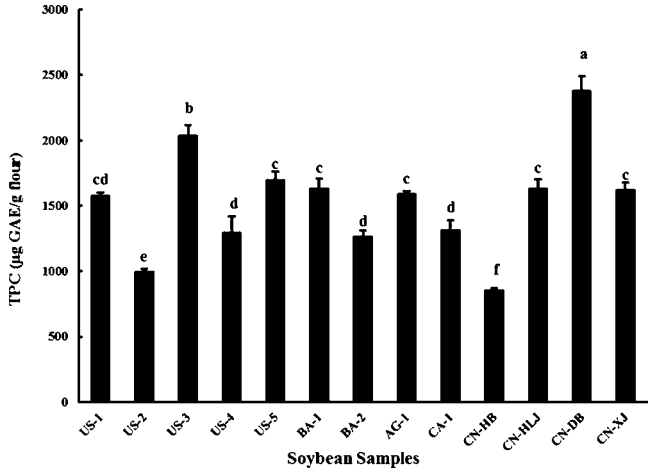

Figure 1. TPC of the soybean samples. GAE stands for gallic acid equivalent. The vertical bars represent the standard deviation ($n = 3$) of each data point. Different letters represent significant differences ($p < 0.05$).

mg of GAE/g. In addition, the TPC value and total isoflavone content of the 13 soybean samples exhibited significant correlation ($r = 0.811$; $p < 0.05$), indicating that the isoflavones might be the primary phenolic compounds in the soybeans.

Antioxidant activity of the soybean flour extracts was measured as their scavenging capacities against peroxyl (ORAC), hydroxyl (HOSC), and $ABTS^{\bullet+}$ radicals. As shown in Figures 1−4, CN-DB soybean from China with the greatest

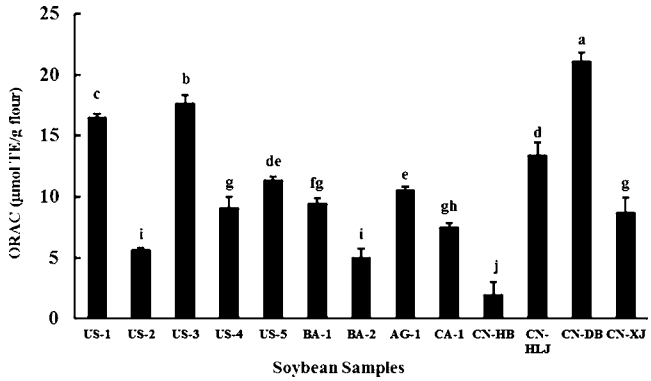

Figure 2. ORAC of the soybean samples. TE stands for Trolox equivalent. The vertical bars represent the standard deviation ($n = 3$) of each data point. Different letters represent significant differences ($p < 0.05$).

TPC exhibited the strongest ORAC of 21.03 μmol of TE/g of flour, the highest HOSC value of 38.52 μmol of TE/g of flour, and the strongest $ABTS^{\bullet+}$ scavenging capacity of 3.97 μmol of TE/g of flour. Among all of the tested soybean samples, TPC showed significant positive correlations with both ORAC ($r = 0.91$; $p < 0.01$) and HOSC ($r = 0.85$; $p < 0.01$) values. These data further confirmed the observations in the previous studies of soybeans.[5,7] Daidzein also showed significant positive correlations with ORAC ($r = 0.89$; $p < 0.01$) and HOSC ($r = 0.566$; $p < 0.05$), indicating that daidzein might play an important role in the overall antioxidant activity of soybeans. No significant correlation was found between any antioxidant capacity and carotenoids or tocopherols. The assays in this study were performed with the extract of defatted soybean

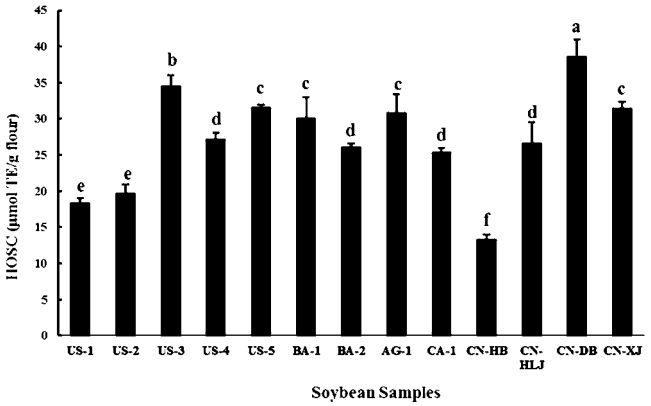

Figure 3. HOSC of the soybean samples. TE stands for Trolox equivalent. The vertical bars represent the standard deviation (n = 3) of each data point. Different letters represent significant differences (p < 0.05).

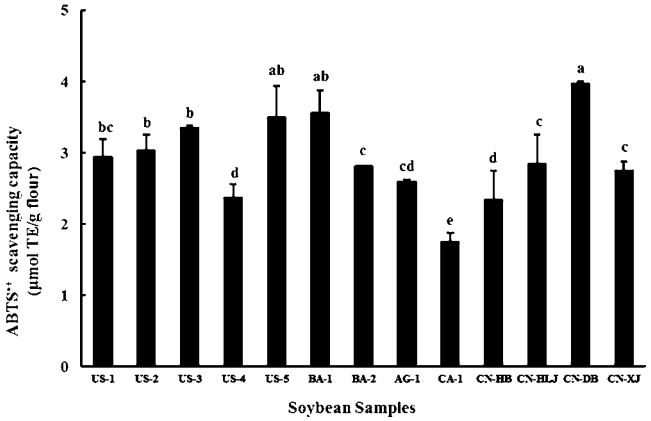

Figure 4. ABTS cation radical scavenging activity of the soybean samples. TE stands for Trolox equivalent. The vertical bars represent the standard deviation (n = 3) of each data point. Different letters represent significant differences (p < 0.05).

flours, and therefore, lipophilic compounds were not expected to significantly contribute to the antioxidant capacities.

Interestingly, the ABTS$^{\bullet+}$ scavenging capacity did not have a significant correlation with the other antioxidant activities. This may be partially explained by the different chemical mechanism involved in these assays. Differences in genotype and growing conditions might also have played a role in the variation.

Anti-inflammatory Effect of Soybean Extracts in RAW 264.7 Mouse Macrophage Cells. Chronic inflammation has been associated with several chronic diseases, such as cardiovascular diseases, cancer, diabetes, arthritis, pulmonary diseases, and Alzheimer's and autoimmune diseases.[33,34] Several cytokines, including interleukin-1β (IL-1β), IL-6, and cyclooxygenase (COX), may induce inflammation and are critical factors in the onset and progress of inflammatory responses. The expression levels of inflammatory cytokine genes are positively associated to the inflammatory responses, and the inhibition of their mRNA expression may lead to alleviation of the inflammatory response. In this study, the expression of IL-1β, IL-6, and COX-2 mRNA was used to reflect the potential anti-inflammation effect of soybean extracts.

Four samples from the 13 commercial soybeans were selected and tested for their potential anti-inflammatory properties. CN-DH and US-3 soybeans had greater TPC, ORAC, HOSC, and ABTS scavenging capacities, whereas CN-HB was low in all tested antioxidant activities, and AG-1 soybean had middle antioxidant activities and TPC value. The extracts from all of the four soybean samples significantly inhibited the IL-1β mRNA expressions at both 10 and 100 μg/mL initial treatment concentrations (Figure 5A). The inhibition

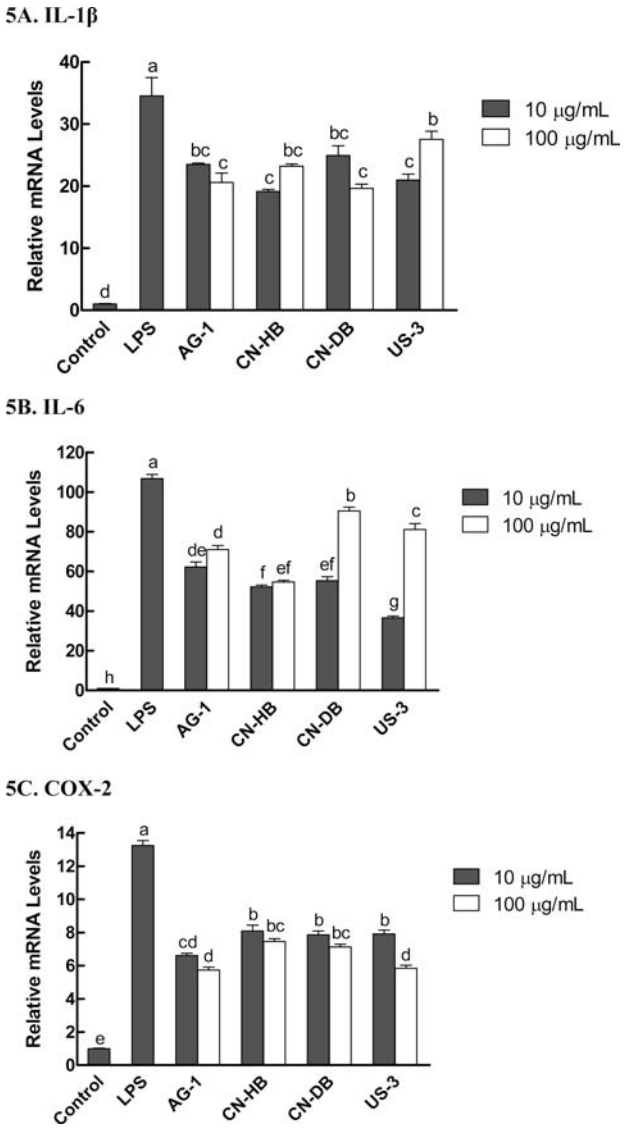

Figure 5. Anti-inflammatory effects of AG-1, CN-HB, CN-DB, and US-3 soybean samples in RAW 264.7 mouse macrophage cells. Control and LPS contained the same concentration of DMSO as all treatment samples. LPS stands for lipopolysaccharide. The vertical bars represent the standard deviation (n = 3) of each data point. Different letters represent significant differences (p < 0.05).

was not dependent upon the soybean extract concentrations, except that the US-3 extract had significantly stronger inhibitory capacity at the lower concentration.

The four soybean extracts also differed in suppressing IL-6 mRNA expression (Figure 5B), although all could significantly suppress IL-6 mRNA expression at both 10 and 100 μg/mL initial treatment concentrations. Both CN-DB and US-3 soybean extracts had significantly stronger inhibitory effects at 10 μg/mL, but no difference in suppressing IL-6 mRNA expression was observed at 10 and 100 μg/mL treatment

concentrations for AG-1 and CN-HB extracts (Figure 5B). Taken together, the results suggested that soybean extracts might contain both inhibitor(s) and inducer(s) for IL-1β and IL-6 mRNA expressions under the experimental conditions. At a certain treatment concentration, either inhibitor or inducer might dominate the overall effect on mRNA expression of IL-1β and IL-6 genes.

As shown in Figure 5C, the four extracts were able to significantly reduce the COX-2 mRNA expression at both 10 and 100 μg/mL initial treatment concentrations. At 10 μg/mL initial concentration, the AG-1 extract had the strongest inhibition on COX-2 mRNA expression, with an approximately 50% reduction in the mRNA expression (Figure 5C), which was significantly stronger than that of CN-HB, CN-DB, and US-3 extracts under the same conditions. At 100 μg/mL initial treatment concentration, AG-1 and US-3 extracts had the same inhibitory activity on COX-2 mRNA expression and the inhibition was significantly stronger than that of CN-HB and CN-DB extracts (Figure 5C). In addition, the US-3 extract had a dose-dependent inhibition on COX-2 mRNA expression under the experimental conditions. These data indicated a possible difference in the chemical compositions of these soybean extracts.

In summary, this study demonstrated possible variations in chemical composition and antioxidant and anti-inflammatory effects of commercial soybeans, although only limited numbers of samples were involved. The results from this study suggested a need of improved quality control and measurement system to better reflect the health properties of soybeans for general consumers and food manufacturers to better use this important food ingredient.

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Notes

The authors declare no competing financial interest.

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