JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Insect-Protected Event DAS-81419-2 Soybean (*Glycine max* L.) Grown in the United States and Brazil Is Compositionally Equivalent to Nontransgenic Soybean

Brandon J. Fast,*[,†] Ariane C. Schafer,[‡] Tempest Y. Johnson,[†] Brian L. Potts,[§] and Rod A. Herman[†]

[†]Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268, United States

[‡]Dow AgroSciences Industrial Ltda., Rod. Anhanguera Km 296, Cravinhos, SP 14140-000, Brazil

[§]Covance Laboratories Inc., 3301 Kinsman Boulevard, Madison, Wisconsin 53704, United States

ABSTRACT: The transgenic soybean event DAS-81419-2 contains genes that encode the Cry1F, Cry1Ac, and PAT proteins. Cry1F and Cry1Ac provide protection against key lepidopteran insect pests, while PAT confers tolerance to the herbicide glufosinate. To satisfy regulatory requirements for the safety evaluation of transgenic crops, studies were conducted in the United States and Brazil to evaluate the nutrient and antinutrient composition of event DAS-81419-2 soybean. On the basis of the results of these studies, event DAS-81419-2 soybean is compositionally equivalent to nontransgenic soybean. This conclusion concurs with numerous other published studies in soybean and other crops where compositional equivalence between the transgenic crop and its nontransgenic comparator has been demonstrated.

KEYWORDS: *crop composition, DAS-81419-2, soybean, safety evaluation, risk assessment*

INTRODUCTION

Soybean event DAS-81419-2 (Conkesta seed) [Conkesta is a registered trademark of The Dow Chemical Company ("DOW") or an affiliated company of Dow] was created via *Agrobacterium*-mediated transformation and expresses the Cry1Ac, Cry1F, and PAT proteins, which are derived from the Gram-positive bacteria *Bacillus thuringiensis* subspecies *kurstaki*, *Bacillus thuringiensis* subspecies *aizawai*, and *Streptomyces viridochromogenes*, respectively. The Cry1Ac and Cry1F proteins provide plant protection against certain lepidopteran insect pests,[1−3] while PAT (used as a selectable marker during the development of event DAS-81419-2 soybean) confers tolerance to the herbicide glufosinate.[4] The protection against key lepidopteran soybean insect pests such as soybean looper (*Chrysodeixis includens*), velvetbean caterpillar (*Anticarsia gemmatalis*), fall armyworm (*Spodoptera frugiperda*), and tobacco budworm (*Heliothis virescens*) that is provided by the Cry1Ac and Cry1F proteins in event DAS-81419-2 will offer soybean producers an invaluable insect management tool that does not require the use of traditional insecticides to control these pests.

Conventional plant breeding involves a multitude of techniques such as direct plant selections, tissue culture, and mutagenesis, and is often associated with mutations, deletions, insertions, and rearrangements with little or no knowledge of the underlying genetic changes that occurred to achieve the desired trait(s).[5] Transgenesis, unlike conventional breeding, consists of the insertion of a known sequence of DNA at a specific insertion point; hence, desirable traits are achieved through a clearly understood mechanism (often the production of a protein that confers insect protection or herbicide tolerance).[6] The development of transgenic crop plants includes extensive research that is conducted to elucidate the genetic changes that are caused by the transgenesis and to investigate any disruption that might occur in native genes or regulatory elements.

The nondisruptive nature of transgenesis has been demonstrated repeatedly. In 86 published studies that were conducted to assess the composition of transgenic crops, compositional equivalence between the transgenic crop and an appropriate nontransgenic comparator was demonstrated.[7−92] Regardless of this amassed body of scientific evidence, government regulations continue to require the conduct of composition studies as part of the safety evaluation of transgenic crops.[93,94]

Previous composition studies have been conducted with soybean expressing Cry1Ac and PAT, cotton expressing Cry1F, Cry1Ac, and PAT, and corn expressing Cry1F; in each of these studies, compositional equivalence between the transgenic crop and the nontransgenic crop was concluded.[11,89,91,95] The objective of this research was to determine whether the composition of forage and seed from event DAS-81419-2 soybean expressing Cry1Ac, Cry1F, and PAT is equivalent to that of nontransgenic soybean.

MATERIALS AND METHODS

Soybean Samples for Compositional Analyses. Field studies were conducted in the United States (U.S.) and Brazil to produce soybean forage and seed samples of event DAS-81419-2, a near-isogenic nontransgenic comparator of the same variety that was never transformed (isoline), and commercially available nontransgenic varieties for compositional analyses. The U.S. study was conducted in 2011 at 10 field sites near Richland, Iowa, Atlantic, Iowa, Carlyle, Illinois, Wyoming, Illinois, Frankfort, Indiana, Fisk, Missouri, La Plata, Missouri, York, Nebraska, Brunswick, Nebraska, and Germansville, Pennsylvania. The Brazil study was conducted in 2012−2013 at two field sites near Indianópolis, Minas Gerais, and Montividiu, Goiás.

Received: October 16, 2014
Revised: January 28, 2015
Accepted: January 31, 2015
Published: February 1, 2015

DOI: 10.1021/jf505015y
J. Agric. Food Chem. 2015, 63, 2063−2073

The experimental design was a randomized complete block with four replicate plots of each entry at each field site in the U.S. study and three replicate plots of each entry at each field site in the Brazil study. In the U.S. study, plots were four rows wide by 7.6 m long with 6 cm seed spacing and 76 cm row spacing. Each four row plot was separated from adjacent plots by two border rows of a commercially available nontransgenic soybean variety of similar maturity. In the Brazil study, plots were 10 rows wide by 10 m long with 10 cm seed spacing and 50 cm row spacing. In addition to DAS-81419-2 and the isoline, a total of five nontransgenic commercial varieties were used (DSR 75213-72, HiSOY 38C60, IL3503, Porter 75148, and Williams 82) in the U.S. study; three out of the five varieties were chosen at random and planted at each field site. In the Brazil study, the nontransgenic commercial variety CD 215 was planted at both field sites. Standard commercial agronomic practices (e.g., insect and weed control) were implemented at each field site (uniformly across the entire trial) to produce a commercially acceptable crop. Forage and seed samples were collected at the R3 (beginning pod) and R8 (full maturity) growth stages, respectively, and shipped to Covance Laboratories Inc. (Madison, Wisconsin) for compositional analysis. Samples were shipped frozen and were placed in freezer storage at the analytical laboratory where they remained until removal for preparation or analysis.

Compositional Analyses. At Covance Laboratories composition samples were cryogenically ground to a homogeneous state using a blender and liquid nitrogen prior to assay. Analytes in forage samples (9 total) included proximates (moisture, carbohydrates, ash, crude fat, and protein), fiber [acid detergent fiber (ADF) and neutral detergent fiber (NDF)], and minerals (calcium and phosphorus); seed analytes (80 total) included proximates (moisture, carbohydrates, ash, crude fat, and protein), fiber (ADF, NDF, crude fiber, and total dietary fiber), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium, and zinc), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine), fatty acids (8:0 caprylic, 10:0 capric, 12:0 lauric, 14:0 myristic, 14:1 myristoleic, 15:0 pentadecanoic, 15:1 pentadecenoic, 16:0 palmitic, 16:1 palmitoleic, 17:0 heptadecanoic, 17:1 heptadecenoic, 18:0 stearic, 18:1 oleic, 18:2 linoleic, 18:3 linolenic, 18:3 γ-linolenic, 20:0 arachidic, 20:1 eicosenoic, 20:2 eicosadienoic, 20:3 eicosatrienoic, 20:4 arachidonic, and 22:0 behenic), vitamins [β-carotene, vitamin B_1 (thiamine hydrochloride), vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_5 (pantothenic acid), vitamin B_6 (pyridoxine hydrochloride), vitamin B_9 (folic acid), vitamin C (ascorbic acid), tocopherols (α, β, γ, δ, total)], and bioactives (lectin, phytic acid, raffinose, stachyose, trypsin inhibitor, total daidzein equivalent, total genistein equivalent, and total glycitein equivalent). With the exception of crude fiber and selenium, samples were assayed using previously published methods.[23] Crude fiber was determined gravimetrically as the loss on ignition of dried residue remaining after digestion of the samples with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions. Selenium samples were closed-vessel microwave digested with nitric acid (HNO_3) and water. After digestion, the solution was brought to a final volume with water. To normalize the organic contribution between samples and standards, a dilution was prepared for analysis that contained methanol. The selenium concentration was determined with Se^{78} using an inductively coupled plasma-mass spectrometer (ICP-MS) with a dynamic reaction cell (DRC) by comparing the counts generated by standard solutions.

Statistical Analysis. At Dow AgroSciences, analysis of variance (ANOVA) was conducted across field sites within the U.S. study and within the Brazil study using a mixed model where entry was considered a fixed effect and location, block within location, and location-by-entry were designated as random effects.[96] Paired contrasts were performed between DAS-81419-2 and the isoline using t tests, and a false-discovery rate adjustment was applied to the P-values to account for multiplicity.[97−99] Differences were considered significant at the 95% confidence level. Analytes were excluded from the statistical analysis if more than 50% of the results were less than the limit of

Table 1. Proximate, Fiber, and Mineral Composition of DAS-81419-2 Soybean Forage

	U.S. study					Brazil study				
analytical component[a]	P value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	P value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	literature range min−max[f]
ash	0.926	8.96 ± 0.31 7.01−16.9	8.99 ± 0.32 7.51−10.9	6.80−15.2	0.955	9.08 ± 0.72 7.63−10.6	8.88 ± 0.72 7.87−11.1	6.37−7.58	4.68−10.782
carbohydrates	0.693	68.2 ± 1.1 57.2−76.4	67.7 ± 1.1 60.3−75.7	50.1−75.6	0.700	64.6 ± 0.6 62.5−66.5	65.9 ± 0.6 64.7−66.8	69.7−71.5	59.8−80.18
crude protein	0.841	20.4 ± 0.8 13.9−29.8	20.6 ± 0.8 15.8−25.8	14.0−35.5	0.700	23.9 ± 0.6 21.7−25.5	22.7 ± 0.6 21.1−24.6	20.0−21.6	11.2−24.71
moisture	0.719	79.7 ± 0.8 75.8−84.3	79.4 ± 0.8 75.5−83.6	75.3−85.5	0.784	81.0 ± 0.8 80.4−81.6	81.8 ± 0.7 79.7−85.0	72.6−76.9	32.05−84.60
crude fat	0.607	2.49 ± 0.19 0.898−3.89	2.70 ± 0.20 0.857−4.32	0.685−5.32	0.955	2.6 ± 0.2 2.30−3.07	2.57 ± 0.25 1.40−3.71	1.78−2.84	1.01−9.87
ADF	0.611	34.7 ± 2.1 22.4−56.7	33.3 ± 2.1 22.2−45.4	19.4−63.3	0.960	25.1 ± 1.6 21.6-31.4	25.6 ± 1.6 19.7−31.3	24.9−29.8	22.72−59.03
NDF	0.609	41.6 ± 2.6 27.8−70.9	39.9 ± 2.6 27.2−59.3	25.2−82.0	0.955	31.3 ± 1.2 27.7−36.4	31.0 ± 1.2 27.5−34.0	31.2−35.8	19.61−73.05
calcium	0.616	1377 ± 64 940−1840	1401 ± 64 908−1740	874−2000	0.700	1134 ± 61 995−1380	1040 ± 61 950−1090	697−988	NR[g]
phosphorus	0.841	266 ± 7 206−327	268 ± 7 201−342	187−381	0.912	254 ± 16 211−296	262 ± 16 223−337	173−238	NR[g]

[a]Moisture = percent fresh weight; calcium and phosphorus = mg/100g dry weight; all others = percent dry weight. [b]P value (false discovery rate adjusted) for t test comparing DAS-81419-2 with isoline. [c]U.S. study: mean across 10 field sites with four replicates per site; Brazil study: mean across two field sites with three replicates per site. [d]Minimum (min) and maximum (max) represent values observed in single replicate plots. [e]U.S. study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots across five commercially available nontransgenic soybean varieties with three varieties planted at each field site. Brazil study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots of one commercially available nontransgenic soybean variety planted at both field sites. [f]See Materials and Methods section for reference citations. [g]NR = not reported.

DOI: 10.1021/jf505015y
J. Agric. Food Chem. 2015, 63, 2063−2073

Table 2. Proximate and Fiber Composition of DAS-81419-2 Soybean Seed

	U.S. study				Brazil study				
analytical component[a]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	literature range min−max[f]
ash	0.233	5.06 ± 0.07 4.62−5.68	5.18 ± 0.07 4.57−6.05	3.79−6.79	0.786	5.35 ± 0.09 5.19−5.45	5.43 ± 0.09 5.14−5.60	4.84−5.15	3.885−6.994
carbohydrates	0.616	38.8 ± 0.7 33.6−41.2	39.0 ± 0.7 33.3−43.3	29.9−40.7	0.700	38.3 ± 2.9 33.7−44.2	32.9 ± 2.9 31.6−34.4	33.8−34.9	29.3−50.2
crude protein	0.662	37.9 ± 0.6 34.3−41.9	38.1 ± 0.6 32.9−42.8	36.5−46.0	0.972	39.9 ± 0.5 39.5−40.6	40.0 ± 0.5 38.9−41.5	38.5−40.4	32−48.4
moisture	0.196	12.3 ± 0.9 8.29−19.2	11.7 ± 0.9 7.56−17.9	7.94−22.7	0.700	36.0 ± 2.9 31.2−42.0	28.7 ± 2.9 25.7−30.6	14.9−18.6	4.7−34.4
crude fat	0.108	18.2 ± 0.5 15.4−21.5	17.7 ± 0.5 14.2−21.0	14.1−21.4	0.700	16.5 ± 2.6 11.1−21.1	21.7 ± 2.6 21.0−22.5	20.6−21.9	8.104−24.7
ADF	0.845	15.3 ± 0.7 10.6−22.8	15.2 ± 0.7 10.5−22.5	10.2−21.0	0.986	11.9 ± 0.9 9.03−15.7	11.9 ± 0.9 10.1−14.1	14.0−16.6	7.81−26.26
NDF	0.719	17.5 ± 0.8 11.7−24.2	17.7 ± 0.8 11.6−25.5	10.6−21.9	0.700	15.0 ± 0.8 12.8−17.6	14.4 ± 0.8 13.0−16.3	14.6−17.2	8.53−23.90
crude fiber	not measured				0.700	6.67 ± 0.99 5.28−8.21	7.72 ± 0.99 6.47−8.82	10.6−12.1	4.12−13.87
total dietary fiber	0.765	23.8 ± 0.9 17.6−29.1	24.0 ± 0.9 17.4−31.3	16.1−29.2	0.700	17.0 ± 0.4 16.4−17.4	17.6 ± 0.4 16.9−18.7	17.5−20.2	NR[g]

[a]Moisture = percent fresh weight; all others = percent dry weight. [b]*P* value (false discovery rate adjusted) for *t* test comparing DAS-81419-2 with isoline. [c]U.S. study: mean across 10 field sites with four replicates per site. Brazil study: mean across two field sites with three replicates per site. [d]Minimum (min) and maximum (max) represent values observed in single replicate plots. [e]U.S. study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots across five commercially available nontransgenic soybean varieties with three varieties planted at each field site. Brazil study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots of one commercially available nontransgenic soybean variety planted at both field sites. [f]See Materials and Methods section for reference citations. [g]NR = not reported.

Table 3. Mineral Composition of DAS-81419-2 Soybean Seed

	U.S. study				Brazil study				
analytical component[a]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	literature range min−max[f]
calcium	0.476	270 ± 8 233−335	267 ± 8 205−328	181−308	0.700	332 ± 12 318−359	322 ± 12 304−341	264−350	116.55−510
copper	0.747	1.32 ± 0.06 0.922−1.64	1.33 ± 0.06 0.894−1.72	0.693−1.86	0.714	0.947 ± 0.140 0.765−1.13	0.911 ± 0.140 0.694−1.07	0.626−1.06	0.632−1.092
iron	0.693	9.56 ± 1.40 6.61−27.3	10.3 ± 1.4 6.21−42.9	6.33−151	0.700	20.1 ± 3.3 14.0−38.8	13.8 ± 3.3 11.4−14.7	6.34−13.5	3.734−10.954
magnesium	0.747	233 ± 4 204−256	232 ± 4 197−257	205−278	0.700	294 ± 8 281−314	267 ± 8 257−276	280−296	219.40−312.84
manganese	0.719	2.64 ± 0.09 2.13−3.09	2.67 ± 0.09 2.01−3.96	2.22−7.18	0.700	2.30 ± 0.27 1.84−2.71	2.48 ± 0.27 2.13−2.79	1.77−2.88	2.52−3.876
phosphorus	0.476	607 ± 14 536−704	619 ± 14 494−708	471−759	0.700	588 ± 29 539−655	620 ± 29 536−670	509−586	506.74−935.24
potassium	0.478	1799 ± 21 1660−1940	1819 ± 21 1490−1980	1650−2050	0.700	1858 ± 21 1790−1930	1977 ± 21 1910−2050	1760−1840	1868.01−2510
selenium	0.616	460 ± 187 <LOQ−2370	498 ± 187 <LOQ−2560	<LOQ−3060	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NR[h]
sodium	NA	NA[g] <LOQ	NA[g] <LOQ	<LOQ−18.5	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	4.05−30
zinc	0.413	4.53 ± 0.15 3.66−5.73	4.63 ± 0.15 3.66−5.83	3.15−6.33	0.700	4.11 ± 0.14 3.72−4.48	4.40 ± 0.14 3.93−5.09	3.66−4.28	4.98−7.578

[a]Selenium = ppb dry weight; all others = mg/100 g dry weight. [b]*P* value (false discovery rate adjusted) for *t* test comparing DAS-81419-2 with isoline. [c]U.S. study: mean across ten field sites with four replicates per site. Brazil study: mean across two field sites with three replicates per site. [d]Minimum (min) and maximum (max) represent values observed in single replicate plots. [e]U.S. study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots across five commercially available nontransgenic soybean varieties with three varieties planted at each field site. Brazil study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots of one commercially available nontransgenic soybean variety planted at both field sites. [f]See Materials and Methods section for reference citations. [g]NA (not available) = analysis not performed; majority of data was <LOQ. [h]NR = not reported.

J. Agric. Food Chem. 2015, 63, 2063−2073
DOI: 10.1021/jf505015y

Table 4. Amino Acid Composition of DAS-81419-2 Soybean Seed

	U.S. study				Brazil study				
analytical component[a]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	literature range min−max[f]
alanine	0.566	4.57 ± 0.03 4.31−4.76	4.59 ± 0.03 4.30−4.82	4.28−4.75	0.760	4.46 ± 0.03 4.39−4.57	4.49 ± 0.03 4.38−4.61	4.35−4.53	4.16−4.74
arginine	0.476	7.49 ± 0.05 7.04−7.79	7.44 ± 0.05 7.05−7.74	7.22−8.20	0.830	7.59 ± 0.05 7.47−7.68	7.57 ± 0.05 7.49−7.66	7.68−8.03	6.41−8.41
aspartic acid	0.476	11.5 ± 0.01 11.3−11.6	11.5 ± 0.01 11.3−11.8	9.99−11.74	0.700	11.5 ± 0.03 11.4−11.6	11.5 ± 0.03 11.4−11.5	11.4−11.6	11.37−12.68
cystine	0.609	1.61 ± 0.03 1.43−1.95	1.63 ± 0.03 1.45−1.86	1.24−1.79	0.700	1.46 ± 0.10 1.33−1.61	1.52 ± 0.10 1.42−1.62	1.23−1.48	1.02−1.87
glutamic acid	0.476	17.3 ± 0.11 16.3−18.0	17.2 ± 0.1 16.3−18.0	17.0−18.6	0.973	18.0 ± 0.1 17.7−18.3	18.0 ± 0.1 17.9−18.1	17.7−18.4	17.71−20.48
glycine	0.845	4.50 ± 0.20 4.34−4.67	4.50 ± 0.02 4.30−4.66	4.14−4.54	0.700	4.34 ± 0.01 4.31−4.40	4.31 ± 0.01 4.27−4.38	4.28−4.36	4.19−4.62
histidine	0.719	2.71 ± 0.02 2.58−2.82	2.70 ± 0.02 2.17−2.84	2.43−2.78	0.896	2.59 ± 0.01 2.55−2.64	2.60 ± 0.01 2.58−2.64	2.45−2.66	2.49−2.89
isoleucine	0.926	4.80 ± 0.02 4.65−4.96	4.80 ± 0.02 4.52−4.99	4.61−4.98	0.700	4.90 ± 0.02 4.82−5.00	4.83 ± 0.02 4.77−4.89	4.72−4.86	4.13−5.11
leucine	0.732	7.65 ± 0.01 7.51−7.83	7.64 ± 0.01 7.49−7.75	7.49−7.98	0.972	7.66 ± 0.02 7.60−7.70	7.67 ± 0.02 7.63−7.73	7.57−7.79	7.46−8.29
lysine	0.476	6.32 ± 0.08 5.90−7.51	6.44 ± 0.08 5.92−7.41	5.61−7.29	0.700	6.46 ± 0.03 6.40−6.63	6.40 ± 0.03 6.34−6.45	5.87−6.61	6.23−7.38
methionine	0.551	1.42 ± 0.01 1.31−1.61	1.44 ± 0.01 1.33−1.61	1.22−1.62	0.700	1.37 ± 0.02 1.33−1.41	1.35 ± 0.02 1.32−1.39	1.22−1.37	1.18−1.71
phenylalanine	0.108	5.15 ± 0.01 4.99−5.25	5.11 ± 0.01 4.92−5.22	4.88−5.37	0.760	5.23 ± 0.04 5.15−5.31	5.21 ± 0.04 5.15−5.25	5.24−5.46	4.91−5.44
proline	0.566	5.14 ± 0.04 4.86−5.97	5.20 ± 0.04 4.91−5.73	4.80−6.02	0.700	5.17 ± 0.04 5.12−5.27	5.24 ± 0.04 5.20−5.30	5.14−5.32	4.75−5.62
serine	0.670	5.13 ± 0.02 4.74−5.37	5.16 ± 0.02 4.85−5.38	4.81−5.53	0.700	5.02 ± 0.04 4.80−5.18	5.16 ± 0.04 5.10−5.22	4.73−5.14	3.25−6.04
threonine	0.616	4.19 ± 0.03 3.99−4.38	4.20 ± 0.03 3.96−4.44	3.86−4.25	0.760	4.03 ± 0.03 3.96−4.08	4.04 ± 0.03 4.00−4.09	3.94−4.05	3.15−4.24
tryptophan	0.747	1.52 ± 0.02 1.42−1.64	1.52 ± 0.02 1.40−1.65	1.27−1.69	0.714	1.38 ± 0.01 1.33−1.43	1.41 ± 0.01 1.37−1.44	1.33−1.38	0.95−1.49
tyrosine	0.638	3.97 ± 0.01 3.88−4.10	3.96 ± 0.01 3.83−4.06	3.82−4.16	0.786	3.94 ± 0.02 3.86−4.00	3.93 ± 0.02 3.89−4.00	3.99−4.07	2.62−3.72
valine	0.693	4.98 ± 0.02 4.74−5.22	4.96 ± 0.02 4.70−5.32	4.63−5.18	0.700	4.85 ± 0.02 4.79−4.91	4.78 ± 0.02 4.70−4.83	4.66−4.88	4.28−5.57

[a]Unit of measure = percent of total amino acids. [b]*P* value (false discovery rate adjusted) for *t* test comparing DAS-81419-2 with isoline. [c]U.S. study: mean across 10 field sites with four replicates per site. Brazil study: mean across two field sites with three replicates per site. [d]Minimum (min) and maximum (max) represent values observed in single replicate plots. [e]U.S. study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots across five commercially available nontransgenic soybean varieties with three varieties planted at each field site. Brazil study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots of one commercially available nontransgenic soybean variety planted at both field sites. [f]See Materials and Methods section for reference citations.

J. Agric. Food Chem. 2015, 63, 2063−2073 DOI: 10.1021/jf505015y

Table 5. Fatty Acid Composition of DAS-81419-2 Soybean Seed

	U.S. study				Brazil study				
analytical component[a]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	literature range min−max[f]
8:0 caprylic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ–0.148
10:0 capric	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ–0.27
12:0 lauric	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ–0.132
14:0 myristic	NA[g]	NA[g] <LOQ[g]	NA[g] <LOQ[g]	<LOQ	0.700	0.0988 ± 0.0018 0.0930–0.103	0.103 ± 0.002 0.094–0.108	0.0724–0.0895	<LOQ–0.238
14:1 myristoleic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ–0.125
15:0 pentadecanoic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ
15:1 pentadecenoic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ
16:0 palmitic	<0.001[h]	11.1 ± 0.1 10.0–11.6	11.6 ± 0.1 10.9–12.3	9.12–11.5	0.749	13.0 ± 0.1 12.8–13.2	12.9 ± 0.1 12.6–13.1	11.3–12.2	1.40–15.77
16:1 palmitoleic	NA[g]	NA[g] <LOQ	NA[g] <LOQ–0.236	<LOQ[g]	0.700	0.108 ± 0.002 0.105–0.111	0.0915 ± 0.0017 0.0871–0.0961	0.111–0.131	<LOQ[g]–0.194
17:0 heptadecanoic	NA[g]	NA[g] <LOQ–0.127	NA[g] <LOQ–0.132	<LOQ[g]–0.133	0.896	0.103 ± 0.003 0.0955–0.111	0.102 ± 0.003 0.0972–0.106	0.0753–0.0877	<LOQ[g]–0.146
17:1 heptadecenoic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ–0.087
18:0 stearic	0.516	4.40 ± 0.12 3.67–5.27	4.46 ± 0.12 3.62–5.22	3.19–5.08	0.760	5.18 ± 0.11 4.85–5.46	5.07 ± 0.11 4.94–5.18	3.65–3.84	0.50–5.88
18:1 oleic	0.439	21.6 ± 0.4 19.6–25.2	21.2 ± 0.4 19.8–25.5	18.8–24.6	0.700	27.7 ± 1.1 26.0–30.4	22.1 ± 1.1 21.5–22.6	25.0–30.0	2.60–45.68
18:2 linoleic	0.476	54.1 ± 0.4 51.2–55.8	53.8 ± 0.4 50.5–55.7	53.6–57.5	0.700	47.5 ± 1.0 45.0–49.2	51.9 ± 1.0 51.5–52.4	47.3–50.7	7.58–58.8
18:3 linolenic	0.314	7.97 ± 0.15 6.91–8.76	8.17 ± 0.15 7.32–8.94	6.58–9.88	0.700	5.14 ± 0.26 4.54–5.64	6.56 ± 0.26 6.06–7.05	6.21–6.95	1.27–12.52
18:3 γ-linolenic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ
20:0 arachidic	0.413	0.319 ± 0.007 0.276–0.370	0.325 ± 0.007 0.282–0.372	0.254–0.383	0.700	0.453 ± 0.012 0.418–0.491	0.471 ± 0.012 0.450–0.484	0.396–0.409	0.038–0.57
20:1 eicosenoic	0.616	0.119 ± 0.019 <LOQ–0.189	0.105 ± 0.019 <LOQ–0.171	<LOQ[g]–0.191	0.700	0.223 ± 0.007 0.210–0.245	0.200 ± 0.006 0.184–0.214	0.205–0.220	0.024–0.35
20:2e icosadienoic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ–0.245
20:3 eicosatrienoic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ
20:4 arachidonic	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	NA[g]	NA[g] <LOQ	NA[g] <LOQ	<LOQ	<LOQ
22:0 behenic	0.476	0.317 ± 0.003 0.283–0.347	0.321 ± 0.003 0.263–0.345	0.281–0.390	0.986	0.466 ± 0.018 0.429–0.499	0.465 ± 0.018 0.438–0.490	0.517–0.534	0.043–0.65

[a]Percent of total fatty acids. [b]*P* value (false discovery rate adjusted) for *t* test comparing DAS-81419-2 with isoline. [c]U.S. study: mean across 10 field sites with four replicates per site. Brazil study: mean across two field sites with three replicates per site. [d]Minimum (min) and maximum (max) represent values observed in single replicate plots. [e]U.S. study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots across five commercially available nontransgenic soybean varieties with three varieties planted at each field site. Brazil study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots of one commercially available nontransgenic soybean variety planted at both field sites. [f]See Materials and Methods section for reference citations. [g]NA (not available) = analysis not performed: majority of data was <LOQ. [h]Statistically significant difference when compared with isoline ($P < 0.05$).

J. Agric. Food Chem. 2015, 63, 2063−2073

DOI: 10.1021/jf505015y

Table 6. Vitamin Composition of DAS-81419-2 Soybean Seed

	U.S. study				Brazil study				
analytical component[a]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	*P* value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	literature range min−max[f]
α-tocopherol (vitamin E)	0.607	14.3 ± 1.3 8.56−29.3	13.6 ± 1.3 9.62−26.6	6.51−25.0	0.700	9.45 ± 0.91 6.98−11.5	12.5 ± 0.9 9.31−15.9	25.2−39.2	1.934−84.9
β-tocopherol	NA[g]	NA[c] <LOQ	NA[c] <LOQ	<LOQ	NA[c]	NA[c] <LOQ	NA[c] <LOQ	<LOQ	NR[g]
γ-tocopherol	0.616	168 ± 8 97−220	172 ± 8 118−219	77.5−204	0.700	39.4 ± 5.2 28.7−49.1	57.0 ± 5.2 36.7−77.9	176−215	NR[g]
δ-tocopherol	0.074	69.4 ± 2.7 41.3−83.1	74.7 ± 2.8 45.4−90.8	49.7−104	0.700	26.7 ± 2.1 22.7−33.5	31.6 ± 2.1 23.8−38.8	62.2−76.6	NR[g]
total tocopherols	0.476	252 ± 8 168−305	261 ± 8 205−301	160−304	not measured				NR[g]
β-carotene	NA	NA[c] <LOQ	NA[c] <LOQ	<LOQ−0.244	not measured				NR[g]
vitamin B_1 (thiamine hydrochloride)	0.662	3.51 ± 0.24 2.35−5.44	3.43 ± 0.24 2.20−5.16	1.82−4.60	0.700	5.69 ± 0.36 5.31−6.07	4.67 ± 0.36 4.08−5.78	3.95−5.40	1.01−2.54
vitamin B_2 (riboflavin)	0.476	3.40 ± 0.08 2.63−4.65	3.51 ± 0.08 2.58−4.64	2.42−5.00	0.700	4.98 ± 0.13 4.51−5.24	5.43 ± 0.13 5.05−5.89	4.53−5.70	1.90−3.21
vitamin B_3 (niacin)	0.413	25.0 ± 0.7 20.2−30.5	25.6 ± 0.7 20.3−32.1	20.7−29.0	0.700	34.3 ± 1.2 31.7−36.4	36.0 ± 1.2 34.3−37.9	18.4−23.0	NR[g]
vitamin B_5 (pantothenic acid)	0.074	14.8 ± 0.5 12.3−19.5	14.0 ± 0.5 11.8−16.8	8.97−18.0	0.700	14.8 ± 0.7 13.7−15.5	16.2 ± 0.72 14.7−18.3	14.4−16.7	NR[g]
vitamin B_6 (pyridoxine hydrochloride)	0.787	5.23 ± 0.11 4.42−6.43	5.18 ± 0.11 4.53−6.00	3.01−6.36	0.700	4.88 ± 0.19 4.71−5.04	4.31 ± 0.19 3.79−4.60	4.49−5.18	NR[g]
vitamin B_9 (folic acid)	0.732	4.21 ± 0.20 3.05−5.62	4.15 ± 0.20 2.75−5.51	2.94−5.59	0.700	5.08 ± 0.23 4.64−5.72	4.50 ± 0.23 4.16−5.06	4.02−4.42	2.386−4.709
vitamin C (ascorbic acid)	0.476	141 ± 13 75.2−231	133 ± 13 74.0−230	49.2−210	0.700	62.2 ± 9.0 48.0−98.8	46.9 ± 9.0 37.0−72.5	53.7−83.8	NR[g]

[a]mg/kg dry weight. [b]*P* value (false discovery rate adjusted) for *t* test comparing DAS-81419-2 with isoline. [c]U.S. study: mean across 10 field sites with four replicates per site. Brazil study: mean across two field sites with three replicates per site. [d]Minimum (min) and maximum (max) represent values observed in single replicate plots. [e]U.S. study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots across five commercially available nontransgenic soybean varieties with three random varieties planted at each field site. Brazil study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots of one commercially available nontransgenic soybean variety planted at both field sites. [f]See Materials and Methods section for reference citations. [g]NR = not reported.

J. Agric. Food Chem. 2015, 63, 2063−2073

DOI: 10.1021/jf505015y

Table 7. Antinutrient and Bioactive Composition of DAS-81419-2 Soybean Seed

	U.S. study				Brazil study				
analytical component[a]	P value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	P value[b]	isoline mean ± SE[c] min−max[d]	DAS-81419-2 mean ± SE[c] min−max[d]	reference variety range min−max[e]	literature range min−max[f]
lectin	0.638	30.8 ± 2.1 13.9−50.1	32.2 ± 2.1 12.4−52.6	7.89−45.2	0.760	9.11 ± 0.86 8.06−10.2	8.50 ± 0.86 6.19−9.98	2.63−6.23	37−323
phytic acid	0.662	1.22 ± 0.06 0.857−2.02	1.24 ± 0.06 0.911−1.52	0.752−1.71	0.972	1.40 ± 0.11 1.22−1.64	1.41 ± 0.11 1.11−1.61	0.998−1.27	0.41−2.68
raffinose	0.674	0.750 ± 0.038 0.505−1.02	0.766 ± 0.038 0.475−0.977	0.570−1.16	0.700	0.840 ± 0.044 0.816−0.903	1.07 ± 0.04 0.930−1.14	0.616−0.773	0.212−1.85
stachyose	0.747	3.68 ± 0.08 3.19−4.14	3.69 ± 0.08 3.15−4.29	3.01−5.28	0.955	3.50 ± 0.04 3.36−3.61	3.49 ± 0.04 3.37−3.54	3.44−3.78	1.21−6.65
total daidzein equivalent	0.609	950 ± 48 462−1200	932 ± 48 504−1190	585−1460	0.760	260 ± 18 207−310	272 ± 18 240−295	504−577	25−2453.5
total genistein equivalent	0.616	1296 ± 63 808−1680	1276 ± 63 922−1620	888−1950	0.760	450 ± 25 358−530	428 ± 25 370−477	614−705	28−2837.2
total glycitein equivalent	0.074	197 ± 6 156−266	180 ± 6 140−237	40.3−259	0.700	160 ± 4 154−171	206 ± 4 196−216	146−168	15.3−349.19
trypsin inhibitor	0.566	29.1 ± 1.2 22.2−46.5	30.2 ± 1.2 21.3−49.9	19.5−41.5	0.700	34.1 ± 4.1 29.3−40.0	38.5 ± 4.1 28.3−46.8	19.0−24.0	18.14−118.68

[a]Lectin = HU/mg protein dry weight; phytic acid, raffinose, and stachyose = % dry weight; isoflavones = μg/g dry weight; trypsin inhibitor = TIU/mg dry weight. [b]P value (false discovery rate adjusted) for t test comparing DAS-81419-2 with isoline. [c]U.S. study: mean across 10 field sites with four replicates per site. Brazil study: mean across two field sites with three replicates per site. [d]Minimum (min) and maximum (max) represent values observed in single replicate plots. [e]U.S. study: reference variety range represents minimum (min) and maximum (max) values observed in single replicate plots across

Figure 1. Site means for ADF and NDF in soybean forage. The shaded area represents the literature range and locations are represented by the following symbols: ○ = Richland, IA; × = Atlantic, IA; + = Carlyle, IL; Δ = Wyoming, IL; □ = Frankfort, IN;◇ = Fisk, MO; ● = La Plata, MO; ▲ = York, NE; ■ = Brunswick, NE; ◆ = Germansville, PA; # = Montividiu, Goiás; ∼ = Indianópolis, Minas Geráis. "Isoline" = near-isogenic nontransgenic line. Note that each nontransgenic commercial variety was included, on average, at only half of the field sites in the U.S. study, so the range of data for these entries is expected to be narrower than that of the isoline and DAS-81419-2 entries, which were present at all 10 sites.

quantitation (<LOQ); said analytes were excluded because data sets where most points are excluded or set to a given nominal value have substantially up-biased data or have improperly underestimated variability, respectively. No forage analytes were excluded, while seed analytes excluded from both the U.S. and Brazil studies were β-tocopherol, sodium, and the fatty acids 8:0 caprylic, 10:0 capric, 12:0 lauric, 14:1 myristoleic, 15:0 pentadecanoic, 15:1 pentadecenoic, 17:1 heptadecenoic, 18:3 γ-linolenic, 20:2 eicosadienoic, 20:3 eicosatrienoic, and 20:4 arachidonic. Additionally, β-carotene and the fatty acids 14:0 myristic, 16:1 palmitoleic, and 17:0 heptadecanoic were excluded from analysis of the U.S. data, and the mineral selenium was excluded from analysis of the Brazil data. The range of values observed in the nontransgenic commercial soybean varieties was included to put any statistically significant differences into context, and ranges for nontransgenic soybean in the literature were also compiled for further reference.[11,20,28,30,35,39,59,85,100−106]

RESULTS AND DISCUSSION

The composition of DAS-81419-2 soybean forage and seed was compared with that of the isoline at 10 field sites in the U.S.

DOI: 10.1021/jf505015y
J. Agric. Food Chem. 2015, 63, 2063−2073

and two field sites in Brazil. In addition to DAS-81419-2 and the isoline, the U.S. study included five commercially available nontransgenic soybean varieties, and the Brazil study included one commercially available nontransgenic soybean variety. Composition analytes evaluated in forage (9) included proximates, fiber, and minerals, while analytes evaluated in seed (80) included proximates, fiber, minerals, amino acids, fatty acids, vitamins, antinutrients, and bioactives. Of the 80 seed analytes, 18 were excluded from the statistical analysis of the U.S. and/or Brazil data sets because more than 50% of the results were <LOQ.

No statistically significant differences were detected between DAS-81419-2 and the isoline in both the U.S. and Brazil studies in all but one (16:0 palmitic acid) of the analytes included in the statistical analysis (Tables 1−7). Moreover, mean results for all analytes fell within the ranges observed in nontransgenic commercial reference varieties and/or the ranges of values for nontransgenic soybean in the literature. In the U.S. study only, a statistically significant difference was detected between the isoline and DAS-81419-2 soybean for 16:0 palmitic acid. Although statistically significant, the difference had no meaningful effect on nutritional value; the mean 16:0 palmitic acid content of DAS-81419-2 was only 4.5% higher than that of the isoline, and the entire range of values observed in DAS-81419-2 was encompassed by the range of values reported in the literature for nontransgenic soybean (Table 5). Furthermore, a difference for 16:0 palmitic acid was not observed in the Brazil study, where the DAS-81419-2 mean was actually slightly lower than the isoline.

While the composition of isoline and DAS-81419-2 soybean was similar both across and within field sites, results within each entry were highly variable across field sites; ADF and NDF in forage provide examples of this site to site variation (Figure 1). The lack of differences between the isoline and DAS-81419-2 coupled with the wide range of results across field sites in these studies indicates that environment has a substantial effect on crop composition and the effect of transgenesis is undetectable, as others have reported.[7,28,85,89,100,107−109]

CONCLUSIONS

On the basis of the absence of meaningful differences between the isoline and DAS-81419-2 soybean in studies that were conducted at multiple field sites in two countries, it is concluded that the composition of DAS-81419-2 soybean forage and seed is equivalent to that of nontransgenic soybean. The conclusion of this study is in concordance with 86 other published studies and over 100 regulatory submissions where compositional equivalence between the transgenic crop and nontransgenic crop was concluded.[7−92,110] Collectively, these results indicate that composition studies to detect unintended effects due to transgenesis with traits that are not expected to alter composition are of dubious value in assessing the safety of transgenic crops.

AUTHOR INFORMATION

Corresponding Author

*Phone: 317-337-3494. E-mail: BJFast@dow.com.

Notes

The authors declare the following competing financial interest(s): BF, AP, TJ, and RH are employed by Dow AgroSciences, which develops and markets transgenic seed. BP is employed by Covance Laboratories Inc., which was contracted by Dow AgroSciences to conduct the composition analytical portion of these studies..

ACKNOWLEDGMENTS

We thank Shane Andersen, David Bennett, Tim Boeker, Jay Deweese, Sue Dorsey, Eric Ehlers, Nathan Goldschmidt, Roger Johnson, Matthew Krause, Daniel Ramsdell, and Renan Gravena for conducting the field phase of these experiments and Michelle Anderson, Jason Dority, and Rebecca Hall for study coordination. We also thank Alyssa Brune, Kathryn Clayton, John Cuffe, Satish Guttikonda, Eric Kraus, Siva Kumpatla, Barry Schafer, and Laura Tagliani for reviewing a draft of this manuscript.

REFERENCES

(1) Aronson, A. I. Why *Bacillus thuringiensis* insecticidal toxins are so effective: unique features of their mode of action. *FEMS Microbiol. Lett.* **2001**, *195*, 1−8.

(2) Höfte, H.; Whiteley, H. R. Insecticidal Crystal Proteins of *Bacillus thuringiensis*. *Microbiol. Rev.* **1989**, *53*, 242−255.

(3) Schnepf, E.; Crickmore, N.; Van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D. R.; Dean, D. H. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 775−806.

(4) Dröge, W.; Broer, I.; Pühler, A. Transgenic plants containing the phosphinothricin-*N*-acetyltransferase gene metabolize the herbicide L-phosphinothricin (glufosinate) differently from untransformed plants. *Planta* **1992**, *187*, 142−151.

(5) Weber, N.; Halpin, C.; Hannah, L. C.; Jez, J. M.; Kough, J.; Parrott, W. Crop genome plasticity and its relevance to food and feed safety of genetically engineered breeding stacks. *Plant Physiol.* **2012**, *160*, 1842−1853.

(6) Schnell, J.; Steele, M.; Bean, J.; Neuspiel, M.; Girard, C.; Dormann, N.; Pearson, C.; Savoie, A.; Bourbonnière, L.; MacDonald, P. A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. *Transgenic Res.* **2014**, 1−17.

(7) Herman, R. A.; Phillips, A. M.; Lepping, M. D.; Fast, B. J.; Sabbatini, J. Compositional safety of event DAS-40278-9 (AAD-1) herbicide-tolerant maize. *GM Crops Food: Biotechnol. Agric.* **2010**, *1*, 294−311.

(8) Abdeen, A.; Schnell, J.; Miki, B. Transcriptome analysis reveals absence of unintended effects in drought-tolerant transgenic plants overexpressing the transcription factor ABF3. *BMC Genomics* **2010**, *11*, 69.

(9) Baker, J.; Hawkins, N.; Ward, J.; Lovegrove, A.; Napier, J.; Shewry, P.; Beale, M. A metabolomic study of substantial equivalence of field-grown genetically modified wheat. *Plant Biotechnol. J.* **2006**, *4*, 381−392.

(10) Berberich, S. A.; Ream, J. E.; Jackson, T. L.; Wood, R.; Stipanovic, R.; Harvey, P.; Patzer, S.; Fuchs, R. L. The composition of insect-protected cottonseed is equivalent to that of conventional cottonseed. *J. Agric. Food Chem.* **1996**, *44*, 365−371.

(11) Berman, K. H.; Harrigan, G. G.; Riordan, S. G.; Nemeth, M. A.; Hanson, C.; Smith, M.; Sorbet, R.; Zhu, E.; Ridley, W. P. Compositions of seed, forage, and processed fractions from insect-protected soybean MON 87701 are equivalent to those of conventional soybean. *J. Agric. Food Chem.* **2009**, *57*, 11360−11369.

(12) Catchpole, G.; Beckmann, M.; Enot, D.; Mondhe, M.; Zywicki, B.; Taylor, J.; Hardy, N.; Smith, A.; King, R.; Kell, D. Hierarchical metabolomics demonstrates substantial compositional similarity between genetically modified and conventional potato crops. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 14458−14462.

(13) Coll, A.; Nadal, A.; Collado, R.; Capellades, G.; Kubista, M.; Messeguer, J.; Pla, M. Natural variation explains most transcriptomic changes among maize plants of MON810 and comparable non-GM varieties subjected to two N-fertilization farming practices. *Plant Mol. Biol.* **2010**, *73*, 349−362.

(14) Drury, S. M.; Reynolds, T. L.; Ridley, W. P.; Bogdanova, N.; Riordan, S.; Nemeth, M. A.; Sorbet, R.; Trujillo, W. A.; Breeze, M. L. Composition of forage and grain from second-generation insect-

DOI: 10.1021/jf505015y
J. Agric. Food Chem. 2015, 63, 2063−2073

protected corn MON 89034 is equivalent to that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2008**, *56*, 4623−4630.
(15) El-Khishin, D. A. A. H.; Amina; El Moghazy, G.; Metry, E. A. Assessment of genetically modified potato lines resistant to potato virus Y using compositional analysis and molecular markers. *Res. J. Agric. Biol. Sci.* **2009**, *5*, 261−271.
(16) Esposito, F.; Fogliano, V.; Cardi, T.; Carputo, D.; Filippone, E. Glycoalkaloid content and chemical composition of potatoes improved with nonconventional breeding approaches. *J. Agric. Food Chem.* **2002**, *50*, 1553−1561.
(17) George, C.; Ridley, W.; Obert, J. Composition of grain and forage from corn rootworm-protected corn event MON 863 is equivalent to that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2004**, *52*, 4149−4158.
(18) Hamilton, K. A.; Pyla, P. D.; Breeze, M.; Olson, T.; Li, M. H.; Robinson, E.; Gallagher, S. P.; Sorbet, R.; Chen, Y. Bollgard II cotton: compositional analysis and feeding studies of cottonseed from insect-protected cotton (*Gossypium hirsutum* L.) producing the Cry1Ac and Cry2Ab2 proteins. *J. Agric. Food Chem.* **2004**, *52*, 6969−6976.
(19) Harrigan, G. G.; Ridley, W. P.; Miller, K. D.; Sorbet, R.; Riordan, S. G.; Nemeth, M. A.; Reeves, W.; Pestert, T. A. The forage and grain of MON 87460, a drought-tolerant corn hybrid, are compositionally equivalent to that of conventional corn. *J. Agric. Food Chem.* **2009**, *57*, 9754−9763.
(20) Harrigan, G. G.; Ridley, W. P.; Riordan, S. G.; Nemeth, M. A.; Sorbet, R.; Trujillo, W. A.; Breeze, M. L.; Schneider, R. W. Chemical composition of glyphosate-tolerant soybean 40-3-2 grown in Europe remains equivalent with that of conventional soybean (*Glycine max* L.). *J. Agric. Food Chem.* **2007**, *55*, 6160−6168.
(21) Herman, R.; Storer, N.; Phillips, A.; Prochaska, L.; Windels, P. Compositional assessment of event DAS-59122-7 maize using substantial equivalence. *Regul. Toxicol. Pharmacol.* **2007**, *47*, 37−47.
(22) Herman, R. A.; Phillips, A. M.; Collins, R. A.; Tagliani, L. A.; Claussen, F. A.; Graham, C. D.; Bickers, B. L.; Harris, T. A.; Prochaska, L. M. Compositional equivalency of Cry1F corn event TC6275 and conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2004**, *52*, 2726−2734.
(23) Herman, R. A.; Phillips, A. M.; Lepping, M. D.; Sabbatini, J., Compositional safety of DAS-68416-4 (AAD-12) herbicide-tolerant soybean. *J. Nutr. Food Sci.* **2011**, *1:2*, DOI 10.4172/215-9600.1000103.
(24) Jiao, Z.; Deng, J.; Li, G.; Zhang, Z.; Cai, Z. Study on the compositional differences between transgenic and non-transgenic papaya (*Carica papaya* L.). *J. Food Compos. Anal.* **2010**, *23*, 640−647.
(25) Khalf, M.; Goulet, C.; Vorster, J.; Brunelle, F.; Anguenot, R.; Fliss, I.; Michaud, D. Tubers from potato lines expressing a tomato Kunitz protease inhibitor are substantially equivalent to parental and transigenic controls. *Plant Biotechnol. J.* **2010**, *8*, 155−169.
(26) Kim, J. K.; Chu, S. M.; Kim, S. J.; Lee, D. J.; Lee, S. Y.; Lim, S. H.; Ha, S.-H.; Kweon, S. J.; Cho, H. S. Variation of glucosinolates in vegetable crops of *Brassica rapa* L. ssp. pekinensis. *Food Chem.* **2010**, *119*, 423−428.
(27) Lin, C. H.; Lu, C. T.; Lin, H. T.; Pan, T. M. Safety assessment and detection method of genetically modified Chinese kale (*Brassica oleracea* cv. *alboglabra*). *J. Agric. Food Chem.* **2009**, *57*, 1876−1881.
(28) Lundry, D. R.; Ridley, W. P.; Meyer, J. J.; Riordan, S. G.; Nemeth, M. A.; Trujillo, W. A.; Breeze, M. L.; Sorbet, R. 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.). *J. Agric. Food Chem.* **2008**, *56*, 4611−4622.
(29) Malowicki, S. M. M.; Martin, R.; Qian, M. C. Comparison of sugar, acids, and volatile composition in raspberry bushy dwarf virus-resistant transgenic raspberries and the wild type 'Meeker' (*Rubus idaeus* L.). *J. Agric. Food Chem.* **2008**, *56*, 6648−6655.
(30) McCann, M. C.; Liu, K. S.; Trujillo, W. A.; Dobert, R. C. Glyphosate-tolerant soybeans remain compositionally equivalent to conventional soybeans (*Glycine max* L.) during three years of field testing. *J. Agric. Food Chem.* **2005**, *53*, 5331−5335.
(31) McCann, M. C.; Rogan, G. J.; Fitzpatrick, S.; Trujillo, W. A.; Sorbet, R.; Hartnell, G. F.; Riodan, S. G.; Nemeth, M. A. Glyphosate-tolerant alfalfa is compositionally equivalent to conventional alfalfa (*Medicago sativa* L.). *J. Agric. Food Chem.* **2006**, *54*, 7187−7192.
(32) McCann, M. C.; Trujillo, W. A.; Riordan, S. G.; Sorbet, R.; Bogdanova, N. N.; Sidhu, R. S. Comparison of the forage and grain composition from insect-protected and glyphosate-tolerant MON 88017 corn to conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2007**, *55*, 4034−4042.
(33) Oberdoerfer, R.; Shillito, R.; de Beuckeleer, M.; Mitten, D. Rice (*Oryza sativa* L.) containing the bar gene is compositionally equivalent to the nontransgenic counterpart. *J. Agric. Food Chem.* **2005**, *53*, 1457−1465.
(34) Obert, J. C.; Ridley, W. P.; Schneider, R. W.; Riordan, S. G.; Nemeth, M. A.; Trujillo, W. A.; Breeze, M. L.; Sorbet, R.; Astwood, J. D. The composition of grain and forage from glyphosate tolerant wheat MON 71800 is equivalent to that of conventional wheat (*Triticum aestivum* L.). *J. Agric. Food Chem.* **2004**, *52*, 1375−1384.
(35) 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.
(36) Poerschmann, J.; Rauschen, S.; Langer, U.; Augustin, J.; Górecki, T. Fatty acid patterns of genetically modified Cry3Bb1 expressing Bt-maize MON88017 and its near-isogenic line. *J. Agric. Food Chem.* **2008**, *57*, 127−132.
(37) Sidhu, R. S.; Hammond, B. G.; Fuchs, R. L.; Mutz, J.-N.; Holden, L. R.; George, B.; Olson, T. Glyphosate-tolerant corn: the composition and feeding value of grain from glyphosate-tolerant corn is equivalent to that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2000**, *48*, 2305−2312.
(38) Tang, M. Z.; Huang, K. L.; Li, X.; Zhou, K.; He, X. Y.; Luo, Y. B. Absence of effect after introducing *Bacillus thuringiensis* gene on nutritional composition in cottonseed. *J. Food Sci.* **2006**, *71*, S38−S41.
(39) 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.
(40) Venneria, E.; Fanasca, S.; Monastra, G.; Finotti, E.; Ambra, R.; Azzini, E.; Durazzo, A.; Foddai, M. S.; Maiani, G. Assessment of the nutritional values of genetically modified wheat, corn, and tomato crops. *J. Agric. Food Chem.* **2008**, *56*, 9206−9214.
(41) Wang, J.; Guo, L. Q.; Lin, J. F. Composition of transgenic *Volvariella volvacea* tolerant to cold stress is equivalent to that of conventional control. *J. Agric. Food Chem.* **2009**, *57*, 2392−2396.
(42) Xin, L.; Xiaoyun, H.; Yunbo, L.; Guoying, X.; Xianbin, J.; Kunlun, H. Comparative analysis of nutritional composition between herbicide-tolerant rice with bar gene and its non-transgenic counterpart. *J. Food Compos. Anal.* **2008**, *21*, 535−539.
(43) Zhu, J.; Patzoldt, W. L.; Shealy, R. T.; Vodkin, L. O.; Clough, S. J.; Tranel, P. J. Transcriptome response to glyphosate in sensitive and resistant soybean. *J. Agric. Food Chem.* **2008**, *56*, 6355−6363.
(44) Ridley, W. P.; Harrigan, G. G.; Breeze, M. L.; Nemeth, M. A.; Sidhu, R. S.; Glenn, K. C. Evaluation of compositional equivalence for multitrait biotechnology crops. *J. Agric. Food Chem.* **2011**, 5865−5876.
(45) Ridley, W. P.; Sidhu, R. S.; Pyla, P. D.; Nemeth, M. A.; Breeze, M. L.; Astwood, J. D. Comparison of the nutritional profile of glyphosate-tolerant corn event NK603 with that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2002**, *50*, 7235−7243.
(46) Wang, Y.; Xu, W.; Zhao, W.; Hao, J.; Luo, Y.; Tang, X.; Zhang, Y.; Huang, K. Comparative analysis of the proteomic and nutritional composition of transgenic rice seeds with Cry1ab/ac genes and their non-transgenic counterparts. *J. Cereal Sci.* **2012**, *55*, 226−233.
(47) Frank, T.; Röhlig, R. M.; Davies, H. V.; Barros, E.; Engel, K.-H. Metabolite profiling of maize kernels—genetic modification versus environmental influence. *J. Agric. Food Chem.* **2012**, *60*, 3005−3012.
(48) Barros, E.; Lezar, S.; Anttonen, M. J.; Van Dijk, J. P.; Röhlig, R. M.; Kok, E. J.; Engel, K.-H. Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics and metabolomics. *Plant Biotechnol. J.* **2010**, *8*, 436−451.

DOI: 10.1021/jf505015y
J. Agric. Food Chem. 2015, 63, 2063−2073

(49) Baudo, M. M.; Lyons, R.; Powers, S.; Pastori, G. M.; Edwards, K. J.; Holdsworth, M. J.; Shewry, P. R. Transgenesis has less impact on the transcriptome of wheat grain than conventional breeding. *Plant Biotechnol. J.* **2006**, *4*, 369−380.
(50) Coll, A.; Nadal, A.; Collado, R.; Capellades, G.; Messeguer, J.; Melé, E.; Palaudelmàs, M.; Pla, M. Gene expression profiles of MON810 and comparable non-GM maize varieties cultured in the field are more similar than are those of conventional lines. *Transgenic Res.* **2009**, *18*, 801−808.
(51) Coll, A.; Nadal, A.; Palaudelmàs, M.; Messeguer, J.; Melé, E.; Puigdomènech, P.; Pla, M. Lack of repeatable differential expression patterns between MON810 and comparable commercial varieties of maize. *Plant Mol. Biol.* **2008**, *68*, 105−117.
(52) Harrigan, G. G.; Lundry, D.; Drury, S.; Berman, K.; Riordan, S. G.; Nemeth, M. A.; Ridley, W. P.; Glenn, K. C. Natural variation in crop composition and the impact of transgenesis. *Nature Biotechnol.* **2010**, *28*, 402−404.
(53) Lehesranta, S.; Davies, H.; Shepherd, L.; Nunan, N.; McNicol, J.; Auriola, S.; Koistinen, K.; Suomalainen, S.; Kokko, H.; Karenlampi, S. Comparison of tuber proteomes of potato varieties, landraces, and genetically modified lines. *Plant Physiol.* **2005**, *138*, 1690−1699.
(54) Park, H.; Lee, S.; Jeong, H.; Cho, S.; Chun, H.; Back, O.; Kim, D.; Lillehoj, H. The nutrient composition of the herbicide-tolerant green pepper is equivalent to that of the conventional green pepper. *Nutr. Res. (N. Y.)* **2006**, *26*, 546−548.
(55) Ricroch, A. E.; Berge, J. B.; Kuntz, M. Evaluation of genetically engineered crops using transcriptomic, proteomic and metabolomic profiling techniques. *Plant Physiol.* **2011**, *155*, 1752−1761.
(56) Kim, J.; Ryu, T.; Sohn, S.; Kim, J.; Chu, S.; Yu, C.; Baek, H. Metabolic fingerprinting study on the substantial equivalence of genetically modified (GM) chinese cabbage to non-GM cabbage. *J. Korean Soc. Appl. Biol. Chem.* **2009**, *52*, 186−192.
(57) Tripathi, S.; Suzuki, J. Y.; Carr, J. B.; McQuate, G. T.; Ferreira, S. A.; Manshardt, R. M.; Pitz, K. Y.; Wall, M. M.; Gonsalves, D. Nutritional composition of Rainbow papaya, the first commercialized transgenic fruit crop. *J. Food Compos. Anal.* **2011**, *24*, 140−147.
(58) Quemada, H.; Zarka, K.; Pett, W.; Bothma, G.; Felcher, K.; Mirendil, H.; Koch, M.; Brink, J.; Douches, D. Safety evaluations of the Cry1Ia1 protein found in the transgenic potato 'SpuntaG2'. *J. Am. Soc. Hortic. Sci.* **2010**, *135*, 325−332.
(59) Berman, K. H.; Harrigan, G. G.; Riordan, S. G.; Nemeth, M. A.; Hanson, C.; Smith, M.; Sorbet, R.; Zhu, E.; Ridley, W. P. Compositions of forage and seed from second-generation glyphosate-tolerant soybean MON 89788 and insect-protected soybean MON 87701 from Brazil are equivalent to those of conventional soybean (*Glycine max*). *J. Agric. Food Chem.* **2010**, *58*, 6270−6276.
(60) Zhou, J.; Harrigan, G. G.; Berman, K. H.; Webb, E. G.; Klusmeyer, T. H.; Nemeth, M. A. Stability in the composition equivalence of grain from insect-protected maize and seed from glyphosate-tolerant soybean to conventional counterparts over multiple seasons, locations, and breeding germplasms. *J. Agric. Food Chem.* **2011**, *59*, 8822−8828.
(61) Noteborn, H. P. J. M.; Bienenmann-Ploum, M. E.; van den Berg, J. H. J.; Alink, G. M.; Zolla, L.; Reynaerts, A.; Pensa, M.; Kuiper, H. A., Safety assessment of the *Bacillus thuringiensis* insecticidal crystal protein CRYIA(b) expressed in transgenic tomatoes. In *Genetically Modified Foods*; American Chemical Society: Washington, DC, 1995; Vol. *605*, pp 134−147.
(62) Qin, F.; Kang, L.; Guo, L.; Lin, J.; Song, J.; Zhao, Y. Composition of transgenic soybean seeds with higher γ-linolenic acid content is equivalent to that of conventional control. *J. Agric. Food Chem.* **2012**, *60*, 2200−2204.
(63) Kim, J.; Park, S.-Y.; Lee, S.; Lim, S.-H.; Kim, H.; Oh, S.-D.; Yeo, Y.; Cho, H.; Ha, S.-H. Unintended polar metabolite profiling of carotenoid-biofortified transgenic rice reveals substantial equivalence to its non-transgenic counterpart. *Plant Biotechnol. Rep.* **2012**, 19−26.
(64) Cheng, K.; Beaulieu, J.; Iquira, E.; Belzile, F.; Fortin, M.; Stromvik, M. Effect of transgenes on global gene expression in soybean is within the natural range of variation of conventional cultivars. *J. Agric. Food Chem.* **2008**, *56*, 3057−3067.
(65) Duke, S. O.; Rimando, A. M.; Pace, P. F.; Reddy, K. N.; Smeda, R. J. Isoflavone, glyphosate, and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosate-resistant soybean. *J. Agric. Food Chem.* **2002**, *51*, 340−344.
(66) Reuter, T.; Aulrich, K.; Berk, A.; Flachowsky, G. Investigations on genetically modified maize (Bt-maize) in pig nutrition: chemical composition and nutritional evaluation. *Arch. Tierernahrung* **2002**, *56*, 23−31.
(67) Beckles, D. M.; Tananuwong, K.; Shoemaker, C. F. Starch characteristics of transgenic wheat (*Triticum aestivum* L.) over-expressing the Dx5 high molecular weight glutenin subunit are substantially equivalent to those in nonmodified wheat. *J. Food Sci.* **2012**, *77*, C437−C442.
(68) Rogan, G. J.; Bookout, J. T.; Duncan, D. R.; Fuchs, R. L.; Lavrik, P. B.; Love, S. L.; Mueth, M.; Olson, T.; Owens, E. D.; Raymond, P. J.; Zalewski, J. Compositional analysis of tubers from insect and virus resistant potato plants. *J. Agric. Food Chem.* **2000**, *48*, 5936−5945.
(69) Roberts, M.; Minott, D. A.; Tennant, P. F.; Jackson, J. C. Assessment of compositional changes during ripening of transgenic papaya modified for protection against papaya ringspot virus. *J. Sci. Food Agric.* **2008**, *88*, 1911−1920.
(70) Park, S.-Y.; Lee, S.; Lee, J.-H.; Ko, H.-S.; Kweon, S.; Suh, S.-C.; Shin, K.-S.; Kim, J. Compositional comparative analysis between insect-resistant rice (*Oryza sativa* L.) with a synthetic *cry1Ac* gene and its non-transgenic counterpart. *Plant Biotechnol. Rep.* **2012**, *6*, 29−37.
(71) Nida, D. L.; Patzer, S.; Harvey, P.; Stipanovic, R.; Wood, R.; Fuchs, R. L. Glyphosate-tolerant cotton: the composition of the cottonseed is equivalent to that of conventional cottonseed. *J. Agric. Food Chem.* **1996**, *44*, 1967−1974.
(72) Cromwell, G. L.; Henry, B. J.; Scott, A. L.; Gerngross, M. F.; Dusek, D. L.; Fletcher, D. W. Glufosinate herbicide-tolerant (LibertyLink) rice vs. conventional rice in diets for growing-finishing swine. *J. Animal Sci.* **2005**, *83*, 1068−1074.
(73) Calsamiglia, S.; Hernandez, B.; Hartnell, G. F.; Phipps, R. Effects of corn silage derived from a genetically modified variety containing two transgenes on feed intake, milk production, and composition, and the absence of detectable transgenic deoxyribonucleic acid in milk in holstein dairy cows. *J. Dairy Sci.* **2007**, *90*, 4718−4723.
(74) Böhme, H.; Aulrich, K.; Daenicke, R.; Flachowsky, G. Genetically modified feeds in animal nutrition. 2nd communication: glufosinate tolerant sugar beets (roots and silage) and maize grains for ruminants and pigs. *Arch. Tierernahrung* **2001**, *54*, 197−207.
(75) Li, X.; Huang, K.; He, X.; Zhu, B.; Liang, Z.; Li, H.; Luo, Y. Comparison of nutritional quality between Chinese indica rice with sck and cry1Ac genes and its nontransgenic counterpart. *J. Food Sci.* **2007**, *72*, S420−S424.
(76) Choi, H.; Moon, J.-K.; Park, B.-S.; Park, H.-W.; Park, S.-Y.; Kim, T.-S.; Kim, D.-H.; Ryu, T.-H.; Kweon, S.-J.; Kim, J.-H. Comparative nutritional analysis for genetically modified rice, Iksan483 and Milyang204, and nontransgenic counterparts. *J. Korean Soc. Appl. Biol. Chem.* **2012**, *55*, 19−26.
(77) Chang, Y.; Zhao, C.; Zhu, Z.; Wu, Z.; Zhou, J.; Zhao, Y.; Lu, X.; Xu, G. Metabolic profiling based on LC/MS to evaluate unintended effects of transgenic rice with *cry1Ac* and *sck* genes. *Plant Mol. Biol.* **2012**, *78*, 477−487.
(78) Kim, J. K.; Park, S.-Y.; Ha, S.-H.; Lee, S. M.; Lim, S.-H.; Kim, H. J.; Ko, H.-S.; Oh, S.-D.; Park, J.-S.; Suh, S.-C. Compositional assessment of carotenoid-biofortified rice using substantial equivalence. *Afr. J. Biotechnol.* **2012**, *11*, 9330−9335.
(79) El Sanhoty, R.; Abd El-Rahman, A. A.; Bögl, K. W. Quality and safety evaluation of genetically modified potatoes Spunta with Cry V gene: compositional analysis, determination of some toxins, antinutrients compounds and feeding study in rats. *Food/Nahrung* **2004**, *48*, 13−18.
(80) Langkilde, S.; Schrøder, M.; Frank, T.; Shepherd, L. V. T.; Conner, S.; Davies, H. V.; Meyer, O.; Danier, J.; Rychlik, M.; Belknap, W. R.; McCue, K. F.; Engel, K.-H.; Stewart, D.; Knudsen, I.; Poulsen,

DOI: 10.1021/jf505015y
J. Agric. Food Chem. 2015, 63, 2063−2073

M. Compositional and toxicological analysis of a GM potato line with reduced α-solanine content—a 90-day feeding study in the Syrian Golden hamster. *Regul. Toxicol. Pharmacol.* **2012**, *64*, 177−185.
(81) Duke, S. O.; Lydon, J.; Koskinen, W. C.; Moorman, T. B.; Chaney, R. L.; Hammerschmidt, R. Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *J. Agric. Food Chem.* **2012**, *60*, 10375−10397.
(82) Bhandari, S.; Basnet, S.; Chung, K.; Ryu, K.-H.; Lee, Y.-S. Comparisons of nutritional and phytochemical property of genetically modified CMV-resistant red pepper and its parental cultivar. *Hortic., Environ. Biotechnol.* **2012**, *53*, 151−157.
(83) Corpillo, D.; Gardini, G.; Vaira, A. M.; Basso, M.; Aime, S.; Accotto, G. P.; Fasano, M. Proteomics as a tool to improve investigation of substantial equivalence in genetically modified organisms: The case of a virus-resistant tomato. *Proteomics* **2004**, *4*, 193−200.
(84) Shepherd, L.; McNicol, J.; Razzo, R.; Taylor, M.; Davies, H. Assessing the potential for unintended effects in genetically modified potatoes perturbed in metabolic and developmental processes. Targeted analysis of key nutrients and anti-nutrients. *Transgenic Res.* **2006**, *15*, 409−425.
(85) Berman, K. H.; Harrigan, G. G.; Nemeth, M. A.; Oliveira, W. S.; Berger, G. U.; Tagliaferro, F. S. Compositional equivalence of insect-protected glyphosate-tolerant soybean MON 87701 × MON 89788 to conventional soybean extends across different world regions and multiple growing seasons. *J. Agric. Food Chem.* **2011**, *59*, 11643−11651.
(86) Takagi, H.; Hirose, S.; Yasuda, H.; Takaiwa, F. Biochemical safety evaluation of transgenic rice seeds expressing T cell epitopes of Japanese cedar pollen allergens. *J. Agric. Food Chem.* **2006**, *54*, 9901−9905.
(87) Ricroch, A. E. Assessment of GE food safety using '-omics' techniques and long-term animal feeding studies. *New Biotechnol.* **2013**, *30*, 349−354.
(88) Gayen, D.; Sarkar, S. N.; Datta, S. K.; Datta, K. Comparative analysis of nutritional compositions of transgenic high iron rice with its non-transgenic counterpart. *Food Chem.* **2013**, *138*, 835−840.
(89) Lundry, D. R.; Burns, J. A.; Nemeth, M. A.; Riordan, S. G. Composition of Grain and Forage from Insect-Protected and Herbicide-Tolerant Corn, MON 89034 × TC1507 × MON 88017 × DAS-59122-7 (SmartStax) is Equivalent to That of Conventional Corn (*Zea mays* L). *J. Agric. Food Chem.* **2013**, *61*, 1991−1998.
(90) Herman, R. A.; Fast, B. J.; Johnson, T. Y.; Sabbatini, J.; Rudgers, G. W. Compositional Safety of Herbicide-Tolerant DAS-81910-7 Cotton. *J. Agric. Food Chem.* **2013**, *61*, 11683−11692.
(91) Lepping, M. D.; Herman, R. A.; Potts, B. L. Compositional Equivalence of DAS-44406-6 (AAD-12 + 2mEPSPS + PAT) Herbicide-Tolerant Soybean and Nontransgenic Soybean. *J. Agric. Food Chem.* **2013**, *61*, 11180−11190.
(92) Venkatesh, T.; Breeze, M. L.; Liu, K.; Harrigan, G. G.; Culler, A. H. Compositional Analysis of Grain and Forage from MON 87427, an Inducible Male Sterile and Tissue Selective Glyphosate-Tolerant Maize Product for Hybrid Seed Production. *J. Agric. Food Chem.* **2014**, *62*, 1964−1973.
(93) EFSA Panel on Genetically Modified Organisms (GMO), Guidance for Risk Assessment of Food and Feed from Genetically Modified Plants. *EFSA J.* **2011**, *9*, 2150−2186.
(94) Commission Implementing Regulation (EU) No 503/2013 on Applications for Authorisation of Genetically Modified Food and Feed in Accordance with Regulation (EC) No 1829/2003. *Official J. Eur Union* **2013**, *56*.
(95) *Application for Commodity Clearance of DAS-24236-5 × DAS-21023-5 (WideStrike) Cotton in the Republic of South Africa*; Dow AgroSciences LLC: Indianapolis, IN, 2013.
(96) *SAS/STAT 9.3 User's Guide*; SAS Institute Inc.: Cary, NC, 2011.
(97) Curran-Everett, D. Multiple comparisons: philosophies and illustrations. *Am. J. Physiol.: Regul., Integr. Compar. Physiol.* **2000**, *279*, R1−R8.
(98) Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B: Methodological* **1995**, *57*, 289−300.
(99) Colquhoun, D. An investigation of the false discovery rate and the misinterpretation of *p*-values. *R. Soc. Open Sci.* **2014**, *1*, 140216.
(100) Zhou, J.; Berman, K. H.; Breeze, M. L.; Nemeth, M. A.; Oliveira, W. S.; Braga, D. P. V.; Berger, G. U.; Harrigan, G. G. Compositional variability in conventional and glyphosate-tolerant soybean (*Glycine max* L.) varieties grown in different regions in Brazil. *J. Agric. Food Chem.* **2011**, *59*, 11652−11656.
(101) Bilyeu, K. D.; Zeng, P.; Coello, P.; Zhang, Z. J.; Krishnan, H. B.; Bailey, A.; Beuselinck, P. R.; Polacco, J. C. Quantitative Conversion of Phytate to Inorganic Phosphorus in Soybean Seeds Expressing a Bacterial Phytase. *Plant Physiol.* **2008**, *146*, 468−477.
(102) Hartwig, E. E.; Kilen, T. C. Yield and Composition of Soybean Seed from Parents with Different Protein, Similar Yield. *Crop Sci.* **1991**, *31*, 290−292.
(103) Iskander, F. Y. Maturation Stage and Mineral Content in Soybeans. *Food Chem.* **1987**, *24*, 29−35.
(104) Kakade, M. L.; Simons, N. R.; Liener, I. E.; Lambert, J. W. Biochemical and Nutritional Assessment of Different Varieties of Soybeans. *J. Agric. Food Chem.* **1972**, *20*, 87−90.
(105) *Environmental Health and Safety, Concensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients*; OECD: Paris, 2001; Vol. *15*, p 30.
(106) *International Life Sciences Institute Crop Composition Database*, version 4.2; International Life Sciences Institute: Washington, DC, 2011; www.cropcomposition.org.
(107) Herman, R. A.; Dunville, C. M.; Juberg, D. R.; Fletcher, D. W.; Cromwell, G. L. Performance of broiler chickens fed diets containing DAS-68416-4 soybean meal. *GM Crops* **2011**, *2*, 169−175.
(108) Harrigan, G. G.; Glenn, K. C.; Ridley, W. P. Assessing the natural variability in crop composition. *Regul. Toxicol. Pharmacol.* **2010**, *58*, S13−S20.
(109) Harrigan, G. G.; Culler, A. H.; Culler, M.; Breeze, M. L.; Berman, K. H.; Halls, S. C.; Harrison, J. M. Investigation of Biochemical Diversity in a Soybean Lineage Representing 35 Years of Breeding. *J. Agric. Food Chem.* **2013**, *61*, 6412−6422.
(110) Herman, R. A.; Price, W. D. Unintended Compositional Changes in Genetically Modified (GM) Crops: 20 Years of Research. *J. Agric. Food Chem.* **2013**, *61*, 11695−11701.

DOI: 10.1021/jf505015y
J. Agric. Food Chem. 2015, 63, 2063−2073