JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 4611–4622 **4611**

Composition of Grain, Forage, and Processed Fractions from Second-Generation Glyphosate-Tolerant Soybean, MON 89788, Is Equivalent to That of Conventional Soybean (*Glycine max* L.)

DENISE R. LUNDRY,*,† WILLIAM P. RIDLEY,† JIAYING J. MEYER,† SUSAN G. RIORDAN,† MARGARET A. NEMETH,† WILLIAM A. TRUJILLO,‡ MATTHEW L. BREEZE,‡ AND ROY SORBET§

Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, Covance Laboratories, Inc., 3301 Kinsman Boulevard, Madison, Wisconsin 53704, and Certus International, Inc., 1422 Elbridge Payne Road, Suite 200, Chesterfield, Missouri 63017

Developments in biotechnology and molecular-assisted breeding have led to the development of a second-generation glyphosate-tolerant soybean product, MON 89788. The MON 89788 event was produced by direct transformation of a *cp4 epsps* (5-enolpyruvylshikimate-3-phosphate synthase) gene cassette derived from *Agrobacterium* sp. strain CP4 into an elite soybean germplasm known for its superior agronomic characteristics and high yielding property. The purpose of this work was to assess whether the nutrient and antinutrient levels in seed and forage tissues of MON 89788 are comparable to those in the conventional soybean variety, A3244, which has background genetics similar to MON 89788 but does not contain the *cp4 epsps* gene cassette. Additional conventional soybean varieties currently in the marketplace were also included in the analysis to establish a range of natural variability for each analyte, where the range of variability is defined by a 99% tolerance interval for that particular analyte. Compositional analyses were conducted on forage, seed and four processed fractions from soybeans grown in ten sites across both the United States and Argentina during the 2004−2005 growing seasons. Forage samples were analyzed for levels of proximates (ash, fat, moisture, and protein) and fiber. Seed samples were analyzed for proximates, fiber, antinutrients, and vitamin E. Defatted, toasted (DT) meal was analyzed for proximates, fiber, amino acids, and antinutrients. Refined, bleached, and deodorized (RBD) oil was analyzed for fatty acids and vitamin E. Protein isolate was analyzed for amino acids and moisture. Crude Lecithin was analyzed for phosphatides. Results of the comparisons indicate that MON 89788 is compositionally and nutritionally equivalent to conventional soybean varieties currently in commerce.

KEYWORDS: Soybean (*Glycine max*); glyphosate-tolerant; biotechnology; composition

INTRODUCTION

Soybean is the main source of plant protein consumed by humans and animals and is the leading source of vegetable oil of all crops produced in the world. The three major soybean commodity products are seeds, oil, and meal, and their broad production and composition makes them an inexpensive source of oil and protein for use as foods and animal feed (*1*). Roundup Ready soybean event 40-3-2 (Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC. Roundup Ready soybean event 40-3-2 is subsequently referred to as Roundup Ready soybean), containing the glyphosate-tolerant trait produced through biotechnology, was commercially introduced to the U.S. in 1996. By the 2006 growing season, 89% of the soybeans or approximately 67 million acres of the soybeans grown in the U.S. were Roundup Ready soybean varieties (*2*). Globally, Roundup Ready soybeans occupied 144 million acres, representing 57% of the total biotechnology-enhanced crop area planted in 2006 (*3*). The total soybean crop value in 2006 for the U.S. alone exceeded $19.7 billion, $17.5 billion (89%) of which is attributed to Roundup Ready soybean (*4*). The rapid adoption of Roundup Ready soybean by growers around the world is attributed to the simplicity of use, cost

* To whom correspondence should be addressed. Telephone: 314-694-1268. Fax: 314-694-8575. E-mail: denise.r.lundry@monsanto.com.
† Monsanto Company.
‡ Covance Laboratories, Inc.
§ Certus International, Inc.

10.1021/jf073087h CCC: $40.75
Published on Web 05/23/2008

efficiencies for weed control, and compatibility with conservation tillage. Because Roundup agricultural herbicides are highly effective against the majority of annual and perennial grasses and broadleaf weeds, growers planting Roundup Ready soybeans are able to reduce the number of herbicides used to control weeds that grow in their field and thereby realize a savings in weed control costs (*5*).

Developments in biotechnology and molecular-assisted breeding have led to the development of a second-generation glyphosate-tolerant soybean product, MON 89788. MON 89788 contains the gene for 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) derived from *Agrobacterium* sp. strain CP4 (*6*). Expression of the gene product CP4 EPSPS renders the plant tolerant to glyphosate, the active ingredient in the Roundup family of agricultural herbicides. The CP4 EPSPS protein produced in MON 89788 is identical to the CP4 EPSPS protein present in the first-generation Roundup Ready soybean, Roundup Ready cotton, and Roundup Ready Flex cotton. The CP4 EPSPS protein is structurally similar and functionally identical to endogenous plant EPSPS enzymes but has a much reduced affinity for glyphosate relative to endogenous plant EPSPS (*7*). In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of shikimate-3-phosphate, thereby depriving plants of aromatic amino acids (*8, 9*). The introduction of the *cp4 epsps* gene into soybeans allows for the production of aromatic amino acids and other metabolites that are essential for plant growth and development in the presence of glyphosate (*10*). MON 89788 was developed by the introduction of the *cp4 epsps* gene cassette into Asgrow soybean variety A3244 (*11*), which is known for its superior agronomic characteristics, including shattering and lodging resistance, heat, drought, cyst nematode, and disease tolerance, emergence, late-season plant intactness, plant height, and high yielding property (*12*). Using elite germplasm as the base genetics, the superior agronomic characteristic of A3244 can be introgressed to other soybean varieties through crosses with MON 89788 containing the *cp4 epsps* cassette. Over 4 years of testing, MON 89788 has been found to have a yield advantage compared to Roundup Ready soybeans in the same elite genetic background (A3244) while maintaining the weed-control and crop-safety benefits of the Roundup Ready soybean system. As a result, MON 89788 is expected to be an excellent agronomic base trait for future breeding improvements and multitrait products.

The nutritional wholesomeness of Roundup Ready soybean and its substantial equivalence to conventional varieties have been well-established (*6, 7, 10, 13–19*) through numerous safety assessment studies. One of the key components of the safety assessment of biotechnology-derived crops is the compositional analysis of the food and feed commodities from that crop. The nutritional evaluation is based on a direct comparison between the biotechnology-derived crop and the relevant conventional counterparts and is designed to identify potential important differences in the levels of key nutrient and antinutrient components (*20*). The statistical significance of any observed difference between MON 89788 and the parental control should be assessed in the context of the range of natural variation for that parameter to determine its biological significance (*21*). This paper describes studies conducted as part of an overall safety assessment of the second-generation glyphosate-tolerant soybean, MON 89788. Compositional evaluations were performed using the guidelines outlined in the Organization for Economic Cooperation and Development (OECD) consensus document for soybean composition (*1*). These guidelines are accepted globally and are consistent with country-specific regulations in the U.S., Canada, European Union (EU), and other countries. The compositional analyses were conducted on seed and forage collected at five locations in each of two distinct geographic regions, U.S. and Argentina, over two growing seasons and processed fractions produced from seed collected at two locations in the U.S. during 2005.

MATERIALS AND METHODS

Soybean Samples for Compositional Analyses. The second-generation glyphosate-tolerant soybean product, MON 89788, was produced by the stable insertion of the *cp4 epsps* coding sequence into the genome of the conventional soybean variety A3244. The control material used for this composition study was the nontransformed parental variety A3244. A wide range of commercially available conventional soybean varieties were grown in the same trials as MON 89788 and A3244 to provide reference materials representative of typical varieties of conventional soybean. These materials were used to establish a range of natural variability in compositional constituents, which is defined by a 99% tolerance interval for each nutrient and antinutrient component analyzed. MON 89788 composition analyses were conducted using materials generated from diverse environmental conditions in the U.S. and Argentina through 2004−2005 seasons.

The field sites where materials for composition analyses were generated consisted of 10 locations across the U.S. and Argentina. The plots in each location were arranged in three randomized complete blocks, and each plot contained MON 89788, A3244, and two or three conventional reference varieties. In the 2004−2005 Argentina trial, three sites were located in the province of Buenos Aires and one site was located in each of the provinces of Córdoba and Santa Fe. In the 2005 U.S. field trial, Arkansas, Nebraska, and Ohio each had one field site and two sites were located in Illinois. The geographic regions selected for field trials are representative of major commercial growing areas in both the northern and southern hemispheres. The field trial from which the soybean seed was harvested to generate the processed fractions was planted without replication at two U.S. sites, one in Illinois and one in Arkansas. Normal agronomic practices were followed for each growing region and for each trial, including the application of registered nonglyphosate-containing maintenance pesticides as required for optimal growth. In addition, plots containing MON 89788 were treated with Roundup herbicide at labeled use rates.

Forage material was collected at approximately the R6 growth stage (full seed) from at least six plants from each plot by cutting at the base (no roots) and compositing the six individual plants into one sample per plot. A3244 samples were collected first, followed by commercial samples and finally MON 89788 samples. The forage samples were transferred to dry ice within 30 min after sampling. Seed was harvested at the R8 growth stage (95% of the pods had reached mature pod color) when the moisture content was approximately 12−15% and was stored at ambient temperature. Forage samples were shipped frozen on dry ice, and seeds were shipped at ambient temperature. At the Monsanto Company, forage and seed samples were homogenized by grinding with dry ice to a fine powder and stored frozen at approximately −20 °C until compositional analysis.

The genetic identity of the forage and processed fraction samples was confirmed by chain of custody and sample-handling records. The genetic identity of the seed was confirmed by sample-handling records and event-specific polymerase chain reaction (PCR) analysis.

Compositional Analysis Methods. Forage samples were analyzed for proximates (ash, fat, moisture, and protein), acid detergent fiber (ADF), and neutral detergent fiber (NDF). Seed samples were analyzed for proximates (ash, fat, moisture, and protein), ADF, NDF, amino acids, fatty acids, isoflavones, lectins, phytic acid, raffinose (U.S. trials only), stachyose (U.S. trials only), trypsin inhibitors, and vitamin E (U.S. trials only). Total carbohydrates in forage and seed were determined by calculation. Defatted, toasted (DT) meal was analyzed for proximates (ash, fat, moisture, and protein), ADF, NDF, amino acids, phytic acid, and trypsin inhibitors. Refined, bleached, and deodorized (RBD) oil was analyzed for fatty acids and vitamin E. Protein isolate was analyzed for amino acids and moisture. Crude lecithin was analyzed for phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, and phosphatidic acid. Compositional analyses were conducted at Covance Laboratories, Inc., in Madison, WI. Samples from each site were analyzed by tissue in a randomized order to minimize assay bias.

Proximate Analysis. Protein levels were determined by the Kjeldahl method (*22–25*). Protein and other nitrogenous compounds in the samples were reduced to ammonia by digestion of the samples with sulfuric acid containing a mercury catalyst mixture. The acid digest was made alkaline, and the ammonia was distilled and titrated with a standard acid. The percent nitrogen was determined and converted to percent protein by multiplication with 6.25. The fat content of the grain was determined using the Soxhlet extraction method (*26*). Seed samples were weighed into cellulose thimbles containing sand or sodium sulfate and dried to remove excess moisture. Pentane was dripped through the samples to remove the fat. Extracts were then evaporated, dried, and weighed. The fat content of the forage was determined by the acid hydrolysis method. The forage was hydrolyzed with hydrochloric acid, and the fat was extracted with ether and hexane (*27, 28*). Ash content was determined by combustion at 550 °C and gravimetric quantitation of the nonvolatile matter remaining (*29*). The moisture content was determined by the loss of weight after drying to a constant weight in a 100 °C vacuum oven (*30, 31*). The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation (*32*):

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

Fiber Analysis. ADF was determined by washing the tissue with an acidic boiling detergent solution to dissolve the protein, carbohydrate, and ash (*33*). An acetone wash was used to remove the fats and pigments. The remaining lignocellulose fraction was determined gravimetrically. NDF was determined by treating the tissue with a neutral boiling detergent solution to dissolve the protein, carbohydrate, enzyme, and ash. Fats and pigments were removed using an acetone wash. The remaining hemicellulose, cellulose, and lignin fractions were measured gravimetrically (*33, 34*).

Amino Acid Composition. The samples were assayed by three methods to obtain the full profile of amino acids (*35*). Tryptophan required a base hydrolysis using sodium hydroxide. Sulfur-containing amino acids required an oxidation using performic acid prior to hydrolysis with hydrochloric acid. Analysis of the remaining amino acids was accomplished through direct hydrolysis with hydrochloric acid. The individual amino acids were quantified using an automated amino acid analyzer.

Fatty Acid Profile. Lipid in seed samples was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% (weight/volume) boron trifluoride/methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation (*36*).

Isoflavone Analysis. Conjugated isoflavones were hydrolyzed in a solution of hydrochloric acid and reagent alcohol into their respective aglycones. Purification of the isoflavones was accomplished on a C-18 solid-phase extraction column (*37*). The levels of the resulting daidzein, genistein, and glycitein aglycones were measured by high-performance liquid chromatography (HPLC) (*38, 39*).

Lectin Analysis. The method used was based on the ability of lectin to bind to specific sugars on the surface of red blood cells (RBCs) of different animals, resulting in RBC agglutination (*40, 41*). The samples were suspended in phosphate-buffered saline (PBS), shaken, and filtered. A 10% hematocrit of lyophilized rabbit blood in PBS was added to each dilution of the filtered extract. The absorbance of each dilution of the sample was measured by a spectrophotometer at 620 nm. One hemagglutinating unit (HU) was defined as the level that caused 50% of the standard cell suspension to sediment in 2.5 h.

Phytic Acid. Phytic acid was extracted using 0.5 M HCl with ultrasonication. Purification and concentration was performed using a silica-based anion-exchange (SAX) column. Sample analysis was conducted using a macroporous polymer HPLC column [PRP-1, 5 μm (150 × 4.1 mm)] connected to a refractive index detector (*42, 43*).

Phosphatide Analysis. The samples were extracted with 98% methylene chloride and 2% methanol. The resulting extracts were analyzed on a HPLC system equipped with an evaporative light-scattering detector (ELSD) (*44*).

Raffinose and Stachyonse Analysis. The method used is based on GC/FID analysis of the silyl derivatives of stachyose and raffinose (*45, 46*). Sugars were extracted from the matrix in water. Aliquots of the extracts were dried and resuspended in hydroxylamine hydrochloride solution in pyridine containing phenyl-β-D-glucoside internal standard. The resulting oximes were converted to silyl derivatives and analyzed quantitavely by gas chromatography with flame ionization detection.

Trypsin Inhibitor Analysis. The ground and defatted samples were suspended in 0.1 N sodium hydroxide solution for 3 h at room temperature. An appropriate dilution of the suspension was made, and an increasing series of aliquots of the diluted suspension was mixed with trypsin and benzoyl-D,L-arginine-*p*-nitroanalide hydrochloride (BAPA). The timed reaction was stopped by the addition of acetic acid, and the filtered reaction mixture was measured at 410 nm. One trypsin unit is arbitrarily defined as an increase of 0.01 absorbance units at 410 nm per 10 mL of the reaction mixture under the conditions used in this method (*47*).

Vitamin E Analysis. The samples were saponified to break down any fat and release any vitamin E. The saponified mixtures were extracted with ethyl ether and then quantitated directly by HPLC on a silica column (*37, 48, 49*).

Statistical Analysis of Compositional Data. To conduct a statistical analysis for a compositional component, at least 50% of the values for the analyte had to be greater than the assay limit of quantitation (LOQ). The following 14 analytes that had >50% of observations below the LOQ for their respective assays were excluded from statistical analysis of results on samples from both field trials and processed fractions: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. For individual measurements below the LOQ, a value equal to half the quantitation limit was assigned prior to statistical analyses. A total of 5 of 24 trypsin inhibitor values in DT meal and 6 of 24 (16:1) palmitoleic values in RBD oil were assigned a value. It may be argued that (16:1) palmitoleic acid in the RBD oil fraction should have been excluded from statistical analyses because all six of the values observed to be less than the LOQ that was from MON 89788 samples. However, more than 50% of the total values were above the LOQ, and the data as a whole fit the acceptance criteria. A studentized PRESS residuals test was applied to the data to identify outliers. A PRESS residual is the difference between any value and its predicted value from a statistical model that excludes the data point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ±3 PRESS residuals. Extreme data points that are also outside of the ±6 studentized PRESS residual range are considered for exclusion, as outliers, from the final statistical analysis. One lectin result for a reference substance from Buenos Aires in the Argentina field trial was identified as an outlier with a PRESS residual value > ±6 and was excluded from statistical analyses.

Statistical analysis of the data from samples collected from each field trial was conducted independently (data not shown). There were a total of 46 components (7 in forage and 39 in seed) for the Argentina trial and 49 components (7 in forage and 42 in seed) for the U.S. trial. Statistical analyses were run using a mixed model analysis of variance for compositional data from each of the five individual sites (individual site data not presented) and from the combination of all sites (combined site) for a given field season in forage and grain. The combined site analysis used the model

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$$

where Y_{ijk} is the unique individual observation, U is the overall mean, T_i is the material effect, L_j is the random location effect, $B(L)_{jk}$ is the random block within the location effect, LT_{ij} is the random location by

Table 1. Fiber and Proximate Composition of Forage from Glyphosate-Tolerant Soybean MON 89788

	2004–2005 Argentina field trials			2005 U.S. field trials			
component[a]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][f]	MON 89788 mean[b] (range)[b]	control mean[d] (range)[d]	commercial (range)[e] [99% TI][f]	literature range
			Fiber				
ADF	30.80 (26.40–37.68)	30.94 (27.17–34.19)	(23.86–36.40) [19.36, 37.79]	36.82 (30.95–45.99)	38.23 (31.18–50.89)	(29.64–50.69) [19.03, 54.55]	32–38[g]
NDF	32.27 (27.67–38.00)	31.77 (25.91–36.10)	(19.61–36.43) [17.49, 42.31]	36.37 (32.77–41.12)	38.25 (32.69–43.14)	(31.43–43.70) [26.89, 46.89]	34–40[h]
			Proximate				
ash	7.81 (6.77–8.56)	8.04 (6.69–9.24)	(6.64–8.91) [5.74, 9.95]	6.76 (5.20–8.45)	6.65 (5.28–7.95)	(5.36–8.36) [3.50, 9.58)	6.718–10.782[i]
carbohydrates	66.32 (64.59–68.29)	66.08 (64.37–67.95)	(62.25–69.28) [59.26, 70.92)]	67.28 (61.61–71.00)	67.40 (64.55–72.30)	(62.57–72.28) [55.96, 77.90]	59.8–74.7[i]
moisture	73.51 (69.70–77.30)	73.81 (70.40–77.40)	(69.80–76.60) [64.50, 81.35]	72.07[j] (67.90–77.60)	73.21 (69.9–77.60)	(68.50–78.40) [60.84, 83.36]	73.5–81.6[i]
protein	21.20 (19.78–23.83)	21.48 (19.24–23.50)	(19.92–24.29) [18.38, 24.98]	20.08 (18.41–23.50)	19.79 (17.47–22.18)	(16.48–22.78) [13.55, 25.95]	14.38–24.71[i]
total fat	4.66 (2.97–6.89)	4.40 (3.35–6.76)	(2.65–7.86) [0, 11.94]	5.87 (4.20–9.49)	6.11 (3.96–8.60)	(3.51–9.87) (0, 14.70)	1.302–5.132[i]

[a] Percent dry weight (moisture = % fresh weight). [b] The mean and range of 15 values (three replicates from each of five field sites). [c] The range of sample values for commercial varieties grown at the same Argentina field sites in 2004–2005. [d] The mean and range of 14 values (three replicates from each of four field sites and two replicates from one field site). [e] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [f] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [g] From ref *1*. [h] From ref *52*. [i] From ref *53*. [j] Statistically significant difference from the control.

Table 2. Fiber and Proximate Composition of Seed from Glyphosate-Tolerant Soybean MON 89788

	2004–2005 Argentina field trials			2005 U.S. field trials			
component [a]	MON 89788 mean[b] (range) [b]	control mean[b] (range) [b]	commercial (range) [c] [99% TI] [f]	MON 89788 mean[b] (range) [c]	control mean[d] (range) [d]	commercial (range) [e] [99% TI] [e]	literature range[g]
			Fiber				
ADF	13.09 (9.64–17.66)	13.73 (10.71–17.97)	(9.22–18.66) [5.61, 19.33]	18.01 (14.64–23.94)	17.46 (14.39–22.44)	(13.30–26.26) [9.62, 28.57]	7.81–18.61
NDF	15.55 (11.28–19.54)	15.84 (12.18–20.56)	(10.79–18.28) [8.49, 20.88]	18.18 (16.38–20.49)	19.11 (15.60–20.73)	(14.41–23.90) [13.26, 26.33]	8.53–21.25
			Proximate				
ash	5.60 (5.05–6.29)	5.58 (5.03–6.25)	(5.01–6.32) [3.87, 7.17]	5.04 (4.66–5.60)	5.03 (4.75–5.46)	(4.61–5.57) [4.00, 6.08]	3.885–6.542
carbohydrates	37.86 (35.81–40.94)	37.94 (35.32–40.94)	(33.84–40.09) [31.36, 43.15]	37.07 (35.01–40.24)	36.88 (35.17–40.74)	(32.75–40.98) [27.86, 45.79)]	29.6–50.2
moisture	11.03 (9.57–12.00)	10.60 (9.41–12.10)	(7.98–11.10) [6.73, 12.59]	7.76 (6.41–9.35)	7.51 (6.51–9.63)	(6.24–9.11) [4.64, 9.94]	5.1–14.9
protein	39.61 (35.72–42.12)	39.61 (36.72–42.32)	(34.78–43.08) [31.13, 47.06]	40.32 (37.31–42.54)	40.38 (36.96–42.44)	(36.48–43.35) [31.50, 47.45]	33.19–45.48
total fat	16.92 (16.22–17.84)	16.86 (15.93–17.71)	(14.62–20.67) [12.16, 24.09]	17.57 (15.35–19.98)	17.72 (14.40–20.91)	(15.97–20.68) [15.38, 21.95]	8.104–23.562

[a] Percent dry weight (moisture = fresh weight). [b] The mean and range of 15 values (three replicates from each of five field sites). [c] The range of sample values for commercial varieties grown at the same Argentina field sites in 2004–2005. [d] The mean and range of 14 values (three replicates from each of four field sites and two replicates from one field site). [e] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [f] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [g] From ref *54*.

the material interaction effect, and e_{ijk} is the residual error. Each individual analyte for MON 89788 was compared to that of the conventional control, A3244, for each of the five sites (data not shown) and for the combined site. The statistical significance is defined at the level of $p < 0.05$.

The overall data set was examined for evidence of biologically relevant changes. On the basis of this evaluation and the results of statistical analyses, analytes for which the levels were not statistically different were deemed to be present at equivalent levels between MON 89788 and A3244.

The data for processed fractions were summarized, determining the simple mean and range values (minimum and maximum) for MON 89788, the parental control, and reference samples. Statistical comparisons were not determined on processed fraction data because these samples were not replicated. There were a total of 26 components in DT meal, 10 components in RBD oil, 19 components in protein isolate, and 4 components in lecithin analyzed from the processed fractions derived from seed produced in the U.S. bulk-up trial.

The conventional reference varieties from each field trial were not analyzed statistically to MON 89788 or its parental control but rather were used to develop a population tolerance intervals for each component analyzed that were expected to contain, with 95% confidence, 99% of the values expressed in the population of commerical soybean varieties. It is important to establish the 99% tolerance interval from representative conventional soybean varieties for each of the analytes, because such data illustrate the compositional variability naturally occurring in commercially grown varieties. By comparison to the 99% tolerance interval, any statistically significant differences between MON 89788 and the control (A3244) may be put into perspective and can be assessed for biological relevance in the context

Table 3. Amino Acid Composition of Seed from Glyphosate-Tolerant Soybean MON 89788

	2004−2005 Argentina field trials			2005 U.S. field trials			
component [a]	MON 89788 mean[b] (range) [b]	control mean[b] (range) [c]	commercial (range) [c] [99% TI] [f]	MON 89788 mean[b] (range) [b]	control mean[d] (range) [d]	commercial (range) [e] [99% TI] [f]	literature range[g]
alanine	1.73 (1.64−1.82)	1.73 (1.65−1.83)	(1.62−1.85) [1.49, 1.99]	1.77 (1.56−1.87)	1.77 (1.71−1.83)	(1.62−1.89) [1.51, 2.00]	1.513−1.851
arginine	2.98 (2.73−3.31)	2.97 (2.73−3.27)	(2.57−3.26) [2.08, 3.66]	3.06 (2.73−3.31)	3.07 (2.76−3.34)	(2.61−3.27) [2.27, 3.60]	2.285−3.358
aspartic acid	4.62 (4.30−5.03)	4.62 (4.33−5.00)	(4.16−5.01) [3.62, 5.47]	4.73 (4.20−5.08)	4.72 (4.42−4.98)	(4.21−5.02) [3.85, 5.44]	3.808−5.122
cystine/cysteine	0.60 (0.55−0.66)	0.60 (0.56−0.66)	(0.52−0.69) [0.44, 0.75]	0.62 (0.58−0.67)	0.62 (0.59−0.65)	(0.57−0.65) [0.55, 0.67]	0.370−0.808
glutamic acid	7.31 (6.76−7.94)	7.28 (6.79−7.86)	(6.52−7.99) [5.57, 8.83]	7.53 (6.69−8.20)	7.49 (6.97−7.90)	(6.62−8.19) [5.86, 8.96]	5.843−8.093
glycine	1.74 (1.65−1.85)	1.73 (1.65−1.84)	(1.59−1.87) [1.41, 2.04]	1.78 (1.58−1.88)	1.78 (1.71−1.86)	(1.62−1.90) [1.46, 2.05]	1.458−1.865
histidine	1.06 (0.98−1.14)	1.05 (1.00−1.13)	(0.96−1.12) [0.87, 1.22]	1.07 (0.95−1.13)	1.07 (1.02−1.13)	(0.96−1.13) [0.90, 1.21]	0.878−1.175
isoleucine	1.81 (1.68−1.94)	1.80 (1.71−1.92)	(1.59−1.95) [1.40, 2.14]	1.83 (1.65−1.97)	1.83 (1.70−1.99)	(1.64−2.00) [1.44, 2.16]	1.563−2.043
leucine	3.10 (2.89−3.31)	3.09 (2.92−3.30)	(2.79−3.31) [2.52, 3.59]	3.18 (2.81−3.39)	3.18 (3.04−3.33)	(2.89−3.42) [2.62, 3.66]	2.590−3.387
lysine	2.58 (2.44−2.74)	2.58 (2.47−2.74)	(2.36−2.76) [2.15, 2.96]	2.62 (2.33−2.76)	2.62 (2.51−2.73)	(2.40−2.77) [2.22, 2.95]	2.285−2.839
methionine	0.58 (0.52−0.62)	0.58 (0.54−0.61)	(0.52−0.63) [0.46, 0.70]	0.52 (0.47−0.56)	0.53 (0.50−0.55)	(0.45−0.56) [0.42, 0.60]	0.443−0.668
phenylalanine	2.06 (1.87−2.21)	2.04 (1.88−2.22)	(1.82−2.21) [1.59, 2.47]	2.10 (1.84−2.24)	2.10 (2.00−2.19)	(1.90−2.29) [1.70, 2.45]	1.632−2.236
proline	2.04 (1.89−2.20)	2.03 (1.88−2.18)	(1.83−2.19) [1.61, 2.40]	2.05 (1.81−2.21)	2.05 (1.95−2.16)	(1.86−2.23) [1.66, 2.38]	1.687−2.284
serine	2.12 (2.01−2.28)	2.12 (1.96−2.27)	(1.95−2.27) [1.72, 2.51]	2.23 (1.93−2.42)	2.21 (2.08−2.28)	(1.99−2.42) [1.84, 2.54]	1.632−2.484
threonine	1.60 (1.50−1.69)	1.60 (1.51−1.70)	(1.49−1.71) [1.35, 1.86]	1.58 (1.42−1.68)	1.59 (1.51−1.66)	(1.44−1.67) [1.38, 1.76]	1.251[g]−1.73[h]
tryptophan	0.44 (0.41−0.48)	0.44 (0.41−0.47)	(0.39−0.48) [0.37, 0.50]	0.39 (0.34−0.44)	0.39 (0.33−0.46)	(0.30−0.47) [0.25, 0.54]	0.3563−0.5016
tyrosine	1.42 (1.34, 1.51)	1.43 (1.34, 1.52)	(1.27−1.53) [1.15, 1.69]	1.41 (1.25−1.48)	1.42 (1.33−1.47)	(1.28−1.51) [1.18, 1.64]	1.016−1.559
valine	1.92 (1.79−2.07)	1.90 (1.80−2.03)	(1.68−2.06) [1.48, 2.26]	1.91 (1.73−2.05)	1.93 (1.77−2.11)	(1.71−2.09) [1.51, 2.27]	1.627−2.204

[a] Percent dry weight. [b] The mean and range of 15 values (three replicates from each of five field sites). [c] The range of sample values for commercial varieties grown at the same Argentina field sites in 2004−2005. [d] The mean and range of 14 values (three replicates from each of four field sites and two replicates from one field site). [e] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [f] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [g] From ref *54*. [h] From ref *13*.

of the natural variability in soybean. Each tolerance interval estimate was based on one observation per unique reference material. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero. SAS software was used to generate all summary statistics and perform all analyses (*50*).

RESULTS AND DISCUSSION

The compositional analysis data and the statistical assessment of these data from two geographically diverse field seasons of soybean trials are presented in **Tables 1–5**. For each component measured from materials harvested from each field trial, least-squares means and the range of observed values are presented for MON 89788 and A3244. As a comparison, the range of observed values and calculated 99% tolerance intervals are presented for the reference soybean varieties. In addition, the range of values contained in the literature is presented for a comparison with test and control ranges.

The compositional analysis data and statistical summaries for the processed fractions are presented in **Tables 6–10**. The range of observed values and calculated 99% tolerance intervals of the fractions derived from conventional references and the values obtained from literature are presented for a comparison. Additional statistical analyses, including analysis of variance and calculation of statistical differences, were not conducted on processed fraction data because of insufficient sample size.

The reproducibility and trends across geographic regions were also examined, and comparisons to conventional soybean varieties using the 99% tolerance intervals were made. There were no analytes that were consistently and statistically different across regions or seasons. Analyses using data from the Argentina combined site indicated that there were no statistical differences in the levels of 100% (46 of 46) of the analytes. Analyses using data from the U.S. combined site indicated that there were no statistical differences in the levels of 92% of the analytes (45 of 49). The four analytes observed to be statistically different between MON 89788 and A3244 included forage moisture and grain daidzein, glycitein, and vitamin E.

Proximate and Fiber Composition in Forage. As presented in **Table 1**, results indicate that, with the exception of moisture in the U.S. trial, there were no statistically significant differences between forage produced by MON 89788 and the control variety for either year. The statistical difference observed for forage moisture from the U.S. trial was not observed across sites, and the mean value was within the 99% tolerance interval and considered to be within the population of commercial soybean.

Table 4. Fatty Acid Composition of Seed from Glyphosate-Tolerant Soybean MON 89788

	2004–2005 Argentina field trials			2005 U.S. field trials			
component[a]	MON 89788 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][f]	MON 89788 mean[b] (range)[b]	control mean[d] (range)[d]	commercial (range)[e] [99% TI][f]	literature range[g]
16:0 palmitic	1.96 (1.86–2.02)	1.95 (1.86–2.05)	(1.50–2.09) [1.14, 2.52]	2.07 (1.84–2.40)	2.07 (1.71–2.46)	(1.66–2.35) [1.32, 2.64]	1.44–2.31
18:0 stearic	0.78 (0.70–0.85)	0.78 (0.72–0.86)	(0.64–1.12) [0.33, 1.33]	0.78 (0.65–0.89)	0.77 (0.61–0.86)	(0.63–1.07) [0.37, 1.28]	0.54–0.91
18:1 oleic	3.30 (2.98–3.56)	3.30 (2.97–3.57)	(2.87–4.75) [2.00, 5.65]	3.53 (3.05–4.24)	3.54 (2.92–4.09)	(2.99–5.29) [2.06, 6.43]	3.15–8.82
18:2 linoleic	8.82 (8.30–9.46)	8.79 (8.27–9.48)	(7.60–11.17) [5.61, 13.37]	9.17 (8.00–10.42)	9.25 (7.42–11.29)	(8.41–10.69) [7.75, 11.22]	6.48–11.6
18:3 linolenic	1.52 (1.36–1.66)	1.53 (1.37–1.62)	(1.28–1.79) [1.06, 2.08]	1.29 (1.09–1.48)	1.30 (1.09–1.60)	(1.02–1.55) [0.84, 1.69]	0.72–2.16
20:0 arachidic	0.059 (0.052–0.063)	0.058 (0.054–0.064)	(0.046–0.082) [0.026, 0.095]	0.061 (0.049–0.071)	0.060 (0.046–0.068)	(0.046–0.076) [0.031, 0.094]	0.04–0.7
20:1 eicosenoic	0.031 (0.030–0.033)	0.031 (0.029–0.034)	(0.026–0.044) [0.019, 0.049]	0.042 (0.032–0.050)	0.042 (0.029–0.053)	(0.030–0.057) [0.021, 0.065]	not available
22:0 behenic	0.056 (0.051–0.059)	0.056 (0.051–0.060)	(0.044–0.070) [0.033, 0.087]	0.063 (0.050–0.072)	0.062 (0.046–0.071)	(0.046–0.073) [0.034, 0.091]	not available

[a] Percent dry weight. [b] The mean and range of 15 values (three replicates from each of five field sites). [c] The range of sample values for commercial varieties grown at the same Argentina field sites in 2004–2005. [d] The mean and range of 14 values (three replicates from each of four field sites and two replicates from one field site). [e] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [f] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [g] From ref *1*.

Table 5. Isoflavone, Antinutrient, and Vitamin E Composition of Seed from Glyphosate-Tolerant Soybean MON 89788

	2004–2005 Argentina field trials			2005 U.S. field trials			
component [a]	MON 89788 mean[b] (range) [b]	control mean[b] (range) [b]	commercial (range) [c] [99% TI] [f]	MON 89788 mean[b] (range) [b]	control mean[d] (range) [d]	commercial (range) [e] [99% TI] [e]	literature range[g]
			Antinutrient				
lectin	1.70 (1.01–4.54)	1.63 (0.86–3.42)	0.60–4.43 (0, 4.75)	4.29 (0.70–9.77)	4.55 (1.44–10.87)	0.45–9.95 (0, 9.72)	0.090–8.460
phytic acid	1.03 (0.64–1.58)	1.05 (0.57–1.58)	0.62–1.92 (0, 2.55)	0.76 (0.58–0.93)	0.75 (0.51–1.07)	0.41–0.96 (0.39, 1.07)	0.634–1.960
raffinose	not available	not available	not available	0.52 (0.40–0.71)	0.54 (0.31–0.83)	0.26–0.84 (0, 1.01)	0.212–0.661
stachyose	not available	not available	not available	2.36 (2.02–2.85)	2.50 (2.12–3.04)	1.53–2.98 (1.19, 3.31)	1.21–3.50
trypsin inhibitor	39.53 (22.07–49.62)	35.73 (25.31–50.67)	28.58–59.03 (19.38, 63.53)	33.69 (24.59–53.85)	31.44 (23.43–41.91)	20.79–55.51 (5.15, 59.34)	19.59–118.68
			Isoflavone				
daidzein	1096.78 (644.77–1478.56)	1136.14 (576.47–1571.91)	224.03–1223.34 (0, 1951.84)	993.67[h] (631.32–1571.41)	1073.57 (747.53–1526.23)	274.88–1485.52 (0, 1925.63)	60.0–2453.5
genistein	1077.37 (674.83–1396.40)	1098.35 (606.46–1426.97)	338.24–1488.89 (0, 2155.90)	797.90 (565.26–996.66)	824.83 (651.01–1003.02)	354.09–984.29 (0, 1387.95)	144.3–2837.2
glycitein	88.02 (51.42–148.94)	83.11 (59.78–121.38)	63.04–203.56 (0, 238.94)	91.77[h] (53.78–162.52)	102.61 (72.93–148.31)	52.72–298.57 (0, 287.45)	15.3–310.4
			Vitamin				
vitamin E	not available	not available	not available	2.71[h] (1.88–3.72)	2.52 (1.58–3.07)	1.29–4.80 (0, 7.00)	0.19–6.17

[a] Isoflavones in μg/g dry weight, lectin in HU/mg FW, phytic acid, raffinose, stachyose and proximates in percent dry weight, trypsin inhibitor in TIU/mg dry weight, and vitamin E in mg/100 g dry weight. [b] The mean and range of 15 values (three replicates from each of five field sites). [c] The range of sample values for commercial varieties grown at the same Argentina field sites in 2004–2005. [d] The mean and range of 14 values (three replicates from each of four field sites and two replicates from one field site). [e] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [f] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [g] From ref *54*. [h] Statistically significant difference from the control.

The levels of all other proximates and fibers in forage fell within the 99% tolerance interval of commercial soybeans and were also considered to be within the population of commercial soybean.

Proximate and Fiber Composition in Seed. The results for proximates and fiber in seed are presented in **Table 2**. There were no statistically significant differences between MON 89788 and the control soybean for either field season. The means and ranges of all proximates, acid detergent fiber, and neutral detergent fiber in MON 89788 and control seed fell within the 99% tolerance interval of the commercial varieties and were considered to be within the population of commercial soybean.

Amino Acid Composition in Seed. Results for amino acids in seed are presented in **Table 3**. No statistically significant differences were observed between MON 89788 and the control soybean for either field season. The levels of all amino acids in

Table 6. Amino Acid Composition of DT Soybean Meal from Glyphosate-Tolerant Soybean MON 89788 Grown in the U.S. in 2005

	MON 89788		conventional control		commercial varieties	literature
component[a]	mean[b]	range[b]	mean[b]	range[b]	(range) [c] [99% TI] [d]	range
alanine	2.38	2.38−2.39	2.37	2.36−2.37	(2.25−2.45) [2.01, 2.70]	2.18−2.59[e]
arginine	4.05	3.97−4.12	4.09	4.01−4.17	(3.73−4.18) [3.16, 4.70]	3.29−4.49[e]
aspartic acid	6.34	6.31−6.37	6.30	6.29−6.30	(5.96−6.54) [5.21, 7.27]	5.18−6.83[e]
cystine/cysteine	0.82	0.80−0.83	0.79	0.78−0.80	(0.74−0.83) [0.64, 0.93]	0.6[f]−0.92[e]
glutamic acid	10.08	10.03−10.13	10.01	9.98−10.04	(9.53−10.55) [8.10, 11.84]	8.05−11.21[e]
glycine	2.35	2.35−2.36	2.34	2.34−2.35	(2.28−2.42) [2.05, 2.62]	2.02−2.40[e]
histidine	1.44	1.44−1.44	1.43	1.42−1.44	(1.34−1.47) [1.20, 1.62]	1.32−1.63 [e]
isoleucine	2.52	2.51−2.52	2.49	2.46−2.51	(2.32−2.57) [2.06, 2.86]	2.11−2.74[e]
leucine	4.29	4.28−4.29	4.26	4.26−4.27	(3.95−4.43) [3.47, 4.97]	3.62−4.72[e]
lysine	3.46	3.44−3.49	3.42	3.41−3.44	(3.28−3.57) [2.87, 3.95]	2.97−3.69[e]
methionine	0.71	0.71−0.71	0.68	0.67−0.69	(0.69−0.74) [0.63, 0.78]	0.5− 0.9[f]
phenylalanine	2.80	2.80−2.81	2.80	2.79−2.80	(2.62−2.95) [2.25, 3.30]	2.39−3.19[e]
proline	2.79	2.78−2.80	2.77	2.75−2.78	(2.63−2.88) [2.31, 3.21]	2.32−3.05[e]
serine	2.92	2.89−2.95	2.91	2.87−2.96	(2.78−3.07) [2.34, 3.45]	1.97[e]−3.3[f]
threonine	2.12	2.11−2.13	2.10	2.09−2.10	(2.01−2.15) [1.82, 2.35]	0.80−2.24[e]
tryptophan	0.68	0.67−0.69	0.69	0.69−0.69	(0.63−0.68) [0.54, 0.77]	0.60[g]−2.08[e]
tyrosine	1.78	1.71−1.84	1.84	1.83−1.85	(1.72−1.91) [1.52, 2.13]	1.68−2.17[e]
valine	2.63	2.62−2.64	2.60	2.55−2.65	(2.47−2.67) [2.24, 2.90]	2.29−2.92[e]

[a] Percent dry weight. [b] The mean and range of 4 values (one replicate analyzed in duplicate from two field sites). [c] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [d] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [e] From ref *55*. [f] From ref *56*. [g] From ref *57*.

Table 7. Fiber, Proximate, and Antinutrient Composition of DT Soybean Meal from Glyphosate-Tolerant Soybean MON 89788 Grown in the U.S. in 2005

	MON 89788		conventional control		commercial varieties	literature
component[a]	mean[b]	range[b]	(range) [c] [99% TI][d]	range	(range) [c] [99% TI][d]	range
			Fiber			
acid detergent fiber	4.80	4.56−5.04	5.30	5.10−5.49	(4.36−7.34) [1.93, 10.42]	5.2−6.7[e]
neutral detergent fiber	5.98	5.42−6.55	6.67	6.62−6.71	(5.32−10.11) [1.11,14.00]	7.4−12.2[e]
			Proximate			
ash	7.18	7.12−7.24	7.00	6.85−7.15	(6.56−7.51) [5.27, 9.03]	5.2−9.1[e]
moisture	10.40	10.35−10.45	9.59	9.28−9.90	(7.76−11.70) [3.09, 15.09]	5.58−11.7[f]
protein	54.35	54.05−54.66	54.53	54.33−54.73	(51.71−56.37) [45.44, 61.53]	47.4−59.5[g]
total fat	0.33	0.27−0.39	0.56	0.56−0.57	(0.18−1.11) [0, 2.37]	0.5[h]−3.30[e]
			Antinutrient			
phytic acid	1.41	1.40−1.42	1.41	1.23−1.59	(1.31−1.70) [0.93, 2.12]	1.3−4.1[f]
trypsin inhibitors	2.06	1.0−3.05	2.18	1.09−3.28	(0.57−3.35) [0, 6.80]	3.8−17.9[f]

[a] Percent dry weight (moisture = fresh weight). [b] The mean and range of 4 values (one replicate analyzed in duplicate from two field sites). [c] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [d] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [e] From ref *1*. [f] From ref *13*. [g] From ref *55*. [h] From ref *58*.

Table 8. Fatty Acid and Vitamin Composition of Refined, Bleached, and Deodorized Soybean Oil from Glyphosate-Tolerant Soybean MON 89788 Grown in the U.S. in 2005

	MON 89788		conventional control		commercial varieties	literature
component [a]	mean[b]	range[b]	mean[b]	range[b]	(range) [c] [99% TI] [d]	range
			Fatty Acid			
16:0 palmitic	11.75	11.70−11.80	11.70	11.70−11.70	(9.47−11.85) [5.93, 15.37]	7−12[e]
16:1 palmitoleic	0.050	0.050−0.050	0.078	0.050−0.11	(0.10−0.13) [0.072, 0.16]	≤0.2[f]
18:0 stearic	4.34	4.22−4.47	4.43	4.30−4.57	(3.94−4.92) [2.71, 5.73]	2−5[e]
18:1 oleic	20.60	20.00−21.20	21.25	20.95−21.55	(21.05−27.35) [12.20, 36.29]	19−34[e]
18:2 linoleic	50.95	50.65−51.25	50.40	50.20−50.60	(45.85−51.10) [40.50, 57.09]	48−60[e]
18:3 linolenic	6.31	5.88−6.74	6.12	5.59−6.65	(5.40−6.86) [3.55, 8.38]	2−10[e]
20:0 arachidic	0.36	0.35−0.36	0.37	0.36−0.38	(0.31−0.37) [0.24, 0.44]	<1.0[e]
20:1 eicosenoic	0.31	0.30−0.31	0.32	0.30−0.33	(0.28−0.32) [0.22, 0.38]	<1.0[g]
22:0 behenic	0.37	0.35−0.39	0.37	0.36−0.39	(0.34−0.43) [0.21, 0.54]	<0.5[g]
			Vitamin			
vitamin E	10.06	9.77−10.35	10.22	8.15−12.30	(2.54−11.35) [0, 24.66]	0.9−35.2[f]

[a] Fatty acids as percent fresh weight of oil, vitamin E as mg/100 g. [b] The mean and range of 4 values (one replicate analyzed in duplicate from two field sites). [c] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [d] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [e] From ref *1*. [f] From ref *51*. [g] From ref *13*.

MON 89788 and control seed fell within the 99% tolerance interval of the commercial varieties and were considered to be within the population of commercial soybean.

Fatty Acid Composition in Seed. Table 4 contains the data for the fatty acid composition in seed. There were no statistically significant differences in fatty acids between MON 89788 and the control soybean for either field season. The levels of all fatty acids in MON 89788 and control seed were within the 99% tolerance interval of the commercial varieties and were considered to be within the population of commercial soybean.

Antinutrient, Isoflavone, and Vitamin E Composition in Seed. The results for lectin, phytic acid, raffinose, stachyose, and trypsin inhibitor are presented in **Table 5**. There were no statistically significant differences between MON 89788 and the control soybean for either field season. The means and ranges of all antinutrients in MON 89788 and control seed fell within the 99% tolerance interval of the commercial varieties. The results for isoflavones and vitamin E in seed are also presented in **Table 5**. Statistically significant differences were observed between MON 89788 and the control soybean for two of the three isoflavones, daidzein and glycitein, and for vitamin E from the U.S. field trial. The differences were not observed across sites and were generally small (7.5−10.6%), and the range of values for MON 89788 fell within the 99% tolerance interval for conventional soybean. All isoflavone and vitamin E values for MON 89788 and control soybean from the Argentina trial fell within the 99% tolerance interval for conventional soybean and were considered to be within the population of commercial soybean.

Amino Acid Composition in DT Meal. The results for amino acid levels in DT meal are presented in **Table 6**. Mean values for these analytes were very similar between MON 89788 and the control soybean. In all cases, the range of values for MON 89788 and control amino acids fell within the 99% tolerance interval calculated for the reference materials, were consistent with the literature range of values, and were considered to be equivalent to commercial soybean.

Fiber, Proximate, and Antinutrient Composition in DT Meal. The results for fiber, proximates, and antinutrients in DT meal are shown in **Table 7**. Mean values for these analytes were very similar between MON 89788 and the control soybean with the exception of total fat, which measured 41% lower in the test material than in the control material. The observed difference is attributed to processing efficiency differences because the levels of fat in the seed were observed to be equivalent between MON 89788 and the control (see **Table 4**). In all cases, the mean and range of values for MON 89788 and control fell within the 99% tolerance interval calculated for the reference materials and were consistent with the literature. Therefore, fiber, proximates, and antinutrients in MON 89788 and control DT meal were considered to be equivalent to commercial soybean varieties.

Fatty Acid and Vitamin E Composition in RBD Oil. Table 8 contains the results for the fatty acid and vitamin E composition in RBD oil. The range of values for MON 89788 and control analytes were similar and fell within the 99% tolerance interval and the literature range of values, with one exception. The exception, (16:1) palmitoleic acid values in both MON 89788 and the control soybean, fell below the tolerance interval. Palmitoleic acid naturally occurs at very low levels in soybean oil, near the method LOQ (*51*), and all test values were below the LOQ (see Statistical Analysis of Compositional Data section) prior to statistical analysis. Furthermore, the test and control values were within the literature range values. On the basis of these observations, the RBD oil from MON 89788 soybean and control soybean are considered equivalent and representative of commercially grown soybean.

Amino Acid and Moisture Composition in Protein Isolate. The results for amino acid and moisture levels in protein isolate

Table 9. Amino Acid and Moisture Composition of Protein Isolate from Glyphosate-Tolerant Soybean MON 89788 Grown in the U.S. in 2005

	MON 89788		conventional control		commercial varieties	literature
component[a]	mean[b]	range[b]	mean[b]	range[b]	(range) [c] [99% TI] [d]	range
			Amino Acid (% DW)			
alanine	4.04	4.01−4.06	4.08	4.06−4.10	(4.02−4.15) [3.87, 4.33]	not available
arginine	7.27	7.17−7.36	7.35	7.32−7.38	(7.32−7.66) [6.97, 7.98]	6.67[e]
aspartic acid	11.07	10.94−11.20	11.28	11.19−11.38	(11.16−11.42) [10.85, 11.70]	not available
cystine/cysteine	1.11	1.10−1.11	1.10	1.08−1.12	(1.04−1.13) [0.95, 1.23]	1.05[e]
glutamic acid	18.51	18.30−18.71	18.90	18.86−18.94	(18.85−19.44) [18.09, 20.14]	not available
glycine	4.03	3.99−4.07	4.08	4.05−4.11	(3.96−4.12) [3.83, 4.32]	not available
histidine	2.44	2.41−2.48	2.47	2.44−2.49	(2.42−2.51) [2.30, 2.66]	2.3[e]
isoleucine	4.47	4.36−4.58	4.43	4.19−4.68	(4.15−4.63) [3.42, 5.31]	4.25[e]
leucine	7.68	7.58−7.79	7.82	7.72−7.92	(7.70−7.92) [7.42, 8.21]	6.78[e]
lysine	6.11	6.03−6.19	6.09	6.04−6.14	(5.97−6.22) [5.69, 6.55]	5.33[e]
methionine	1.27	1.27−1.28	1.29	1.28−1.31	(1.22−1.34) [1.11, 1.43]	1.13[e]
phenylalanine	5.09	5.02−5.16	5.18	5.14−5.21	(5.15−5.29) [4.95, 5.46]	4.59[e]
proline	4.74	4.70−4.78	4.83	4.80−4.85	(4.82−4.95) [4.64, 5.07]	not available
serine	5.14	5.14−5.15	5.26	5.15−5.37	(5.23−5.45) [4.91, 5.78]	not available
threonine	3.42	3.41−3.43	3.47	3.44−3.51	(3.42−3.56) [3.28, 3.72]	3.14[e]
tryptophan	1.16	1.13−1.20	1.14	1.14−1.14	1.04−1.18 [0.88, 1.35]	1.12[e]
tyrosine	3.62	3.56−3.67	3.64	3.64−3.64	(3.57−3.71) [3.43, 3.85]	not available
valine	4.51	4.39−4.63	4.47	4.18−4.77	(4.15−4.66) [3.35, 5.45]	4.1[e]
			Proximate (% FW)			
moisture	4.91	4.89−4.94	6.06	6.00−6.13	(4.15−6.30) [1.12, 8.77]	3.9−7.0[f]

[a] Percent dry weight (moisture = fresh weight). [b] The mean and range of 4 values (one replicate analyzed in duplicate from two field sites). [c] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [d] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [e] From ref *1*. [f] From ref *59*.

Table 10. Phosphatide Composition of Crude Lecithin from Glyphosate-Tolerant Soybean MON 89788 Grown in the U.S. in 2005

	MON 89788		conventional control		commercial varieties	literature
component [a]	mean[b]	range[b]	mean[b]	range[b]	(range) [c] [99% TI] [d]	range[e]
l-α-phosphatidic acid	3.19	3.12−3.25	3.64	3.30−3.99	(3.19−5.75) [0, 9.44]	0.2−14.0
l-α-phosphatidylcholine	15.58	15.00−16.15	16.38	16.10−16.65	(14.00−17.50) [10.43, 20.35]	12.0−46.0
l-α-phosphatidylethanolamine	14.18	13.95−14.40	14.50	14.15−14.85	(11.10−14.60) [6.96, 19.13]	8.0−34.0
l-α-phosphatidiylinositol	10.29	9.82−10.75	11.03	10.85−11.20	(9.67−12.00) [6.97, 14.67]	1.7−21.0

[a] Percent fresh weight. [b] The mean and range of 4 values (one replicate analyzed in duplicate from two field sites). [c] The range of sample values for commercial varieties grown at the same U.S. field sites in 2005. [d] TI = tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [e] From ref *60*.

are shown in **Table 9**. In all cases, the range of values for MON 89788 and control analytes were similar and all values fell within the 99% tolerance interval and within the literature range of values where data has been published. On the basis of these results, the protein isolate from MON 89788 soybean and control soybean are considered equivalent and representative of commercially grown soybean.

Phosphatide Composition in Lecithin. The results for phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol are shown in **Table 10**. In all cases, the range of values for MON 89788 and control analytes were similar and fell within the 99% tolerance interval and the literature range of values. On the basis of these results, the crude lecithin from MON 89788 soybean and control soybean are considered equivalent and representative of commercially grown soybean.

The results of these compositional analyses show that no significant difference ($p < 0.05$) was observed between MON 89788 and A3244 in all 46 components measured from the Argentina field trial and 45 of the 49 components analyzed in the U.S. field trial. Of the four analytes, forage moisture, seed daidzein, seed glycitein, and seed vitamin E, from the U.S. trials that were observed to be significantly different ($p < 0.05$) between MON 89788 and the control, the differences were within the 99% tolerance interval calculated from the commercial varieties of soybean produced and analyzed in the same study. For forage moisture, the magnitude of differences was small between MON 89788 and A3244 (less than 3% across both seasons) and there was no consistent trend for the observed differences. A similar lack of consistent trend was also observed for daidzein, glycitein, and vitamin E. Other analytes that were statistically different in either season were only observed sporadically, and no cross-season pattern was observed. These data confirm the observation from the single-season analysis and indicate that there is no biologically relevant differences between MON 89788 and A3244. The 59 components measured in the processed fractions were all similar between MON 89788 and A3244, and all component levels fell within the 99% tolerance intervals for the reference materials produced and analyzed in the same study. The one exception observed was for the low abundance fatty acid palmitoleic acid in the RBD oil from MON 89788 and A3244, which were both below the 99% tolerance interval. This observation was not considered biologically meaningful because the test values were assigned and the statistical values were within the literature range, which is representative of the natural population of conventional soybean varieties.

In all, 108 nutrients and antinutrients in seed, forage, meal, RBD oil, protein isolates, and lecithin from MON 89788 soybean were compared to conventional soybean. Examination of both data sets indicated that there are no analytes that show a consistent trend of difference between MON 89788 and A3244 within a season or across both seasons. These data demonstrated that component levels were similar, and the few statistically significant differences observed were not observed consistently across sites, were within the natural population variation for conventional soybean as defined by the in-study 99% tolerance interval and/or data reported in the literature, and were not deemed biologically relevant. Therefore, it is concluded that forage, seed, meal, RBD oil, protein isolates, and lecithin from MON 89788 soybean are compositionally equivalent to that of conventional soybean.

ACKNOWLEDGMENT

We thank the Monsanto Agronomy and Sample Management groups for generation and preparation of the samples used in this study, the Monsanto Product Characterization group for the molecular analysis of the seed, and Raymond Dobert, Phillip Eppard, Roy Fuchs, and Beryl Packer for their critical review of the manuscript.

LITERATURE CITED

(1) Organisation for Economic Co-operation and Development (OECD). OECD consensus document on compositional considerations for new varieties of soybean: Key food and feed nutrients and antinutrients. OECD Environmental Health and Safety Publications, Paris, France, 2001.

(2) The American Soybean Association. Soy stats, domestic utilization, 2007, http://www.soystats.com/2007.

(3) James, C. Global status of commercialized Biotech/GM Crops: 2006. In ISAAA Brief 35, ISAAA: Ithaca, NY, 2006; p 3 and 5.

(4) The American Soybean Association. Soy stats, introduction, 2007, http://www.soystats.com/2007.

(5) Carpenter, J. E. *Case Studies in Benefits and Risks of Agricultural Biotechnology: Roundup Ready Soybeans and Bt Field Corn*; National Center for Food and Agricultural Policy: Washington, D.C., 2001; pp 1−54.

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

(7) Padgette, S. R.; Kolacz, K. H.; Delannay, X.; Re, D. B.; LaValle, B. J.; Tinius, C. N.; Rhodes, W. K.; Otero, Y. L.; Barry, G. F.; Eichholtz, D. A.; Peschke, V. M.; Nida, D. L.; Taylor, N. B.; Kishore, G. M. Development, identification and characterization of a glyphosate-tolerant soybean line. Crop Sci. **1995**, *35*, 1451–1461.

(8) Haslam, E. *Shikimic Acid: Metabolism and Metabolites*; John Wiley and Sons: Chichester, U.K., 1993.

(9) Steinrucken, H.; Amerhein, N. The herbicide glyphosate is a potent inhibitor of 5-enolypyruvylshikimic acid-3-phosphate synthase. Biochem. Biophys. Res. Commun. **1980**, *94*, 1207–1212.

(10) Padgette, S. R.; Re, D. B.; Barry, G. F.; Eichholtz, D. A.; Delannay, X.; Fuchs, R. L.; Kishore, G. M.; Fraley, R. T. New weed control oportunities: Development of soybeans with a Roundup Ready gene. *Herbicide-Resistant Crops*; Duke, S. O., Ed.; CRC Lewis Publishers: Boca Raton, FL, 1996; pp 53−84.

(11) Paschal, E. H. Soybean cultivar 88154622393, 1997.

(12) Tylka, G. L.; Marett, C. C. Evaluation of soybean varieties resistant to soybean cyst nematode in Iowa 1999. Iowa State University Extension Nematology Publication on the web, 1999.

(13) 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.

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

(15) Harrison, L. A.; Bailey, M. R.; Naylor, M. W.; Ream, J. E.; Hammond, B. G.; Nida, D. L.; Burnette, B. L.; Nickson, T. E.; Mitsky, T. A.; Taylor, M. L.; Fuchs, R. L.; Padgette, S. R. The expressed protein in glyphosate-tolerant soybean, 5-enolypyruvylshikimate-2-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. *J. Nutr.* **1996**, 728–740.

(16) Cromwell, G. L.; Lindermann, M. D.; Randolph, H. H.; 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.

(17) Goda, Y.; Akiyama, H.; Suiyama, E.; Takahashi, S.; Kinjo, J.; Nohara, T.; Toyodo, M. Comparison of soyasponin and isoflavone contents between genetically modified GM- and non-GM soybeans. J. Food Hyg. Soc. Jpn. **2002**, *43* (6), 339–347.

(18) McCann, M. C.; Liu, K.; Trujillo, W. A.; Dobert, R. C. Glyphosate-tolerant soybeans remain compositionaly equivalent to conventional soybeans (*Glycine max* L.) during three years of testing. J. Agric. Food Chem. **2005**, *53* (13), 5331–5335.

(19) Sanden, M.; Krogdahl, Å.; Bakke-Mckellep, A. M.; Buddington, R. K.; Hemre, G.-I. Growth performance and organ development in Atlantic salmon. *Salmo salar* L. parr fed genetically modified (GM) soybean and maize. *Aquacult. Nutr.* **2006**, *12* (1), 1–14.
(20) World Health Organization (WHO). WHO strategies for assessing the safety of foods produced by biotechnology. WHO, Geneva, 1991.
(21) Codex. Principles for the risk analysis of foods derived from modern biotechnology. In Codex Alimentarius Commission, 2003; Vol. CAC/GL 44-2003.
(22) Bradstreet, R. B. *The Kjeldahl Method for Organic Nitrogen*; Academic Press: New York, 1965.
(23) Kalthoff, I. M.; Sandell, E. B. *Quantitative Inorganic Analysis*; MacMillian: New York, 1948.
(24) Association of Official Analytical Chemists (AOAC). Nitrogen (total) in fertilizers. Methods 955.04. In *Official Method of Analysis of AOAC International*, 17th ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapter 2, pp 14−15.
(25) Association of Official Analytical Chemists (AOAC). Protein in grains. Method 979.09. In *Official Method of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; pp 30−34.
(26) Association of Official Analytical Chemists (AOAC). Fat (crude) or ether extract in meat. Method 960-39. In *Official Method of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapter 39, p 2.
(27) Association of Official Analytical Chemists (AOAC). Fat in flour. Method 922.06. In *Official Method of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapter 32, p 5.
(28) Association of Official Analytical Chemists (AOAC). Fat (crude) or ether extract in pet food. Method 954.02. In *Official Method of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapter 4, p 33.
(29) Association of Official Analytical Chemists (AOAC). Ash of flour. Method 923.03. In *Official Method of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapter 32, p 2.
(30) Association of Official Analytical Chemists (AOAC). Moisture in cheese. Method 926.08. In *Official Method of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; p 70.
(31) Association of Official Analytical Chemists (AOAC). Solids (total) and moisture in flour. Method 925.09. In *Official Method of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapter 32, p 1.
(32) United States Department of Agriculture (USDA). Agriculture handbook. Energy value of foods. In *Agricultural Handbook Number 74*; United States Department of Agriculture: Washington, D.C., 1973; pp 2−11.
(33) United States Department of Agriculture (USDA). Forage fiber analysis. In *Agricultural Handbook Number 379*; United States Department of Agriculture: Washington, D.C., 1970.
(34) American Association of Cereal Chemists (AACC). Method 32.20. In *American Association of Cereal Chemists*, 9th ed.; AACC: Minneapolis, MN, 1998.
(35) Association of Official Analytical Chemists (AOAC). Protein efficiency ratio. Method 982.30. In *Official Method of Analysis of AOAC International*, 18th ed.; Horwitz, W., Ed.; The Association of Official Analytical Chemists International: Gaithersburg, MD, 2005; Chapter 45, pp 63−66.
(36) American Oil Chemists Society (AOCS). Fatty acid composition by gas chromatography. Method Ce 1-62. In *Official Methods and Recommended Practices of the American Oil Chemists Society*, 5th ed.; Firestone, D., Ed.; AOCS: Champaign, IL, 1997.
(37) Speek, A. J.; Schijver, J.; Schreurs, W. H. P. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric deterction. *J. Food Sci.* **1985**, *50*, 121–124.
(38) Seo, A.; Morr, C. V. Improved high-performance liquid chromatographic analysis of phenolic acid and isoflavones from soybean protein products. *J. Agric. Food Chem.* **1984**, *32*, 530–533.
(39) Pettersson, H.; Kiessling, K. H. Liquid chromatographic determination of the plant estrogens coumestrol and isoflavones in animal feed. *J. AOAC* **1984**, *67*, 503–506.
(40) Klurfeld, D. M.; Kritchevsky, D. Isolation and quantitation of lectins from vegetable oils. *Lipids* **1987**, *22*, 667–668.
(41) Liener, I. E. The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. *Arch. Biochem. Biophys.* **1955**, 223–231.
(42) Lehrfeld, J. High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. *Cereal Chem.* **1989**, *66*, 510–515.
(43) Lehrfeld, J. HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: Problem and solutions. *J. Agri. Food Chem.* **1994**, *42*, 2726–2731.
(44) American Oil Chemists Society (AOCS). Determination of lecithin phospholipids by HPLC. Method Ja 7b-91. In *Official Methods and Recommended Practices of the American Oil Chemists Society*, 5th ed.; Firestone, D., Ed.; AOCS: Champaign, IL, 1997.
(45) Mason, B. S.; Slover, H. T. A gas chromatographic method for the determination of sugars in foods. *J. Agri. Food Chem.* **1971**, *19*, 551–554.
(46) Brobst, K. M. Gas−liquid chromatography of trimethylsilyl derivatives. In *Methods in Carbohydrate Chemistry*; Academic Press: New York, 1972; Vol. 6.
(47) American Oil Chemists Society (AOCS). Method Ba 12-75. In *Official Methods and Recommended Practices of the American Oil Chemists Society*, 5th ed.; Firestone, D., Ed.; AOCS: Champaign, IL, 1997.
(48) Cort, W. M.; Vincente, T. S.; Waysek, E. H.; Williams, B. D. Vitamin E content of feedstuffs determined by high-perforamance liquid chromatographic fluorescence. *J. Agric. Food Chem.* **1983**, *31*, 1330–1333.
(49) McMurray, C. H.; Blanchflower, W. J.; Rice, D. A. Influence of extraction techniques on determination of α-tocopherol in animal feedstuffs. *J. AOAC* **1980**, *63*, 1258–1261.
(50) SAS Software Release 9.1 (TS1M3). SAS Institute, Inc.: Cary, NC, 2002− 2003, http://support.sas.com/documentation/onlinedoc/91pdf/index.html.
(51) Codex. Standard for named vegetable oils. In CODEX-Stan 210, 2005, http://www.codexalimentarius.net/download/standards/336/CXS_210e.pdf.
(52) International Life Sciences Institute (ILSI). ILSI Crop Composition Database, version 2.0, Jan 18, 2006, http://www.cropcomposition.org; Search criteria: soybean forage, all locations, all years, all fiber.
(53) International Life Sciences Institute (ILSI). ILSI Crop Composition Database, version 2.0, Jan 18, 2006, http://www.cropcomposition.org; Search criteria: soybean forage, all locations, all years, all proximates, dry weight.
(54) International Life Sciences Institute (ILSI). ILSI Crop Composition Database, version 2.0, Jan 18, 2006, http://www.cropcomposition.org; Search criteria: soybean seed, all locations, all years.
(55) Karr-Lilienthal, L. K.; Grieshop, C. M.; Merchen, N. R.; Mahan, D. C.; Fahey, G. C. Chemical composition and protein quality comparisons of soybeans and soybean meals from five leading soybean-producing countires. *J. Agri. Food Chem.* **2004**, 6193–9199.
(56) Grieshop, C. M.; Kadzere, C. T.; Clapper, G. M.; Flickinger, E. A.; Bauer, L. L.; Frazier, R. L.; Fahey, G. C. Chemical and nutritional characteristics of United States soybeans and soybean meals. *J. Agri. Food Chem.* **2003**, 7684–7691.

(57) Iowa Soybean Association. Global Soybean Meal Analysis Project. In *Soybean Meal Info Center*, 2001.
(58) Orthoefer, F. T. Processing and utilization. In *Soybean Physiology, Agronomy, and Utilization*; Norman, A. G., Ed.; Academic Press: New York, 1978; pp 219−246.
(59) Mattil, K. F. Composition, nutritional, and functional properties, and quality criteria of soy protein isolates. *J. AOCS* **1974**, 81A–84A.
(60) Szuhaj, B. F. Lecithins. In *Bailey's Industrial Oil and Fat Products*, 6th ed.; Shahidi, F., Ed.; John Wiley and Sons, Inc.: New York, 2005; pp 361−441.

Received for review October 19, 2007. Revised manuscript received February 6, 2008. Accepted March 25, 2008.

JF073087H