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Glyphosate-Tolerant Soybeans Remain Compositionally Equivalent to Conventional Soybeans (*Glycine max* L.) during Three Years of Field Testing

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Previous studies have shown that the composition of glyphosate-tolerant soybeans (GTS) and selected processed fractions was substantially equivalent to that of conventional soybeans over a wide range of analytes. This study was designed to determine if the composition of GTS remains substantially equivalent to conventional soybeans over the course of several years and when introduced into multiple genetic backgrounds. Soybean seed samples of both GTS and conventional varieties were harvested during 2000, 2001, and 2002 and analyzed for the levels of proximates, lectin, trypsin inhibitor, and isoflavones. The measured analytes are representative of the basic nutritional and biologically active components in soybeans. Results show a similar range of natural variability for the GTS soybeans as well as conventional soybeans. It was concluded that the composition of commercial GTS over the three years of breeding into multiple varieties remains equivalent to that of conventional soybeans.

KEYWORDS: Soybean (*Glycine max* L.); glyphosate-tolerant; composition; genetically modified; natural variability

INTRODUCTION

The first agricultural biotechnology crops were commercially introduced in the early 1990s. Among the first of these products was glyphosate-tolerant soybeans (GTS, marketed under the trade name Roundup Ready (Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC) soybean) that were available in the U.S. in 1995. Since then, numerous agronomically and nutritionally enhanced traits have been introduced in more than 70 different crop species, and 50 products have been approved in numerous countries around the world (*1*−*3*). Before commercialization, a new biotechnology crop undergoes a thorough safety assessment (*4*, *5*). The comparative safety assessment process of a food or feed derived from biotechnology crops is based upon the concept that there is a "reasonable certainty of no harm" from its intended use (*5*−*8*). This process compares the phenotypic characteristics and composition of the biotechnology-derived crop to that of its conventional counterpart crop that has a history of safe use (*9*). The process is known as substantial equivalence, which is utilized by many regulatory and food safety authorities worldwide as a key part of assessing the safety of food or feed derived from biotechnology crops. Beyond this compositional comparison, additional safety assessments are made including the agronomic performance of the crop, the safety of the source gene, toxic and allergenic potential of the protein, unintended effects due to the insertion of the gene, and nutritional equivalence (*5*, *7*, *10*).

GTS was genetically engineered to produce a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein as encoded by the *Agrobacterium* sp. strain *cp4 epsps* gene. The CP4 EPSPS protein is functionally similar to plant EPSPS enzymes but has a much reduced affinity for glyphosate, a selective EPSPS inhibitor and the active ingredient in Roundup agricultural herbicides (*11*). The continued activity of CP4 EPSPS, even when treated with glyphosate, allows the plant to continue to produce aromatic amino acids and other secondary metabolites that are required for normal growth and development (*12*). The safety of the CP4 EPSPS protein in GTS has been well documented (*12*−*14*).

The composition of GTS was determined in seed and various processed fractions from a multi-location trial over two years in previous studies (*15*, *16*). In these studies, a comprehensive assessment of the components measured in seed included proximates, amino acids, fatty acids, antinutrients, and isoflavones. The conclusion was that the composition of GTS and selected processed fractions was substantially equivalent to that of conventional soybeans over a wide range of analytes. Furthermore, the nutritional equivalence was also determined by growth and performance studies in chicken, dairy cattle, and swine (*17*, *18*). These additional studies demonstrated that

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animals fed GTS had growth performance and feed efficiency similar to animals fed conventional soybeans.

The benefit of GTS is that it allows the soybean farmer to control a broad spectrum of weeds using glyphosate herbicides with minimal impact on the plant and environment (*19*). GTS was grown in eight countries with 102 million acres (41.4 million hectares) cultivated in 2003 (*20*), indicating the widespread adoption of this technology. An important consideration for any new technology is to demonstrate consistent and reliable performance over time. Performance can be defined for agronomic traits as the stability of the intended trait in diverse genetic backgrounds.

In this paper, consistent performance of GTS was assessed by comparing the composition of representative GTS seed in diverse genetic backgrounds harvested during three different years in the United States and Canada to conventional soybean varieties. The composition of GTS seed that are commercially available in each year tested was compared to the composition of seed from conventional soybean varieties. The GTS and conventional soybean varieties were not matched for similar genetic backgrounds as in previous composition studies (*15*, *16*). Analyses measured the levels of proximates, lectin, trypsin inhibitor, and isoflavones in soybean seed. These selected analytes represent basic nutritional and biologically active components in soybeans. Comparisons of the values of the GTS and conventional seed were then made to those values published in the literature and the International Life Science Institute (ILSI) Crop Composition Database (*21*).

MATERIALS AND METHODS

Soybean Seed Samples. Samples were collected from Monsanto soybean seed stations during the 2000, 2001, and 2002 field seasons in the United States and Canada. All of the soybean varieties were commercially available or near commercial germplasms reflective of a broad range of maturity groups (I−IX). The GTS varieties were treated in-season with Roundup agricultural herbicide applications according to the labeled rates and timing. The soybean seed samples were stored at ambient temperature until ground. The ground samples were stored in a −20 °C freezer until analysis, and appropriate chain-of-custody accompanied all shipments.

2000 Field Season. Seed from 25 different varieties of GTS and 25 different varieties of conventional soybeans were harvested in 2000. The GTS varieties were: AG0801, AG2101, AG2301, AG3503, AG3702, AG3903, AG4901, AG5401, AG5501, CSR3623, CSR3922N, CSR4003, CSR4122N, CSR4812, CSR2523, CSR2900, CSR3112N, CSR6212, CSRX124, CSRX58, H4998RR, H5999RR, H6686RR, H7550RR, and H8001RR. The conventional soybean varieties were: A1900, A2069, A2104, A2247, A2553, A2704, A2804, A2869, A3469, A3904, A4341, A4604, A4922, A5547, A5959, A6297, A6961, CX284C, CX393C, CX400, CX420C, CX470C, H4994, H6255, and H6686.

2001 Field Season. Seed from 25 different varieties of GTS and 25 different varieties of conventional soybeans were harvested in 2001. The GTS varieties were: AG1401, AG1701, AG1902, AG2102-14, AG2403, AG2705, AG3303, AG3401, AG3702, AG3703, AG4201, AG4603, AG5001, AG5301, AG5903, AG6202, AG6701, CSR5952N, DKB17-51, DKB25-51, DKB27-51, DKB46-51, H5110RR, H5223RR, and H5887RR. The conventional soybean varieties were: A2824, A3244, A3834, A3836, A5427, A5547, A6297, CBN2001D0C, CCP4301A1C, CEI3900E0C, CGL5200B0C, CJW1901B1C, CMA5801B0C, CMA5901C0C, COX3501B0C, COX3501C0C, CRM2401H1C, CST321N, CST341, CX229, CX232, FP24960, H518, QR5282B, and WP25920.

2002 Field Season. Seed from 16 different varieties of GTS and 16 different varieties of conventional soybeans were harvested in 2002. The GTS varieties were: H5333RR, AG5402, AG5605, DKB64-51, AG1401, DKB19-52, DKB22-51, AG2403, DKB37-51, AG2403, DKB44-52, AG3801, DKB009-51, AG0101, FLS2602R, and AG0601. The conventional soybean varieties were: H922, A4922, A5547, CMA5801B0C, A2225, CST231N, DJW2501B0C, FP29930, A3525, COX3501B0C, CEI3900E0C, ACP4301A1C, Opal, Exeter, FL0062, and BJB2100D0C.

Compositional Analysis. Soybean seed samples harvested during 2000, 2001, and 2002 were ground and analyzed for the levels of proximates (ash, carbohydrates, moisture, protein, and total fat), lectin, trypsin inhibitor, and isoflavones (expressed as aglycones: daidzein, genistein, and glycitein). Covance Laboratories Inc. (Madison, WI) generated the analytical data for these samples.

Each method conducted utilized a standard or a quality control sample with known analyte content, and each sample was analyzed once. Brief descriptions of the analytical methods utilized are below. All laboratory activities followed Good Laboratory Practices (*22*).

Proximate Analysis. The moisture content was estimated by loss of weight upon drying the sample in an oven to a constant weight (*23*, *24*). Protein concentration was estimated by determining the total nitrogen using the Kjeldahl method, previously described (*25*, *26*). The total fat content was estimated using a Soxhlet extraction method (*27*, *28*). The ash content was estimated by the ignition of a sample with a furnace and determining the percent ash gravimetrically (*29*). The carbohydrate content was calculated using the following equation (*30*):

$$\%\ \text{carbohydrates} = 100\% - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{ash} + \%\ \text{moisture})$$

Antinutrient and Isoflavone Analysis. The lectin content was measured by suspending the sample in a phosphate buffered saline solution, adding lyophilized rabbit blood (Sigma, St. Louis, MO), and reading the absorbance at 650 nm (*31*, *32*). Total trypsin inhibitor activity was measured by suspending the sample in a sodium hydroxide solution, adding trypsin (Sigma, St. Louis, MO) and benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (Sigma, St. Louis, MO), and reading the absorbance at 410 nm (*33*). The isoflavone content was measured as the three aglycones daidzein, genistein, and glycitein. The sample was extracted with a hydrochloric acid/alcohol solution. Isoflavone aglycones were purified by passage through a C18 solid-phase extraction column. Analysis was conducted on a high-performance liquid chromatography system with ultraviolet spectrophotometric quantitation as previously described (*34*, *35*).

Data Reduction and Statistical Analysis. Data generated on the samples were measured on a fresh weight basis. Using the moisture content that was determined for each sample, the data were converted to dry weight. Simple means and ranges for the GTS and conventional soybean seed were determined on the dry weight values of each analyte. Standard error (SE) of the mean was calculated for each simple mean as the standard deviation divided by the square root of the number of values. The means and ranges were compared to published values and those values in the ILSI Crop Composition Database (*21*) to place the values in the perspective of natural variability.

RESULTS AND DISCUSSION

Proximate Analysis of Soybean Seed from 2000, 2001, and 2002. The data for the proximate analysis of GTS and conventional soybean seed for the 2000, 2001, and 2002 field seasons are found in **Table 1**. The means and ranges of the levels of proximates measured in GTS seed are similar to the conventional soybean seed means and ranges. Across all three years, the moisture content ranged from 5.1% to 7.5% fresh weight (fw) for GTS seed and 5.1−8.8% fw for conventional soybean seed. The protein content ranged from 33.4% to 43.0% dry weight (dw) for GTS seed and 34.4−45.3% dw for conventional soybean seed. The total fat content ranged from 14.6% to 21.2% dw in GTS seed and 14.4−22.3% dw for conventional soybean seed. The ash content ranged from 4.9% to 6.0% dw for GTS seed and 5.0−6.0% dw for conventional soybean seed. The carbohydrate content ranged from 31.5 to 42.9% dw for GTS seed and 31.0−42.1% dw for conventional

Table 1. Proximate Analysis of Seed from GTS and Conventional Soybeans Harvested in 2000, 2001, and 2002

	2000 field season		2001 field season		2002 field season			
component[a]	GTS mean[c] ± SE[d] (range)[f]	conven.[b] mean[c] ± SE[d] (range)[f]	GTS mean[c] ± SE[d] (range)[f]	conven.[b] mean[c] ± SE[d] (range)[f]	GTS mean[e] ± SE[d] (range)[f]	conven.[b] mean[e] ± SE[d] (range)[f]	literature range[a]	ILSI[g] range[a]
moisture	5.4 ± 0.03 (5.1–5.7)	5.3 ± 0.03 (5.1–5.6)	6.6 ± 0.03 (6.3–6.9)	6.6 ± 0.03 (6.3–6.9)	6.0 ± 0.21 (5.2–7.5)	6.3 ± 0.28 (5.3–8.8)	5.2–14.3[h]	5.1–14.9
protein	39.7 ± 0.32 (37.0–43.0)	39.8 ± 0.30 (36.4–42.6)	37.8 ± 0.38 (33.4–41.5)	38.3 ± 0.31 (34.4–40.6)	40.2 ± 0.25 (38.0–42.4)	41.2 ± 0.55 (37.7–45.3)	32.9–43.6[i] 36.0–48.4[j]	33.2–45.5
total fat	18.5 ± 0.27 (15.8–21.2)	18.9 ± 0.31 (15.5–22.3)	18.2 ± 0.23 (14.6–20.0)	17.9 ± 0.31 (14.4–20.9)	20.4 ± 0.13 (19.5–21.2)	19.7 ± 0.31 (17.8–22.1)	12.0–24.0[k] 19.8–27.7[i]	8.1–23.6
ash	5.2 ± 0.04 (4.9–5.5)	5.4 ± 0.05 (5.0–5.8)	5.6 ± 0.05 (4.9–6.0)	5.5 ± 0.06 (5.0–6.0)	5.6 ± 0.07 (5.0–6.0)	5.6 ± 0.06 (5.0–6.0)	4.3–5.9[l]	3.9–6.5
carbohydrates	36.6 ± 0.30 (33.7–39.1)	36.0 ± 0.32 (32.6–39.7)	38.4 ± 0.40 (35.3–42.9)	38.3 ± 0.36 (34.8–42.1)	33.8 ± 0.27 (31.5–35.9)	33.5 ± 0.46 (31.0–36.4)	29.3–41.3[l]	29.6–50.2

[a] All data expressed as percent dry weight of sample, except moisture, which is percent fresh weight of sample. [b] Conventional soybean seed samples. [c] The simple mean of 25 values. [d] The standard error (SE) of the mean. [e] The simple mean of 16 values. [f] Range denotes the lowest and highest individual values across samples. [g] ILSI Crop Composition Database, ref 21. [h] Reference 16. [i] Reference 36. [j] Reference 37. [k] Reference 41. [l] Reference 15.

Table 2. Lectin, Trypsin Inhibitor, and Isoflavone Analysis of Seed from GTS and Conventional Soybeans Harvested in 2000, 2001, and 2002

	2000 field season		2001 field season		2002 field season			
component[a] (unit)	GTS mean[b] ± SE[d] (range)[f]	conven.[c] mean[b] ± SE[d] (range)[f]	GTS mean[b] ± SE[d] (range)[f]	conven.[c] mean[b] ± SE[d] (range)[f]	GTS mean[e] ± SE[d] (range)[f]	conven.[c] mean[e] ± SE[d] (range)[f]	literature range[a]	ILSI[g] range[a]
lectin (HU/mg)	1.05 ± 0.06 (0.46–1.62)	1.07 ± 0.09 (0.55–2.34)	2.8 ± 0.24 (1.1–6.5)	3.4 ± 0.41 (1.1–9.0)	0.79 ± 0.12 (0.15–1.69)	1.82 ± 0.38 (0.57–6.13)	0.8–2.4[h,i] 37–426[j,k]	0.11–9.0
total trypsin inhibitor (TIU/mg)	45.9[l] ± 1.8 (24.6–64.6)[l]	44.4[l] ± 1.9 (30.1–65.8)[l]	41.8 ± 2.6 (23.7–72.9)	41.7 ± 1.9 (27.9–75.5)	51.0 ± 3.4 (36.1–90.6)	50.5 ± 2.8 (34.7–75.0)	33.2–54.5[h]	19.6–119
				Isoflavones (μg/g)				
daidzein	397 ± 32 (145–680)	377 ± 40 (25–946)	677 ± 66 (238–1797)	600 ± 69 (218–1530)	542 ± 52 (189–928)	538 ± 64 (175–1236)	98.8–1242[m,n]	60–2453
genistein	544 ± 35 (255–838)	562 ± 48 (28–1033)	875 ± 52 (399–1572)	805 ± 64 (390–1477)	630 ± 36 (414–998)	640 ± 48 (323–919)	130–1501[m,n]	144–2837
glycitein	148 ± 9.4 (75–243)	142[o] ± 8.1[o] (73–229)[o]	156 ± 13 (85–363)	154 ± 11 (90–309)	137 ± 11 (69–204)	130 ± 12 (45–228)	42.2–204[m,n]	15–310

[a] All data expressed as dry weight of sample. [b] The simple mean of 25 values. [c] Conventional soybean seed samples. [d] The standard error (SE) of the mean. [e] The simple mean of 16 values. [f] Range denotes the lowest and highest individual values across samples. [g] ILSI Crop Composition Database, ref 21. [h] Reference 15. [i] Data expressed as fresh weight of sample. [j] Data expressed as hemagglutinating unit per milligam of protein. [k] Reference 42. [l] Data expressed as defatted weight of sample. [m] Data expressed as edible portion. [n] Reference 38. [o] One data point that was below the limit of quantitation of the assay was removed.

soybean seed. The SE for the above assays was very low ranging from 0.03% to 0.55% of the values. The ranges of the values from GTS and conventional soybean seed across all three years are similar to ranges in the published literature data (*15*, *16*, *36*, *37*) and the ILSI Crop Composition Database (*21*). These data show that the proximate values for GTS are within the range of natural variability observed in conventional soybean seed.

Lectin, Trypsin Inhibitor, and Isoflavone Composition in Soybean Seed from 2000, 2001, and 2002. The lectin, trypsin inhibitor, and isoflavone data are presented in **Table 2**. The ranges of values for lectin, trypsin inhibitor, and isoflavones show significant variability across all three years (i.e., large range of values). The mean and range of the GTS seed values, however, are similar to the mean and range of conventional soybean seed values for the levels of trypsin inhibitor and isoflavones (daidzein, genistein, glycitein) within each given year. Across all three years, the lectin values show a significant variability for both GTS and conventional soybean seed comparing year to year. However, the lectin range across all three years is similar between GTS seed (0.15−6.5 hemagglutinating unit (HU)/mg dw), conventional soybean seed (0.55−9.0 HU/mg dw), and the ILSI Crop Composition Database (0.11−9.0 HU/mg dw) (*21*).

The trypsin inhibitor content across all three years ranged from 23.7 to 90.6 trypsin inhibitor unit (TIU)/mg dw for GTS seed and 27.9−75.5 TIU/mg dw for conventional soybean seed with a SE ranging from 1.8 to 3.4. The trypsin inhibitor values for the GTS and conventional soybean seed are also within or similar to the range reported in the literature (33.2-54.5 TIU/mg dw) (*15*) and ILSI Crop Composition Database range (19.6−119 TIU/mg dw) (*15*, *21*).

The daidzein content ranged from 145 to 1797 μg/g dw for GTS seed and 25−1530 μg/g dw for conventional soybean seed with a SE ranging from 32 to 69. The genistein content ranged from 255 to 1572 μg/g dw for GTS seed and 28−1477 μg/g dw for conventional soybean seed with a SE ranging from 35 to 64. The glycitein content ranged from 69 to 363 μg/g dw for GTS seed and 45−309 μg/g dw for conventional soybean seed with a SE ranging from 8.1 to 13. The isoflavone values for the GTS and conventional soybean seed are also within or similar to the range reported in the literature (*38*) and the ILSI Crop Composition Database (*21*).

It is typical for the measured levels of all nutrients and antinutrients in crops to vary depending on the environmental conditions, cultivar grown, and method used (*39*, *40*). Because the GTS and conventional soybean seed were not matched for genetic backgrounds and were not grown at the same field site,

during the same field season, and under the same environmental conditions, the variation observed for the isoflavone values of GTS and conventional soybeans was expected.

The comparative safety assessment process of a food or feed derived from biotechnology crops is based upon the concept that there is a "reasonable certainty of no harm" from its intended use. This process compares the phenotypic characteristics and composition of the biotechnology-derived crop to that of conventional crops with a known history of safe use. Previous studies have shown that the composition of GTS is substantially equivalent to that of conventional soybeans in previous studies. The results of this study further indicate that the composition of commercialized GTS over three years of breeding into multiple genetic backgrounds remains substantially equivalent to that of conventional soybeans. In conclusion, the nutritional and biologically active levels of GTS reported here are similar to the natural variability of nutritional and biologically active levels in soybean seed of conventional varieties and those values reported in the ILSI Crop Composition Database.

ABBREVIATIONS USED

dw, dry weight; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; fw, fresh weight; HU, hemagglutinating unit; ILSI, International Life Science Institute; GTS, glyphosate-tolerant soybeans; SE, standard error; TIU, trypsin inhibitor unit.

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LITERATURE CITED

(1) Fuchs, R. L. Feeds derived from genetically modified crop plants. In *Nutritional Toxicology*; Kotsonis, F. N., Mackey, M. A., Eds.; Taylor and Francis, Inc.: New York, 2002; pp 75−92.

(2) James, C.; Krattiger, A. F. "Global review of the field testing and commercialization of transgenic plants, 1986 to 1995: The first decade of crop biotechnology," International Service of the Acquisition of Agri-biotech Applications, 1996.

(3) Harlander, S. K. The evolution of modern agriculture and its future with biotechnology. *J. Am. Coll. Nutr.* **2002**, *21*, 161S−165S.

(4) Chassy, B. M. Food safety evaluation of crops produced through biotechnology. *J. Am. Coll. Nutr.* **2002**, *21*, 166S−173S.

(5) World Health Organization "Strategies for assessing the safety of foods produced by biotechnology," Joint FAO/WHO Consultations, 1991.

(6) World Health Organization "Application of the principles of substantial equivalence to the safety evaluation of foods and food components from plants derived by modern biotechnology," WHO, 1995.

(7) Food and Agriculture Organization "Biotechnology and food safety," Joint FAO/WHO Consultation, 1996.

(8) Food and Drug Administration Title 21, Part 170, Section 3, Subsection i. In *Code of Federal Regulations*, 2004.

(9) Nair, R. S.; Fuchs, R. L.; Schuette, S. A. Current methods for assessing safety of genetically modified crops as exemplified by data on glyphosate-tolerance soybeans. *Toxicol. Pathol.* **2002**, *30*, 117−125.

(10) König, A.; Cockburn, A.; Crevel, R. W. R.; Debruyne, E.; Grafstroem, R.; Hammerling, U.; Kimber, I.; Knudsen, I.; Kuiper, H. A.; Peijnenburg, A. A. C. M.; Penninks, A. H.; Poulsen, M.; Schauzu, M.; Wal, J. M. Assessment of the safety of food derived from genetically modified (GM) crops. *Food Chem. Toxicol.* **2004**, *42*, 1047−1088.

(11) Steinrucken, J.; Amrhein, N. The herbicide glyphosate is a potent inhibitor of 5-enolypyryvylshikimic acid-2-phosphate synthase. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1207−1212.

(12) Padgette, S. R.; Kolacz, K.; Delannay, X.; Re, D.; LaVallee, B.; Tinius, C.; Rhodes, W.; Otero, Y.; Barry, G.; Eichholtz, D.; Peshke, V.; Nida, D.; Taylor, N. B. Development, identification, and characterization of a glyphosate-tolerant soybean line. *Crop Sci.* **1995**, *35*, 1451−1461.

(13) Harrison, L.; Bailey, M.; Naylor, M.; Ream, J.; Hammond, B. G.; Nida, D.; Burnette, B. The expressed protein in glyphosate-tolerant soybean, 5-enolypyryvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested *in vitro* and is not toxic to acutely gavaged mice. *J. Nutr.* **1996**, *126*, 728−740.

(14) Burks, A.; Fuchs, R. L. Assessment of the endogenous allergens in glyphosate tolerant and commercial soybean varieties. *J. Allergy Clin. Immunol.* **1995**, *96*, 1008−1010.

(15) Padgette, S. R.; Taylor, N. B.; Nida, D. L.; Bailey, M. R.; MacDonald, J.; Holden, L. R.; Fuchs, R. L. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *J. Nutr.* **1996**, *126*, 702−716.

(16) Taylor, N. B.; Fuchs, R. L.; MacDonald, J.; Shariff, A. R.; Padgette, S. R. Compositional analysis of glyphosate-tolerance soybeans treated with glyphosate. *J. Agric. Food Chem.* **1999**, *47*, 4469−4473.

(17) Hammond, B. G.; Vicini, J. L.; Hartnell, G. F.; Naylor, M. W.; Knight, C. D.; Robinson, E.; Fuchs, R. L.; Padgette, S. R. The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by incorporation of glyphosate tolerance. *J. Nutr.* **1996**, *126*, 717−727.

(18) Cromwell, G. L.; Lindemann, M. D.; Randolph, J. H.; Parker, G. R.; Coffey, R. D.; Laurent, K. M.; Armstrong, C. L.; Mikel, W. B.; Stanisiewski, E. P.; Hartnell, G. F. Soybean meal from Roundup Ready or conventional soybeans in diets for growing-finishing swine. *J. Anim. Sci.* **2002**, *80*, 708−715.

(19) Padgette, S. R.; Re, D.; Barry, G.; Eichholtz, D.; Delannay, X.; Fuchs, R. L.; Kishore, G.; Fraley, R. T. *New weed control opportunities: Development of soybeans with a Roundup Ready gene*; CRC Press: Boca Raton, FL, 1996; pp 53−84.

(20) James, C. "Global status of commercialized transgenic crops," International Service for the Acquisition of Agri-biotech Applications, 2003.

(21) Ridley, W. P.; Shillito, R. D.; Coats, I.; Steiner, H.-Y.; Shawgo, M.; Phillips, A.; Dussold, P.; Kurtyka, L. Development of the International Life Sciences Institute crop composition database. *J. Food Compos. Anal.* **2004**, *17*, 423−438.

(22) U.S. EPA Federal Insecticide, Fungicide and Rodenticide Act: Good Laboratory Practice Standards. *Code of Federal Regulations* 40 CFR Part 160, Washinton, DC, 1999.

(23) Association of Official Analytical Chemists Method 926.08 − Moisture in cheese. In *Official Methods of Analysis*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; Vol. II, Chapter 33, p 70.

(24) Association of Official Analytical Chemists Method 925.09 − Solids (total) and moisture in flour. In *Official Methods of Analysis*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; Vol. II, Chapter 32, p 1.

(25) Association of Official Analytical Chemists Method 955.04 − Nitrogen (total) in fertilizers. In *Official Methods of Analysis*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; Vol. I, Chapter 2, pp 14−15.

(26) Association of Official Analytical Chemists Method 979.09 − Protein in grains. In *Official Methods of Analysis*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; Vol. II, Chapter 32, pp 30−34.

(27) Association of Official Analytical Chemists Method 922.06 − Fat in flour. In *Official Methods of Analysis*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; Vol. II, Chapter 32, p 5.

(28) Association of Official Analytical Chemists Method 954.02 − Fat (crude) or ether extract in pet food. In *Official Methods of Analysis*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; Vol. I, Chapter 4, p 33.
(29) Association of Official Analytical Chemists Method 923.03 − Ash of flour. In *Official Methods of* Analysis; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; Vol. II, Chapter 32, p 2.
(30) United States Department of Agriculture Energy value of foods. *Agriculture Handbook 74*; USDA: Washington, DC, 1973; pp 2−11.
(31) Liener, I. E. The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. *Arch. Biochem. Biophys.* **1955**, *54*, 223−231.
(32) Klurfeld, D. M.; Kritchevsky, D. Isolation and quantitation of lectins from vegetable oils. *Lipids* **1987**, *22*, 667−668.
(33) American Oil Chemists' Society Method Ba 12−75 − Trypsin inhibitor activity. *Official Methods and Recommended Practices of the American Oil Chemists' Society*; AOCS: Champaign, IL, 1997.
(34) Pettersson, H.; Kiessling, K.-H. Liquid chromatographic determination of the plant estrogen coumestrol and isoflavones in animal feed. *J. AOAC Int.* **1984**, *67*, 503−506.
(35) Seo, A.; Morr, C. V. Improved high-performance liquid chromatographic analysis of phenolic acids and isoflavonoids from soybean protein products. *J. Agric. Food Chem.* **1984**, *32*, 530−533.
(36) Maestri, D. M.; Labuckas, D. O.; Meriles, J. M.; Lamarque, A. L.; Zygadlo, J. A.; Guzman, C. A. Seed composition of soybean cultivars evaluated in different environmental regions. *J. Sci. Food Agric.* **1998**, *77*, 494−498.
(37) Hartwig, E. E.; Kilen, T. C. Yield and composition of soybean seed from parents with different protein, similar yield. *Crop Sci.* **1991**, *31*, 290−292.
(38) USDA-ISU Isoflavones Database. United States Department of Agriculture, Iowa State University, 2002.
(39) Setchell, K. D. R.; Cole, S. J. Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling. *J. Agric. Food Chem.* **2003**, *51*, 4146−4155.
(40) De Mejía, E. G.; Guzmán-Maldonado, S. H.; Acosta-Gallegos, J. A.; Reynoso-Chamacho, R.; Ramírez-Rodríguez, E.; Pons-Hernández, J. L.; González-Chavira, M. M.; Castellanos, J. Z.; Kelly, J. D. Effect of cultivar and growing location on the trypsin inhibitors, tannins, and lectins of common beans (*Phaseolus vulgaris* L.) grown in the Semiarid Highlands of Mexico. *J. Agric. Food Chem.* **2003**, *51*, 5962−5966.
(41) Orthoefer, F. T. Processing and utilization. In *Soybean Physiology, Agronomy, and Utilization*; Norman, A. G., Ed.; Academic Press: New York, 1978; pp 219−246.
(42) Kakade, M. L.; Simons, N. R.; Liener, I. E.; Lambert, J. W. Biochemical and nutritional assessment of different varieties of soybeans. *J. Agric. Food Chem.* **1972**, *20*, 87−90.

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