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ARTICLE

Compositions of Seed, Forage, and Processed Fractions from Insect-Protected Soybean MON 87701 Are Equivalent to Those of Conventional Soybean

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Monsanto Co. has developed biotechnology-derived, insect-protected soybean MON 87701 that produces the Cry1Ac insecticidal crystal (δ-endotoxin) protein derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki*. Cry1Ac provides protection from feeding damage caused by certain targeted lepidopteran pests. The purpose of this work was to assess whether the compositions of seed, forage, and processed fractions (meal, oil, protein isolate, and lecithin) of MON 87701 are comparable to those of conventional soybean. Compositional analyses were conducted on seed and forage tissues harvested from MON 87701 and conventional soybean grown in multiple replicated sites in the United States during the 2007 growing season and in Argentina during the 2007−2008 growing season. Seed, forage, and processed fractions from conventional soybean varieties currently in the marketplace were included in the analyses to establish a range of natural variability for each compositional component; the range of variability was defined by a 99% tolerance interval. Additional seed was collected from soybean grown in a separate U.S. production during the 2007 season. This seed and processed fractions (meal, oil, protein isolate, and crude lecithin) derived from it were also subjected to compositional analyses. Forage samples were analyzed for levels of proximates (ash, fat, moisture, and protein), carbohydrates by calculation, and fiber. Seed samples were analyzed for proximates, carbohydrates by calculation, fiber, amino acids, fatty acids, antinutrients, and vitamin E. Toasted, defatted (TD) 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. Overall, results demonstrated that the seed, forage, and processed fractions of MON 87701 are compositionally equivalent to those of conventional soybean.

KEYWORDS: Soybean (*Glycine max*); insect-protected; biotechnology; composition

INTRODUCTION

The composition of soybean, as well as the ease and geographical range of its agricultural production, makes it an inexpensive source of oil and protein for use as food and as animal feed (*1*). Indeed, soybean is the main source of consumable plant protein and the leading source of vegetable oil worldwide. The United States, Brazil, and Argentina are the three top soybean-producing countries, contributing approximately 82% of world soybean production in the 2006−2007 season (*2*). The total soybean crop value in 2007 for the United States alone exceeded $26.8 billion (*2*). Most of the soybean acreage is focused on cultivation of the glyphosate-tolerant Roundup Ready soybean (*2*). The rapid global adoption of Roundup Ready soybean is attributed to the effectiveness and significant cost efficiencies in weed control. Through modern agricultural biotechnology, Monsanto has developed soybean varieties that contain the *cry1Ac* gene derived from *Bacillus thuringiensis* (*3*, *4*). Expression of the Cry1Ac protein confers resistance to certain targeted lepidopteran pests. MON 87701 contains the *cry1Ac* gene derived from *B. thuringiensis* and therefore offers improved insect management strategies for soybean growers and is an important base trait for future breeding improvements and multitrait products.

This paper reports data from a comprehensive compositional assessment of insect-protected soybean MON 87701. This assessment followed globally accepted guidelines outlined in Organization for Economic Cooperation and Development (OECD) consensus documents (*1*). The OECD consensus document emphasizes direct comparisons between the new biotechnology-derived crop and a near-isogenic control to identify potential differences in the levels of key nutrient and antinutrient

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components (5). Compositional analyses were conducted on seed and forage from soybean grown at five locations in each of two distinct soybean-producing geographic regions, in the United States and Argentina, over two seasons (2007 and 2007−2008, respectively), and on seed and processed fractions (meal, oil, protein isolate, and crude lecithin) from soybean grown at two locations in a separate U.S. production during 2007, referred to as a second U.S. production.

MATERIALS AND METHODS

Soybean Samples for Compositional Analyses. MON 87701 was produced by the stable insertion of the *cry1Ac* coding sequence into the genome of the conventional soybean variety A5547. The control substance used in this composition study was the nontransformed parental variety A5547. Compositional analyses were conducted on soybean samples from a total of three field productions. Two of these field productions generated seed and forage for direct analysis, and the third generated seed that was subsequently used to develop processed fractions (meal, oil, protein isolate, and crude lecithin) upon which further analyses were performed. Seed and forage were harvested from a U.S. 2007 field trial that included five replicate sites in Baldwin County, Alabama; Jackson County, Arkansas; Clarke County, Georgia; Jackson County, Illinois; and Wayne County, North Carolina; and from an Argentinean 2007−2008 field trial that included three replicated sites in the province of Buenos Aires (Tacuari, Gahan, and Berdier) and one replicated site in each of the provinces of Córdoba (Alejo Ledesma) and Santa Fe (San Francisco). At each site, plants were grown in three randomized complete blocks, and each block contained MON 87701, A5547, and four different conventional commercial soybean varieties (the reference substances) grown in separate plots. Conventional commercial soybean varieties included in both U.S. and Argentinean field trials were A5843, A5959, CMA 5804AOC, UA 4805, Ozark, Anand, Hornbeck C5894, A5560, CMC 5901COC, LEE 74, A5403, A4922, H4994, H5218, A5427, DP 5989, Hutcheson, USG 5601T, and Fowler. H6686 was included in the U.S. field trials, and USG 5002T was included in the Argentinean field trials. The field trial from which the soybean seed was harvested to generate processed fractions was conducted in 2007 at two replicated U.S. sites; one in Jackson County, Arkansas, and one in Jackson County, Illinois. The geographic regions selected for all field trials were representative of major commercial growing areas in the United States and Argentina. Normal agronomic practices were followed for each growing region.

For the forage sample, at least six plants from each plot were collected at approximately the R6 growth stage (full seed) by cutting at the base and then composited. The forage samples were transferred to dry ice within 30 min after sampling. Seed was harvested at the R8 growth stage (full maturity) and stored at ambient temperature. Forage samples were shipped from the field to Monsanto Co. and frozen on dry ice; seeds were shipped at ambient temperature. At Monsanto Co., forage and seed samples were homogenized by grinding with dry ice and stored frozen at approximately −20 °C until used for compositional analysis. The seed harvested for processed fractions was sent to GLP Technologies in Navasota, TX, to obtain the following fractions; toasted, defatted (TD) meal; refined, bleached, and deodorized (RBD) oil; protein isolate; and crude lecithin.

The genetic identity of the seed, forage, and processed fraction samples was verified by chain-of-custody and sample-handling records. The genetic identity of the seed was further confirmed by event-specific Polymerase Chain Reaction (PCR) analysis.

Compositional Analysis Methods. Forage samples were analyzed for proximates (ash, fat, moisture, and protein), carbohydrates by calculation, acid detergent fiber (ADF), and neutral detergent fiber (NDF). Seed samples were analyzed for proximates (ash, fat, moisture, and protein), carbohydrates by calculation, ADF, NDF, amino acids, fatty acids, trypsin inhibitors, phytic acid, lectin, isoflavones, vitamin E, raffinose, and stachyose. TD meal was analyzed for proximates (ash, fat, moisture, and protein), carbohydrate by calculation, ADF, NDF, amino acids, phytic acid, and trypsin inhibitors. RBD oil was analyzed for fatty acids and vitamin E. Protein isolate was analyzed for amino acids and moisture. Crude lecithin was analyzed for phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid. Compositional analyses were conducted at EPL-Bio-Analytical Services (EPL-BAS) in Niantic, IL. Samples from each site were analyzed by tissue in a randomized order to minimize assay bias. Analyses used established methods; brief summaries of the analytical methods used are presented below.

Proximate Analysis. Protein levels were determined according to the Kjeldahl method (*6*−*9*). Samples were manually digested on a heating block using sulfuric acid and a selenium catalyst and then transferred to the analyzer unit, where the digests were distilled and titrated. The protein content was calculated by multiplying the amount of nitrogen in the sample by 6.25.

Fat content of the seed was determined using Soxhlet extraction (*10*). Seed samples were extracted for 16 h with pentane. The pentane extract was evaporated to dryness, and the crude fat residue was determined gravimetrically. The fat content of the forage was determined by acid hydrolysis (*11*). The forage samples were dried in an oven for at least 2 h. The crude fat content was determined gravimetrically after acid hydrolysis and extraction with mixed ethers.

Ash content was determined by combustion at 650 °C, and the weight of the ash residue remaining after ignition was determined gravimetrically (*12*).

Moisture content of the forage was determined by loss of weight after drying to a constant weight in a forced-air oven at 135 °C for at least 2 h (*13*). Seed was dried to a constant weight in a vacuum oven at 100 °C and 25 in. of mercury pressure for 15 h (*14*).

Carbohydrates by calculation were determined using the fresh weight-derived data and the formula presented below (*15*):

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

Fiber Analysis. ADF was determined by digesting with an acid detergent solution and washing with water. The remaining residue was dried and weighed to determine ADF content (*16*). NDF was determined by digesting with a neutral detergent solution, sodium sulfite, and α-amylase. The remaining residue was dried and weighed to determine NDF content (*16*, *17*). ADF and NDF analyses used the ANKOM^{200} Fiber Analyzer (Ankom Technology, Fairport, NY).

Amino Acid Composition. The samples were assayed by three methods to obtain the full profile of amino acids. Levels of tryptophan were determined by hydrolyzing the sample with 4 M LiOH. Samples were diluted with deionized water, filtered, and analyzed by reversed-phase HPLC with UV detection (*18*). After acid oxidation and hydrolysis, cystine and methionine were measured as the 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate derivatives using reversed-phase UPLC with UV detection (*19*). Analysis of the remaining amino acids was accomplished by converting the free acids, after acid hydrolysis, to the 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate derivatives followed by reversed-phase UPLC with UV detection (*19*).

Fatty Acid Profile. Lipid in the seed samples was extracted by Soxhlet and saponified with 2% (w/v) sodium hydroxide in methanol. The saponification mixture was methylated with 12−15% boron trifluoride/methanol. The resulting methyl esters were extracted with hexane. The methyl esters of the fatty acids were analyzed by gas chromatography with flame ionization detection using external standards for quantitation method (*20*−*22*).

Isoflavone Analysis. The sample was extracted using a solution of hydrochloric acid and reagent alcohol heated on steam baths or hot plates. The extract was brought to volume, diluted, and centrifuged. An aliquot of the supernatant was placed onto a C_{18} solid-phase extraction column. Unwanted components of the matrix were rinsed off with 20% methanol, and then the isoflavones were eluted with 80% methanol. The sample was analyzed on a high-performance reversed-phase liquid chromatography system with ultraviolet spectrophotometric detection (*23*, *24*).

Lectin Analysis. The sample was suspended in 10% phosphate-buffered saline (pH 7.4), stirred, and then centrifuged at 3000 rpm for 10 min. An aliquot of the resulting extract was serially diluted at minimum 10 cuvettes containing PBS. A 10% hematocrit of lyophilized rabbit blood in PBS was added to each dilution. After incubation at room temperature for 2.5 h, the absorbance of each dilution of the sample and lectin control was read by a spectrophotometer at 620 nm, using PBS to zero the

instrument. One hemagglutinating unit (HU) was defined as the level that caused 50% of the standard cell suspension to sediment in 2.5 h (*25*).

Phytic Acid Analysis. Phytic acid was extracted with dilute hydrochloric acid (2.4%) and isolated using an ion-exchange solid phase extraction column. Once isolated and eluted, the phytic acid was analyzed for elemental phosphorus by inductively coupled plasma optical emission spectroscopy (ICP-OES). The phytic acid content was then calculated from the phosphorus concentration (*26*).

Phosphatide Analysis. The sample was weighed and diluted using 95:5 chloroform/methanol (with 1% formate and 0.05% triethylamine) using sonication and stirring. The dilute sample was directly injected onto a Diol HPLC column under normal phase conditions. The resulting extract was analyzed on an HPLC system equipped with an evaporative light-scattering detector (*27*).

Raffinose and Stachyose Analysis. The sample was extracted with methanol/deionized water (65:35), followed by partitioning with chloroform and evaporation to dryness. The sample residue was redissolved in deionized water and analyzed by reversed-phase HPLC with refractive index detection (*28*, *29*).

Trypsin Inhibitor Analysis. The sample was defatted with petroleum ether. The sample was then extracted for 3 h with 0.01 N sodium hydroxide. Varying aliquots of the sample suspension were exposed to a known amount of trypsin and then benzoyl-DL-arginine-*p*-nitroanalide hydrochloride. The sample was allowed to react for 10 min at 37 °C. After 10 min, the reaction was halted by the addition of acetic acid. The solution was centrifuged, and then the absorbance was determined at 410 nm. Trypsin inhibitor activity was determined by photometrically measuring the inhibition of trypsin's reaction with benzoyl-DL-arginine-*p*-nitroanalide hydrochloride (*30*).

Vitamin E (α-Tocopherol) Analysis. The sample was saponified to break down fat and release vitamin E. The saponified mixture was extracted with hexane. The hexane extract was concentrated and then analyzed by normal phase HPLC on a silica column with fluorescence detection (*31*).

Statistical Analysis of Compositional Data. In all, a total of 261 analytical components were measured (7 in forage and 57 in grain for both the United States and Argentina, 58 in grain for the second United States, 27 in meal, 25 in oil, 19 in protein isolate, and 4 in crude lecithin). To conduct a statistical analysis for a compositional component, at least 50% of the values for any given analyte had to be greater than the assay limit of quantitation (LOQ). Analytes excluded from analysis of seed samples from all field trials and from RBD oil excluded 8:0 caprylic acid, 12:0 lauric acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 18:3 γ-linolenic, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, and 22:1 erucic acid. Additionally, 10:0 capric acid and 17:1 heptadecenoic acid from Argentinean and second-year U.S. seed samples were excluded. 20:2 eicosadienoic acid from RBD oil was excluded. For individual measurements below the LOQ, a value equal to half of the LOQ was assigned prior to statistical analyses. Fatty acid values in seed from all field trials were assigned a value for 20:2 eicosadienoic acid, from U.S. production 2007, for 17:1 heptadecenoic acid, and for 24:0 lignoceric acid from the second U.S. production. Two fatty acid values in RBD oil were assigned a value for 16:1 palmitoleic acid and 24:0 lignoceric acid.

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 within ±3 PRESS residuals. Data points that were outside the ±6 studentized PRESS residual range were considered for exclusion from the final statistical analysis. For forage, one moisture value for one reference substance from the first U.S. field production and one fat value for one reference substance from the Argentinean field trial were excluded from statistical analyses. For seed, one fat value for MON 87701 from the first U.S. field trial was excluded from statistical analyses.

Statistical analyses were run using a mixed model analysis of variance for compositional data from each of the five individual sites for Argentinean and U.S. trials (individual site data not presented) and from the combination of all sites (combined site analysis) for a given field season. The combined site analysis used the model

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

where Y_{ijk} = unique individual observation, U = overall mean, T_i = material effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by material interaction effect, and e_{ijk} = residual error. Each individual analyte for MON 87701 was compared to that of the conventional control, A5547, and statistical significance was defined at the level of $p < 0.05$. For each analyte, least-squares means were generated for MON 87701 and A5547.

The commercial conventional soybean varieties from the first U.S. 2007 production and the Argentinean 2007−2008 production were not analyzed statistically to MON 87701 or its parental control. They were used to develop a population tolerance interval for each component analyzed that was expected to contain, with 95% confidence, 99% of the values expressed in the population of commercial soybean varieties. By comparison to the 99% tolerance interval, any statistically significant differences between MON 87701 and the control (A5547) may be put into perspective and can be assessed for biological relevance in the context of the natural variability in soybean. Each tolerance interval estimate was based upon one observation per unique reference material. Because negative quantities were not possible, calculated negative lower tolerance bounds were set to zero. SAS software was used to generate all summary statistics and perform all analyses (*32*).

RESULTS AND DISCUSSION

This paper compares the levels of key nutritional and antinutritional components in seed and forage from insect-protected MON 87701 soybean to that of a conventional control grown in field trials in the United States during 2007 and in Argentina during 2007−2008. The comparative assessment was conducted using a mixed model analysis of variance with statistical significance assigned at the $p < 0.05$ level. In addition, the compositional profile of MON 87701 was compared to those of commercial conventional soybean varieties grown in the same field trials by calculating a 99% tolerance interval to address compositional variability in commercially available conventional soybean (*33*). Composition values for MON 87701 were also compared with values derived from the published literature on commercial conventional soybean. The paper further compared the levels of key nutritional and antinutritional components in seed and processed fractions (meal, oil, protein isolate, and crude lecithin) grown in field trials in a second U.S. production during 2007.

Proximate and Fiber Composition in Forage. There were no statistically significant differences ($p > 0.05$) between forage produced by MON 87701 and the control for either year (**Table 1**). The mean levels of all proximates and fibers in the forage of MON 87701 from the U.S. and Argentinean production fell within the 99% tolerance interval established from the reference substances grown in the respective regions and were therefore considered to be within that of the commercial conventional soybean population.

Proximate and Fiber Composition in Seed. With the exception of carbohydrates by calculation and protein in the U.S. trial, there were no statistically significant differences ($p > 0.05$) between the proximate and fiber composition of MON 87701 and the control (**Table 2**). The relative magnitude differences between MON 87701 and control mean values for carbohydrates by calculation and protein, expressed as difference of the control, were small (~6.1% for carbohydrates by calculation and ~3.9% for protein) and not considered to be meaningful from a food/feed safety or nutritional perspective. In addition, the mean levels of all proximates and fibers in the seed of MON 87701 from the U.S. and Argentinean production fell within the 99% tolerance interval established from the reference substances grown in the respective

Table 1. Fiber and Proximate Composition of Forage from Insect-Protected Soybean MON 87701

	2007 U.S. field trials			2007−2008 Argentinean field trials			
component[a]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][e]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[d] [99% TI][e]	literature range[f]
fiber							
ADF	37.17 (30.04−58.25)	36.53 (27.42−42.06)	(27.99−47.33) [14.93, 56.87]	34.87 (30.51−40.14)	34.70 (29.71−46.90)	(25.49−44.75) [24.42, 44.95]	32−38
NDF	47.16 (37.02−55.99)	45.57 (34.23−64.19)	(30.96−54.55) [21.51, 66.01]	39.53 (32.80−44.60)	41.46 (35.55−50.64)	(32.28−50.79) [30.47, 51.81]	34−40
proximate							
ash	5.84 (5.05−7.46)	6.32 (5.10−8.13)	(4.77−8.54) [2.46, 10.14]	6.02 (5.45−6.84)	6.09 (5.56−6.53)	(5.15−7.34) [4.34, 7.99]	6.72−10.78
carbohydrates	71.43 (68.29−76.73)	70.97 (63.68−74.26)	(60.61−77.26) [56.93, 85.88]	70.18 (65.73−74.36)	70.70 (66.44−73.87)	(63.46−75.76) [61.22, 78.33]	59.8−74.7
moisture	72.86 (70.10−76.80)	73.41 (69.40−78.10)	(66.50−80.20) [57.84, 88.56]	69.10 (59.81−73.17)	69.78 (62.76−72.79)	(32.05−73.16) [26.09, 106.20]	73.5−81.6
protein	17.39 (13.56−20.03)	17.07 (14.20−23.29)	(12.68−22.92) [7.05, 27.27]	18.78 (15.87−21.34)	18.31 (14.93−20.38)	(13.72−23.76) [11.40, 26.39]	14.38−24.71
total fat	5.30 (3.60−6.82)	5.65 (4.23−7.23)	(3.48−7.88) [1.11, 9.11]	5.01 (3.45−6.99)	4.91 (3.73−7.22)	(2.96−7.07) [2.72, 7.40]	1.30−5.13

[a] Percent dry weight (moisture = % fresh weight, carbohydrates by calculation). [b] The least-squares 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 U.S. field sites in 2007. [d] The range of sample values for commercial varieties grown at the same Argentinean field sites in 2007−2008. [e] TI, tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [f] Reference *34*.

Table 2. Fiber and Proximate Composition of Seed from Insect-Protected Soybean MON 87701

	2007 U.S. field trials			2007−2008 Argentinean field trials			
component[a]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][e]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[d] [99% TI][e]	literature range[f]
fiber							
ADF	15.58 (13.53−17.05)	15.62 (14.00−19.02)	(12.79−17.98) [11.13, 20.21]	15.06 (13.30−20.51)	14.88 (12.61−19.45)	(11.81−18.19) [12.19, 17.09]	7.81−18.61
NDF	17.33 (15.06−21.80)	17.28 (15.02−22.45)	(13.32−23.57) [7.24, 28.70]	16.12 (14.22−19.09)	16.23 (15.00−17.53)	(13.64−19.01) [13.27, 18.44]	8.53−21.25
proximate							
ash	5.20 (4.70−5.90)	5.14 (4.70−5.88)	(4.32−5.62) [3.74, 6.45]	4.86 (4.57−5.24)	4.92 (4.46−5.25)	(4.39−5.28) [4.04, 5.67]	3.89−6.99
carbohydrates	34.22[g] (21.58−39.61)	36.44 (29.88−43.48)	(31.97−38.00) [28.17, 40.99]	38.62 (36.92−39.92)	38.78 (36.73−40.36)	(34.33−41.87) [33.33, 43.02]	29.6−50.2
moisture	7.52 (5.86−10.70)	6.84 (5.44−8.74)	(5.48−11.70) [1.45, 12.81]	10.05 (9.53−11.25)	10.04 (8.70−11.63)	(7.32−10.45) [6.88, 10.06]	4.7−34.4
protein	39.27[g] (36.49−42.23)	37.80 (32.29−41.87)	(38.14−42.66) [35.30, 45.38]	38.05 (36.35−39.62)	37.89 (36.05−39.20)	(34.70−42.19) [34.14, 43.15]	33.2−45.5
total fat	20.29 (17.33−23.08)	20.12 (17.24−22.55)	(17.90−23.56) [14.74, 25.18]	18.49 (18.05−19.35)	18.41 (17.59−19.28)	(15.10−20.28) [14.79, 21.86]	8.10−23.6

[a] Percent dry weight (moisture = % fresh weight, carbohydrates by calculation). [b] The least-squares 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 U.S. field sites in 2007. [d] The range of sample values for commercial varieties grown at the same Argentinean field sites in 2007−2008. [e] TI, tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [f] Reference *35*. [g] Statistically significant difference from control.

regions and were therefore considered to be within that of the commercial conventional soybean population.

Amino Acid Composition in Seed. For the U.S. production, levels of amino acids were higher in MON 87701 (**Table 3**), consistent with the increased protein content noted above. Differences in mean levels reached statistical significance for nine amino acids (alanine, glycine, histidine, isoleucine, leucine, lysine, serine, threonine, and valine); relative magnitude differences, expressed as difference from the control, were generally small, however (∼5%). With the exception of tryptophan, there were no significant differences ($p > 0.05$) in levels of amino acids between MON 87701 and the control in the Argentinean production. For tryptophan, the relative magnitude difference, expressed as difference from the control, was small, however (∼5.8%). The mean levels of all amino acids in the seed of MON 87701 from the U.S. and Argentinean production fell within the 99% tolerance interval established from the reference substances grown in the respective regions and were therefore considered to be within that of the commercial conventional soybean population.

Fatty Acid Composition in Seed. With the exception of 22:0 behenic acid in the U.S. production and 18:3 linolenic acid in the Argentinean production, there were no statistically significant differences ($p > 0.05$) in levels of fatty acids between MON 87701 and the control soybean (**Table 4**). The relative magnitude differences between MON 87701 and control mean values for these two components, expressed as difference of the control, were small (∼4.3% for 22:0 behenic acid and ∼4.0% for 18:3 linolenic acid) and not considered to be meaningful from

Table 3. Amino Acid Composition of Seed from Insect-Protected Soybean MON 87701

	2007 U.S. field trials			2007−2008 Argentinean field trials			
component[a]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][e]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[d] [99% TI][e]	literature range[f]
alanine	1.72[g] (1.66−1.84)	1.69 (1.59−1.82)	(1.66−1.93) [1.49, 2.02]	1.63 (1.55−1.78)	1.62 (1.43−1.75)	(1.43−1.87) [1.46, 1.89]	1.51−2.10
arginine	2.68 (2.36−3.00)	2.58 (2.37−2.89)	(2.54−2.99) [2.22, 3.25]	2.70 (2.41−3.03)	2.67 (2.34−2.97)	(2.15−3.05) [2.26, 3.22]	2.29−3.40
aspartic acid	4.90 (4.61−5.26)	4.85 (4.46−5.34)	(4.74−5.50) [4.22, 5.96]	4.79 (4.22−5.62)	4.75 (4.01−5.18)	(4.18−5.72) [4.04, 5.83]	3.81−5.12
cystine/cysteine	0.62 (0.57−0.67)	0.61 (0.56−0.69)	(0.53−0.68) [0.45, 0.77]	0.54 (0.41−0.70)	0.57 (0.46−0.69)	(0.41−0.71) [0.40, 0.72]	0.37−0.81
glutamic acid	7.65 (7.25−8.21)	7.53 (6.89−8.26)	(7.53−8.72) [6.60, 9.37]	7.31 (6.35−8.41)	7.19 (5.49−7.82)	(6.20−8.62) [6.33, 8.77]	5.84−8.20
glycine	1.75[g] (1.63−1.89)	1.70 (1.64−1.85)	(1.67−1.99) [1.49, 2.09]	1.64 (1.49−1.79)	1.63 (1.42−1.77)	(1.41−1.88) [1.42, 1.91]	1.46−2.00
histidine	1.12[g] (1.05−1.18)	1.08 (1.03−1.15)	(1.04−1.24) [0.94, 1.31]	1.04 (0.92−1.17)	1.04 (0.90−1.19)	(0.86−1.16) [0.86, 1.21]	0.88−1.18
isoleucine	1.81[g] (1.68−1.99)	1.76 (1.64−1.96)	(1.73−2.02) [1.54, 2.14]	1.71 (1.49−1.84)	1.69 (1.41−1.84)	(1.49−1.92) [1.48, 2.01]	1.54−2.08
leucine	3.04[g] (2.82−3.36)	2.94 (2.73−3.29)	(2.93−3.32) [2.64, 3.52]	2.84 (2.61−3.06)	2.81 (2.41−3.01)	(2.39−3.15) [2.50, 3.27]	2.59−3.62
lysine	2.74[g] (2.48−2.99)	2.62 (2.42−2.91)	(2.35−3.15) [2.05, 3.47]	2.53 (2.28−2.85)	2.49 (2.22−2.75)	(2.19−3.00) [2.21, 3.00]	2.29−2.84
methionine	0.53 (0.48−0.58)	0.53 (0.47−0.59)	(0.49−0.62) [0.42, 0.68]	0.47 (0.39−0.55)	0.50 (0.42−0.59)	(0.39−0.65) [0.38, 0.63]	0.43−0.68
phenylalanine	2.15 (1.91−2.48)	2.04 (1.91−2.38)	(1.97−2.44) [1.66, 2.64]	1.93 (1.75−2.17)	1.90 (1.63−2.10)	(1.62−2.14) [1.65, 2.23]	1.63−2.35
proline	2.01 (1.86−2.16)	1.96 (1.85−2.12)	(1.92−2.25) [1.73, 2.35]	1.94 (1.80−2.12)	1.93 (1.71−2.06)	(1.63−2.18) [1.70, 2.22]	1.69−2.28
serine	2.03[g] (1.90−2.19)	1.96 (1.87−2.13)	(1.96−2.30) [1.75, 2.38]	1.91 (1.74−2.07)	1.89 (1.51−2.06)	(1.63−2.18) [1.69, 2.19]	1.11−2.48
threonine	1.60[g] (1.50−1.72)	1.55 (1.49−1.68)	(1.54−1.74) [1.40, 1.83]	1.48 (1.35−1.59)	1.47 (1.23−1.60)	(1.28−1.72) [1.29, 1.73]	1.14−1.86
tryptophan	0.51 (0.47−0.54)	0.50 (0.46−0.53)	(0.47−0.55) [0.43, 0.59]	0.52[g] (0.49−0.56)	0.49 (0.41−0.51)	(0.45−0.56) [0.44, 0.57]	0.36−0.50
tyrosine	1.13 (0.96−1.33)	1.10 (0.98−1.22)	(1.04−1.31) [0.85, 1.48]	0.99 (0.85−1.26)	1.01 (0.74−1.22)	(0.79−1.25) [0.73, 1.28]	1.02−1.61
valine	1.92[g] (1.80−2.07)	1.86 (1.76−2.04)	(1.83−2.13) [1.64, 2.22]	1.83 (1.59−2.00)	1.81 (1.50−1.94)	(1.57−2.03) [1.61, 2.10]	1.60−2.20

[a] Percent dry weight. [b] The least-squares 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 U.S. field sites in 2007. [d] The range of sample values for commercial varieties grown at the same Argentinean field sites in 2007−2008. [e] TI, tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [f] Reference *35*. [g] Statistically significant difference from control.

a food/feed safety or nutritional perspective. The mean levels of all fatty acids in the seed of MON 87701 from the U.S. and Argentinean production fell within the 99% tolerance interval established (when available) from the reference substances grown in the respective regions and were therefore considered to be within that of the commercial conventional soybean population.

Antinutrient Composition in Seed. The results for the key antinutrients lectin, phytic acid, raffinose, stachyose, and trypsin inhibitor are presented in **Table 5**. With the exception of trypsin inhibitor in the U.S. production and stachyose in the Argentinean production, there were no statistically significant differences ($p > 0.05$) between MON 87701 and the control. The relative magnitude differences between MON 87701 and control mean values for these two components, expressed as difference from the control, were small (~8.8% for trypsin inhibitor and ~5.0% for stachyose) and not considered to be meaningful from a food/feed safety or nutritional perspective. The mean levels of these antinutrients in the seed of MON 87701 from the U.S. and Argentinean production fell within the 99% tolerance interval established from the reference substances grown in the respective regions and were therefore considered to be within that of the commercial conventional soybean population.

Isoflavone and Vitamin E Composition in Seed. The results for isoflavones (daidzein, genistein, and glycitein) and vitamin E in seed are presented in **Table 5**. Statistically significant differences ($p < 0.05$) were observed between MON 87701 and the control for daidzein from the U.S. field production and for vitamin E from both the U.S. and Argentinean productions. The relative magnitude differences between MON 7701 and control mean values for daidzein, expressed as difference of the control, was small (~10.4%) and not considered to be meaningful from a food/feed safety or nutritional perspective. The relative magnitude differences for vitamin E were greater [~23.3% (United States) and ~28.9% (Argentina)]; however, this difference is substantially less than the differences observed across the two productions (**Table 5**), and therefore the enhanced levels of vitamin E in MON 87701 relative to the control are probably not meaningful from a food/feed safety or nutritional perspective. The mean levels of isoflavones and vitamin E in the seed of MON 87701 from the U.S. and Argentinean production fell within the 99% tolerance interval established from the reference substances grown in the respective regions and were therefore considered to be within that of the commercial conventional soybean population.

Table 4. Fatty Acid Composition of Seed from Insect-Protected Soybean MON 87701

	2007 U.S. field trials			2007−2008 Argentinean field trials			
component[a]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][e]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[d] [99% TI][e]	literature range[f]
10:0 capric	0.20	0.21	(0.15−0.27)	not available	not available	not available	not available
	(0.14−0.25)	(0.16−0.26)	[0.065, 0.34]	not available	not available	not available	
14:0 myristic	0.093	0.094	(0.064−0.097)	0.086	0.084	(0.063−0.097)	0.071−0.238
	(0.082−0.10)	(0.083−0.11)	[0.052, 0.12]	(0.080−0.096)	(0.075−0.096)	[0.051, 0.11]	
16:0 palmitic	11.80	11.88	(9.80−12.38)	11.49	11.43	(9.90−12.63)	9.55−15.77
	(11.32−12.30)	(11.50−12.13)	[8.88, 13.53]	(11.11−11.97)	(10.80−12.04)	[9.32, 13.12]	
16:1 palmitoleic	0.092	0.095	(0.073−0.14)	0.084	0.087	(0.055−0.12)	0.086−0.194
	(0.073−0.11)	(0.078−0.11)	[0.037, 0.15]	(0.063−0.10)	(0.070−0.10)	[0.047, 0.13]	
17:0 heptadecanoic	0.094	0.093	(0.076−0.10)	0.11	0.11	(0.092−0.13)	0.085−0.146
	(0.084−0.10)	(0.082−0.099)	[0.066, 0.11]	(0.095−0.12)	(0.096−0.11)	[0.081, 0.13]	
17:1 heptadecenoic	0.041	0.041	(0.020−0.064)	not available	not available	not available	0.073−0.087
	(0.023−0.048)	(0.019−0.047)	[0.0058, 0.083]	not available	not available	not available	
18:0 stearic	4.59	4.70	(3.21−5.24)	4.98	4.98	(3.81−5.50)	2.70−5.88
	(3.97−5.36)	(4.03−5.36)	[1.88, 6.25]	(4.76−5.35)	(4.59−5.63)	[2.97, 6.03]	
18:1 oleic	22.35	22.71	(16.69−35.16)	18.64	18.73	(17.22−22.96)	14.3−32.2
	(19.21−26.64)	(20.34−28.78)	[5.01, 42.01]	(17.84−19.93)	(17.69−19.99)	[14.88, 24.59]	
18:2 linoleic	52.16	51.76	(44.17−57.72)	54.17	54.51	(51.51−56.73)	42.3−58.8
	(49.32−54.63)	(47.18−54.07)	[38.57, 66.94]	(53.26−54.80)	(53.20−55.53)	[50.10, 59.06]	
18:3 linolenic	7.24	7.11	(4.27−8.81)	9.34[g]	8.97	(7.59−9.60)	3.00−12.52
	(5.55−8.41)	(5.34−8.26)	[2.69, 10.81]	(8.58−9.91)	(8.32−9.90)	[6.57, 10.73]	
20:0 arachidic	0.51	0.51	(0.36−0.55)	0.46	0.46	(0.35−0.52)	0.163−0.482
	(0.41−0.58)	(0.41−0.57)	[0.23, 0.64]	(0.42−0.52)	(0.42−0.55)	[0.32, 0.52]	
20:1 eicosenoic	0.24	0.23	(0.21−0.30)	0.15	0.15	(0.13−0.22)	0.140−0.350
	(0.19−0.28)	(0.18−0.28)	[0.16, 0.33]	(0.13−0.17)	(0.14−0.16)	[0.094, 0.22]	
20:2 eicosadienoic	0.040	0.042	(0.016−0.054)	0.042	0.043	(0.017−0.064)	0.077−0.245
	(0.020−0.054)	(0.020−0.047)	[0.0029, 0.083]	(0.019−0.062)	(0.018−0.071)	[0.017, 0.068]	
22:0 behenic	0.56[g]	0.54	(0.38−0.59)	0.45	0.44	(0.35−0.50)	0.277−0.595
	(0.46−0.65)	(0.45−0.65)	[0.30, 0.67]	(0.40−0.50)	(0.39−0.49)	[0.32, 0.53]	

[a] Percent total fatty acid. [b] The least-squares 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 U.S. field sites in 2007. [d] The range of sample values for commercial varieties grown at the same Argentinean field sites in 2007−2008. [e] TI, tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [f] Reference *35*. [g] Statistically significant difference from control.

Table 5. Isoflavone, Antinutrient, and Vitamin Composition of Seed from Insect-Protected Soybean MON 87701

	2007 U.S. field trials			2007−2008 Argentinean field trials			
component[a]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[c] [99% TI][e]	MON 87701 mean[b] (range)[b]	control mean[b] (range)[b]	commercial (range)[d] [99% TI][e]	literature range[f]
antinutrient							
lectin	0.96	0.72	(0.090−2.47)	2.70	3.46	(1.32−11.18)	0.09−8.46
	(0.062−2.01)	(0.28−1.28)	[0, 3.40]	(1.48−5.03)	(1.60−8.39)	[0, 7.03]	
phytic acid	1.85	1.97	(1.10−2.32)	1.46	1.55	(0.81−2.27)	0.63−1.96
	(1.39−2.29)	(1.31−2.66)	[0.54, 3.05]	(0.90−1.92)	(1.02−2.10)	[0.61, 2.54]	
raffinose	1.33	1.34	(0.52−1.62)	1.14	1.15	(0.58−1.44)	0.21−0.66
	(0.49−1.70)	(0.43−1.85)	[0.038, 2.24]	(0.95−1.35)	(0.98−1.29)	[0.36, 1.56]	
stachyose	4.59	4.93	(1.97−5.55)	3.82[g]	4.02	(2.91−4.84)	1.21−3.50
	(1.83−6.42)	(2.27−6.65)	[0.99, 7.93]	(3.35−4.21)	(3.14−4.38)	[2.10, 5.35]	
trypsin inhibitor	26.06[g]	28.57	(20.84−37.24)	27.77	27.21	(18.14−42.51)	19.59−118.68
	(21.65−32.53)	(22.49−34.20)	[13.58, 46.02]	(18.89−33.26)	(23.45−30.96)	[12.07, 46.95]	
isoflavone							
daidzein	667.54[g]	604.88	(213.98−1273.94)	960.33	934.74	(361.48−1458.24)	60.0−2453.5
	(188.96−983.26)	(198.95−830.65)	[0, 1585.14]	(846.15−1090.89)	(826.96−1095.41)	[0, 1773.24]	
genistein	655.57	594.58	(148.06−1024.50)	886.75	858.71	(505.88−1095.57)	144.3−2837.2
	(214.73−863.84)	(244.95−760.87)	[0, 1352.86]	(778.99−960.43)	(757.45−976.36)	[182.37, 1390.68]	
glycitein	164.87	156.93	(32.42−208.45)	200.02	184.78	(49.40−255.94)	15.3−310.4
	(61.08−228.79)	(61.28−227.25)	[0, 272.12]	(143.11−252.60)	(136.52−217.42)	[0, 294.10]	
vitamin							
vitamin E	7.69[g]	6.24	(1.65−8.08)	4.40[g]	3.42	(1.12−6.94)	0.19−6.17
	(6.36−9.62)	(4.88−7.94)	[0, 11.09]	(3.61−5.29)	(2.87−4.11)	[0, 7.03]	

[a] Isoflavones in mg/kg dry weight; lectin in HU/mg of fresh weight; phytic acid, raffinose, and stachyose in percent dry weight; trypsin inhibitor in TIU/mg of dry weight; and vitamin E in mg/100 g of dry weight. [b] The least-squares 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 U.S. field sites in 2007. [d] The range of sample values for commercial varieties grown at the same Argentinean field sites in 2007−2008. [e] TI, tolerance interval, specified to contain 99% of the commercial variety population with 95% confidence; negative limits set to zero. [f] Reference *35*. [g] Statistically significant difference from control.

Table 6. Fiber and Proximate Composition of Soybean Seed from Insect-Protected Soybean MON 87701 Grown in the United States in 2007 and Used To Generate Processed Fractions

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
fiber					
ADF	16.67	15.13−18.34	16.16	15.76−16.47	7.81−18.61
NDF	16.72	15.40−17.91	16.54	16.26−17.04	8.53−21.25
proximate					
ash	5.09	4.89−5.32	5.07	4.85−5.30	3.89−6.99
carbohydrates	37.00	36.90−37.11	37.00	36.56−37.95	29.6−50.2
moisture	6.72	6.51−6.92	6.73	6.58−6.83	4.7−34.4
protein	38.55	38.06−38.83	38.76	38.02−39.33	33.2−45.5
total fat	19.35	18.91−19.52	19.18	18.81−19.37	8.10−23.6

[a] Percent dry weight (moisture = % fresh weight, carbohydrates by calculation). [b] The least-squares mean and range of eight values (two replicates analyzed in duplicate from two field sites). [c] Reference *35*.

Composition of Seed Used in Generation of Processed Fractions. Levels of proximates, fiber, amino acids, fatty acids, antinutrients, isoflavones, and vitamin E (**Tables 6−9**) were also assessed in MON 87701 grown in U.S. 2007 production and used for the generation of processed fractions. Results were broadly consistent with the analyses from the U.S. and Argentinean productions and confirmed that the seed composition of MON 87701 is equivalent to that of conventional soybean. Overall, 44 of 47 comparisons were not statistically significantly different ($p > 0.05$) from the control. Components that were significantly different ($p < 0.05$) included tyrosine, 16:0 palmitic acid, and 20:1 eicosenoic acid. The relative magnitude differences (when expressed as difference from the control) for tyrosine, 16:0 palmitic acid, and 20:1 eicosenoic acid were small (~1.6, ~2.2, and ~6.7%, respectively) and not considered to be meaningful from a food/feed safety or nutritional perspective. No significant differences ($p > 0.05$) in these components were observed between MON 87701 and control in the U.S. 2007 and Argentinean 2007−2008 productions, and values for these components in MON 87701 fell within literature ranges reported for conventional soybean. In all cases, the range of values for the MON 87701 and control components were similar, although in several cases these values were outside that reported in the literature. On the basis of these results, the seeds from MON 87701 and control soybean are considered to be equivalent and representative of commercial conventional soybean population.

Fiber, Proximate, and Antinutrient Composition in TD Meal. With the exception of ADF and ash, there were no statistically significant differences ($p > 0.05$) between MON 87701 and the control (**Table 10**) for TD meal. The relative magnitude differences between MON 87701 and control mean values for ADF and ash, expressed as difference of the control, were small (~15.7% for ADF and ~3.5% for ash) and not considered to be meaningful from a food/feed safety or nutritional perspective. With the exception of ADF and NDF, the mean values for fiber, proximates, and antinutrients for MON 87701 were within literature values reported for these components derived from commercial conventional soybean or within the range of values established for the control and therefore within that of the population of commercial conventional soybean. Mean values of ADF and NDF for MON 87701 and the control were outside the literature range but close in magnitude to each other. On the basis of these results the fiber, proximate, and antinutrient compositions of MON 87701 soybean and control are considered to be equivalent and representative of that of commercial conventional soybean population.

Table 7. Amino Acid Composition of Soybean Seed from Insect-Protected Soybean MON 87701 Grown in the United States in 2007 and Used To Generate Processed Fractions

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
alanine	1.62	1.53−1.67	1.64	1.60−1.68	1.51−2.10
arginine	2.54	2.44−2.61	2.57	2.50−2.64	2.29−3.40
aspartic acid	4.65	4.35−4.82	4.75	4.58−4.90	3.81−5.12
cystine	0.59	0.57−0.61	0.60	0.58−0.62	0.37−0.81
glutamic acid	7.39	6.93−7.68	7.56	7.32−7.80	5.84−8.20
glycine	1.67	1.62−1.69	1.68	1.66−1.69	1.46−2.00
histidine	1.14	1.11−1.18	1.13	1.11−1.15	0.88−1.18
isoleucine	1.76	1.64−1.83	1.78	1.74−1.83	1.54−2.08
leucine	2.89	2.74−2.99	2.92	2.85−2.99	2.59−3.62
lysine	2.43	2.30−2.53	2.47	2.39−2.53	2.29 −2.84
methionine	0.54	0.52−0.56	0.55	0.53−0.57	0.43−0.68
phenylalanine	1.93	1.86−1.98	1.93	1.89−1.97	1.63−2.35
proline	1.92	1.84 −1.96	1.94	1.91−1.99	1.69−2.28
serine	1.93	1.87−1.97	1.95	1.91−1.99	1.11−2.48
threonine	1.52	1.47−1.55	1.53	1.51−1.54	1.14−1.86
tryptophan	0.38	0.37−0.39	0.38	0.37−0.39	0.3−0.50
tyrosine	0.97[d]	0.95−1.00	0.96	0.93−0.98	1.02−1.61
valine	1.85	1.77−1.89	1.86	1.82−1.89	1.60−2.20

[a] Percent dry weight. [b] The least-squares mean and range of 8 values (two replicates analyzed in duplicate from two field sites). [c] Reference *35*. [d] Statistically significant difference from control.

Table 8. Fatty Acid Composition of Seed from Insect-Protected Soybean MON 87701 Grown in the United States in 2007 and Used To Generate Processed Fractions

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
14:0 myristic	0.087	0.082−0.089	0.090	0.084−0.099	0.071−0.238
16:0 palmitic	11.58[d]	11.52−11.65	11.84	11.76−12.02	9.55−15.77
16:1 palmitoleic	0.082	0.074−0.088	0.092	0.081−0.10	0.086−0.194
17:0 heptadecanoic	0.098	0.092−0.11	0.093	0.090−0.094	0.085−0.146
18:0 stearic	4.33	3.89−4.78	4.33	3.99−4.75	2.70−5.88
18:1 oleic	20.70	19.11−22.02	20.24	19.30−21.18	14.3−32.2
18:2 linoleic	53.30	51.95−54.66	53.95	52.76−55.26	42.3−58.8
18:3 linolenic	8.09	8.00−8.24	8.12	7.91−8.33	3.00−12.52
20:0 arachidic	0.87	0.43−2.09	0.44	0.41−0.48	0.163−0.482
20:1 eicosenoic	0.20[d]	0.19−0.20	0.18	0.17−0.19	0.140−0.350
20:2 eicosadienoic	0.040	0.027−0.055	0.039	0.036−0.047	0.077−0.245
22:0 behenic	0.48	0.45−0.50	0.45	0.42−0.48	0.277−0.595
24:0 lignoceric	0.15	0.15−0.17	0.14	0.079−0.16	not available

[a] Percent total fatty acid. [b] The least-squares mean and range of 8 values (two replicates analyzed in duplicate from two field sites). [c] Reference *35*. [d] Statistically significant difference from control.

Amino Acid Composition in TD Meal. The results for amino acid in meal are presented in **Table 11**. With the exception of four amino acids (arginine, histidine, phenylalanine, and tryptophan), there were no statistically significant differences ($p > 0.05$) between MON 87701 and the control. The relative magnitude differences between MON 87701 and control mean values for these amino acids, expressed as difference of the control, were small (all < 4.6%) and not considered to be meaningful from a food/feed safety or nutritional perspective. With the exception of alanine, lysine, and tyrosine, the mean values for all amino acids for MON 87701 were within literature values reported for these components derived from commercial conventional soybean and therefore within that of the population of commercial conventional soybean. On the basis of these results the amino acid

Table 9. Isoflavone, Antinutrient, and Vitamin Composition of Soybean Seed from Insect-Protected Soybean MON 87701 Grown in the United States in 2007 and Used To Generate Processed Fractions

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
antinutrient					
lectin	0.94	0.26−1.62	0.75	0.45−0.99	0.09−8.46
phytic acid	1.37	1.21−1.51	1.34	1.19−1.47	0.63−1.96
raffinose	1.06	1.05−1.07	1.07	1.04−1.10	0.21−0.66
stachyose	4.91	4.71−5.18	5.07	4.79−5.44	1.21−3.50
trypsin inhibitor	31.96	30.29−34.07	33.48	31.33−35.15	19.59−118.68
isoflavone					
daidzein	808.94	737.72−917.70	772.14	692.04−839.36	60.0−2453.5
genistein	760.30	710.68−788.81	724.94	700.60−748.24	144.3−2837.2
glycitein	169.65	124.92−194.99	182.94	167.52−212.79	15.3−310.4
vitamin					
vitamin E	6.82	6.28−7.54	5.08	4.70−5.48	0.19−6.17

[a] Isoflavones in mg/kg dry weight; lectin in HU/mg of fresh weight; phytic acid, raffinose, and stachyose in percent dry weight; trypsin inhibitor in TIU/mg of dry weight; and vitamin E in mg/100 g of dry weight. [b] The least-squares mean and range of eight values (two replicates analyzed in duplicate from two field sites). [c] Reference *35*.

Table 10. Fiber, Proximate, and Antinutrient Composition of Soybean Meal from Insect-Protected Soybean MON 87701

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
fiber					
ADF	4.25[e]	3.94−4.42	5.04	4.66−5.27	5.2−6.7
NDF	6.72	6.36−7.07	7.18	6.82−7.64	7.4−12.2
proximate					
ash	6.65[e]	6.35−6.94	6.42	6.12−6.74	5.2−9.1
carbohydrates	39.04	38.01−39.57	39.59	38.99−40.12	32.0[d]−38.0[d]
moisture	5.59	3.57−7.84	3.94	2.40−5.54	5.58−11.7
protein	52.97	52.51−53.98	51.82	49.83−53.27	47.4−59.5
total fat	1.34	1.01−1.68	2.16	1.43−3.52	0.5−3.30
antinutrient					
phytic acid	2.04	1.77−2.38	1.93	1.75−2.15	1.3−4.1
trypsin inhibitor	3.08	2.97−3.22	3.25	3.01−3.56	3.8−17.9

[a] Percent dry weight (moisture = fresh weight, carbohydrates by calculation) and trypsin inhibitor in TIU/mg of dry weight. [b] The least-squares mean and range of four values (one replicate analyzed in duplicate from two field sites). [c] Reference *34*. [d] Reference *36*. [e] Statistically significant difference from control.

compositions of MON 87701 soybean and control are considered to be equivalent and representative of commercial conventional soybean population.

Fatty Acid and Vitamin E Composition in RBD Oil. With the exception of 20:1 eicosenoic acid, 22:0 behenic acid, and vitamin E, there were no statistically significant differences ($p > 0.05$) between MON 87701 and control (**Table 12**). The relative magnitude differences between MON 87701 and control mean values for the fatty acid components, expressed as difference from the control, were small (~6.6% for 20:1 eicosenoic acid and ~5.8% for behenic acid) and not considered to be meaningful from a food/feed safety or nutritional perspective. The relative magnitude difference for vitamin E was greater (~36.8%), consistent with the enhanced levels of this nutrient observed in the seed of MON 87701. With the exception of 16:0 palmitic acid, the mean values for all fatty acids and for vitamin E for MON 87701 were within literature values reported for these components derived from commercial conventional soybean and therefore within that of the population of commercial conventional soybean. Both MON 87701 and control values for 16:0 palmitic acid were outside reported literature values, although the range of

Table 11. Amino Acid Composition of Meal from Insect-Protected Soybean MON 87701

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
alanine	2.13	2.10−2.14	2.16	2.01−2.36	2.18−2.59
arginine	3.47[d]	3.40−3.54	3.40	3.26−3.52	3.29−4.49
aspartic acid	6.09	6.00−6.15	6.22	5.78−6.78	5.18−6.83
cystine	0.79	0.77−0.80	0.80	0.78−0.84	0.6−0.92
glutamic acid	9.70	9.52−9.83	9.86	9.16−10.64	8.05−11.21
glycine	2.28	2.25−2.31	2.22	2.16−2.25	2.02−2.40
histidine	1.51[d]	1.45−1.55	1.46	1.43−1.49	1.32−1.63
isoleucine	2.36	2.28−2.41	2.37	2.22−2.48	2.11−2.74
leucine	3.88	3.80−3.97	3.86	3.63−4.01	3.62−4.72
lysine	2.96	2.89−3.03	3.09	2.84−3.46	2.97−3.69
methionine	0.74	0.72−0.76	0.73	0.70−0.75	0.5−0.9
phenylalanine	2.65[d]	2.60−2.71	2.57	2.48−2.66	2.39−3.19
proline	2.58	2.55−2.60	2.56	2.44−2.66	2.32−3.05
serine	2.58	2.53−2.61	2.52	2.43−2.59	1.97−3.3
threonine	2.04	2.01−2.05	2.00	1.92−2.08	0.80−2.24
tryptophan	0.71[d]	0.69−0.74	0.68	0.67−0.70	0.60−2.08
tyrosine	1.30	1.26−1.33	1.29	1.27−1.31	1.68−2.17
valine	2.46	2.38−2.51	2.46	2.32−2.59	2.29−2.92

[a] Percent dry weight. [b] The least-squares mean and range of eight values (two replicates analyzed in duplicate from two field sites). [c] Reference *34*. [d] Statistically significant difference from control.

Table 12. Fatty Acid and Vitamin E Composition of Oil from Insect-Protected Soybean MON 87701

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
14:0 myristic	0.090	0.090−0.091	0.089	0.085−0.093	ND−0.2[d]
16:0 palmitic	12.20	12.12−12.27	12.23	12.17−12.29	7−12
16:1 palmitoleic	0.056	0.039−0.061	0.073	0.059−0.087	ND−0.2[d]
17:0 heptadecanoic	0.098	0.095−0.099	0.096	0.094−0.10	ND−0.1[d]
18:0 stearic	4.62	4.20−5.14	4.64	4.25−5.05	2−5
18:1 oleic	21.91	20.67−22.97	21.93	21.29−22.59	19−34
18:2 linoleic	54.00	52.26−55.68	53.98	52.87−55.11	48−60
18:3 linolenic	5.60	5.57−5.65	5.60	5.39−5.73	2−10
20:0 arachidic	0.50	0.47−0.56	0.49	0.46−0.53	0.1−0.6[d]
20:1 eicosenoic	0.25[e]	0.25−0.25	0.24	0.23−0.24	ND−0.5[d]
22:0 behenic	0.52[e]	0.49−0.54	0.49	0.48−0.51	ND−0.7[d]
24:0 lignoceric	0.15	0.12−0.18	0.15	0.12−0.17	ND−0.5[d]
vitamin E	29.44[e]	26.95−32.75	21.51	19.70−24.00	0.9−35.2

[a] Fatty acids as percent total fatty acid; vitamin E as mg/100 g of fresh weight; literature range in percent fresh weight; ND, nondetectable, defined as ≤0.05%. [b] The least-squares mean and range of four values (one replicate analyzed in duplicate from two field sites. [c] Reference *34*. [d] Reference *37*. [e] Statistically significant difference from control.

values observed for MON 87701 and control components were similar. On the basis of these results the fatty acid and vitamin composition of MON 87701 soybean and control are considered to be equivalent and representative of commercial conventional soybean population.

Amino Acid and Moisture Composition in Protein Isolate. There were no statistically significant differences ($p > 0.05$) in levels of amino acids and moisture from protein isolate from MON 87701 and control (**Table 13**). In all cases the range of values for the MON 87701 and control components were similar, although in many cases values for both MON 87701 and the control were outside that reported in the literature. On the basis of these results the protein isolate from MON 87701 and control are considered to be equivalent and representative of commercial conventional soybean population.

Table 13. Amino Acid and Moisture Composition of Protein Isolate from Insect-Protected Soybean MON 87701

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
alanine	3.33	3.31−3.36	3.35	3.31−3.39	4.02−4.15
arginine	6.80	6.66−6.97	6.90	6.73−7.00	7.32−7.66
aspartic acid	12.35	12.03−12.69	12.30	12.10−12.58	11.16−11.42
cystine/cysteine	1.40	1.26−1.48	1.46	1.39−1.49	1.04−1.13
glutamic acid	20.87	20.21−21.44	20.88	20.48−21.33	18.85−19.44
glycine	3.65	3.61−3.69	3.70	3.63−3.77	3.96−4.12
histidine	2.31	2.26−2.33	2.33	2.27−2.36	2.42−2.51
isoleucine	4.22	4.16−4.29	4.24	4.19−4.30	4.15−4.63
leucine	6.93	6.78−7.05	6.93	6.89−7.02	7.70−7.92
lysine	5.74	5.61−5.85	5.72	5.61−5.85	5.97−6.22
methionine	1.13	1.01−1.22	1.19	1.12−1.24	1.22−1.34
phenylalanine	4.77	4.63−4.87	4.83	4.76−4.90	5.15−5.29
proline	4.82	4.75−4.89	4.84	4.74−4.90	4.82−4.95
serine	4.63	4.53−4.70	4.64	4.55−4.69	5.23−5.45
threonine	3.05	3.02−3.09	3.08	3.05−3.14	3.42−3.56
tryptophan	1.16	1.14−1.18	1.16	1.12−1.21	1.04−1.18
tyrosine	2.86	2.79−2.93	2.88	2.81−2.97	3.57−3.71
valine	4.08	4.04−4.11	4.12	4.10−4.14	4.15−4.66
moisture	2.36	2.08−2.77	2.63	2.12−3.45	3.9−7.0

[a] Percent dry weight (moisture = fresh weight). [b] The least-squares mean and range of four values (one replicate analyzed in duplicate from two field sites). [c] Reference *34*.

Table 14. Phosphatide Composition of Crude Lecithin from Insect-Protected Soybean MON 8770

	MON 87701		control		
component[a]	mean[b]	range[b]	mean[b]	range[b]	literature range[c]
l-α-phosphatidic acid	3.32	2.67−4.52	3.12	2.63−4.06	0.2−14.0
l-α-phosphatidylcholine	5.91	3.61−8.18	5.20	3.24−6.95	12.0−46.0
l-α-phosphatidylethanolamine	3.73	2.34−5.07	3.37	2.48−4.19	8.0−34.0
l-α-phosphatidylinositol	3.51	2.42−4.60	3.27	2.27−4.09	1.7−21.0

[a] Percent fresh weight. [b] The least-squares mean and range of four values (one replicate analyzed in duplicate from two field sites). [c] Reference *34*.

Phosphatide Composition in Lecithin. There were no statistically significant differences ($p > 0.05$) in levels of phosphatides (phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol) from lecithin from MON 87701 and control (**Table 14**). In all cases the ranges of values for MON 87701 and control components were similar. On the basis of these results the crude lecithin from MON 87701 soybean and control are considered to be equivalent and representative of commercial conventional soybean population.

Concluding Remarks. Overall, the results of the analyses of seed and forage samples from the first U.S. 2007 production showed that there were no significant differences ($p > 0.05$) between MON 87701 and the control for 38 of 53 comparisons from the combined site analysis. Differences included values for carbohydrates by calculation, protein, nine amino acids, 22:0 behenic acid, trypsin inhibitor, daidzein, and vitamin E. Overall, the results of the analyses of seed and forage samples from the Argentinean 2007−2008 production showed that there were no significant differences ($p > 0.05$) between MON 87701 and the control for 49 of 53 comparisons from the combined site analysis. Differences included tryptophan, 18:3 linolenic acid, stachyose, and vitamin E. From the second U.S. production used to generate seed and processed fractions, analysis showed that only 3 of the 47 seed components, 6 of the 27 TD meal components, and 3 of the 13 RBD oil components were observed to be significantly different ($p < 0.05$) between MON 87701 and the control. No differences were observed in the protein isolate and crude lecithin fractions. When differences were observed, relative magnitude differences were generally small and the test mean values fell within the 99% tolerance interval or literature values for commercial conventional soybean. Therefore, it is concluded that seed, forage, and processed fractions (meal, oil, protein isolate, and crude lecithin) from MON 87701 are compositionally equivalent to those of conventional soybean.

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