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.)

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ABSTRACT: Monsanto Company and Dow AgroSciences LLC have developed the combined-trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 (SmartStax, a registered trademark of Monsanto Technology LLC). The combination of four biotechnology-derived events into a single corn product (stacking) through conventional breeding provides broad protection against lepidopteran and corn rootworm insect pests as well as tolerance to the glyphosate and glufosinate—ammonium herbicide families. The purpose of the work described here was to assess whether the nutrient, antinutrient, and secondary metabolite levels in grain and forage tissues of the combined-trait product are comparable to those in conventional corn. Compositional analyses were conducted on grain and forage from SmartStax, a near-isogenic conventional corn hybrid (XE6001), and 14 conventional reference hybrids, grown at multiple locations across the United States. No statistically significant differences between SmartStax and conventional corn were observed for the 8 components analyzed in forage and for 46 of the 52 components analyzed in grain. The six significant differences observed in grain components ($p < 0.05$) were assessed in context of the natural variability for that component. These results demonstrate that the stacked product, SmartStax, produced through conventional breeding of four single-event products containing eight proteins, is compositionally equivalent to conventional corn, as previously demonstrated for the single-event products.

KEYWORDS: *Corn (Zea mays), herbicide-tolerant, insect-protected, biotechnology, composition*

■ INTRODUCTION

The adoption of biotechnology-derived (biotech) crops has occurred more rapidly than any other modern agricultural innovation. In 2011, global planting of biotech crops increased for the 16th consecutive year and now extends to a total of 160 million hectares across 29 developing and industrialized countries.[1] The United States continues to produce the world's largest share of biotech crops, followed by Brazil, Argentina, India, Canada, and China.

Advances in plant breeding and transformation technology (biotech) have facilitated the introduction of multiple biotech traits into a single crop variety. These combined-trait stacked products have been increasingly preferred by farmers over the single-trait products. Double stacks in maize, conferring two types of insect resistance or one insect resistance trait plus herbicide tolerance, and triple stacks, conferring two types of insect resistance plus herbicide tolerance, have been the fastest growing stacked products in recent years.[1] In 2011, planting of stacked traits grew by 31% over the previous year compared to the single-trait product increases of 5% for herbicide tolerance and 10% for insect resistance. In addition to the U.S., many other countries have commercialized stacked products, and this trend is expected to increase over time with increasingly complex stacks.

Monsanto Company (St. Louis, MO) and Dow AgroSciences LLC (Indianapolis, IN) have developed the combined-trait corn product MON 89034 × TC1507 × MON 88017 × DAS59122-7 (SmartStax, a registered trademark of Monsanto Technology LLC) through the conventional, progressive cross-hybridization of four individual biotech products that each confer specific benefits of insect protection and/or herbicide tolerance. The four biotech products used to create this combined-trait breeding stack are MON 89034 (YieldGard VT Pro), TC1507 (HERCULEX I Insect Protection), MON 88017 (YieldGard VT Rootworm/ 56RR2), and DAS-59122-7 (HERCULEX RW Insect Protection). YieldGard VT Pro and YieldGard VT Rootworm/RR2 are trademarks of Monsanto Technology LLC. HERCULEX is a trademark of Dow AgroSciences LLC.[2] Together, these single-event products produce eight unique biotechnology-derived proteins within the stacked product SmartStax. Each of these single-event products within SmartStax has been independently reviewed by numerous international regulatory organizations,[2] including the United States Department of Agriculture (USDA), the United States Food and Drug Administration (U.S. FDA), and the European Food Safety Authority (EFSA), and has been registered by the United States Environmental Protection Agency (U.S. EPA). The registration and subsequent commercialization of these single-trait products was contingent on and preceded by the rigorous safety and characterization studies that are typically used in the evaluation of new varieties of crops derived through biotechnology.[3−13] These assessments, including composition and animal feeding studies, can

be applied to determine if the compositional and nutritional parity of single-trait products to a conventional comparator extends to the combined-trait product.[14]

Compositional analysis is a major component of the comparative safety assessment process. It involves comparisons of levels of key nutrients, antinutrients, and secondary metabolites in the new biotech crop to a conventional comparator.[15−17] Assessments of differences typically use significance testing. When no differences are observed, the biotech crop is considered to be as nutritious and safe as the conventional counterpart for food and feed uses. Differences identified in these studies are followed by a more detailed evaluation of the biological relevance of the difference in the context of natural variability in corn hybrids with a history of safe consumption.

The importance of assessing the biological relevance of a statistically significant difference in the context of natural variability was originally described by a joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) expert consultation on foods derived from biotechnology[15] and endorsed by other groups, such as the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, to develop the Codex guideline.[18] The Codex guideline states: "The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance". Approaches must, therefore, include additional data considerations, such as the magnitude of the difference, the distribution of component ranges, and the impact of environment and genetics (natural variability) on levels of the components of interest.

The purpose of this study was to evaluate the composition of the combined-trait product, SmartStax, compared to the near isogenic conventional corn hybrid XE6001. Although several studies have been published that assess the composition of crops with biotech traits,[3,6,7,10,19−21] this is the first published study to evaluate forage and grain composition of a biotechnology-derived breeding stack product comprised of four single events, producing eight unique proteins.

■ MATERIALS AND METHODS

Corn Samples for Compositional Analyses. The experimental test hybrid material in this study was the combined-trait product, MON 89034 × TC1507 × MON 88017 × DAS-59122-7 (SmartStax). The control was a conventional corn hybrid (XE6001) with a genetic background similar to SmartStax. A total of 15 commercially available conventional reference hybrids were planted concurrently with the test and control hybrids to provide in-study data on the natural variability of each component based on genetics and environment. Other hybrid corn materials were included in the field design and the statistical model but are not relevant to this report.

Materials for composition analyses were generated in 2006 at five locations within the primary corn-growing regions of the United States: two sites were located in Iowa; two sites were located in Illinois; and one site was located in Nebraska. At each location, corn hybrids were planted in a randomized complete block design with three blocks. Within each block, plots (6 rows × 6.1 m) were assigned randomly to SmartStax, XE6001, or three of the conventional reference hybrids. A total of 15 references were planted across the 5-site multi-location study (3/site), and these hybrids were non-genetically modified (GM) and were selected for each site as typical of what is grown in that area.

Standard agronomic practices for each geographic region were followed, including the application of registered non-glyphosate- and non-glufosinate-containing maintenance pesticides. In addition, a single application of Roundup WeatherMAX (Roundup and Roundup WeatherMAX are registered trademarks of Monsanto Technology LLC) was applied, according to the label, at a rate of 0.75 lb a.e./acre to the SmartStax plots at approximately the V2−V4 growth stages. After 7−14 days, a single application of Liberty (Liberty is a registered trademark of Bayer CropScience) herbicide was applied, according to the label, at a rate of 30 oz/acre to the SmartStax plots. The genetic purity of SmartStax, XE6001, and commercial reference corn was maintained by bagging the tassels and ear shoots at anthesis and hand-pollinating each plant.

Forage samples were collected at the late dough/early dent stage (R4−R5) from two plants of each plot by cutting at the base above the ground and compositing the individual plants into one sample per plot. Forage samples were transferred to dry ice within 30 min after sampling. The grain was collected as a composite sample from all remaining ears in the plot. XE6001 samples were collected first, followed by commercial reference samples, and finally, SmartStax samples. Grain was harvested at normal kernel maturity (R6 growth stage) when the moisture content measured approximately 12−15% and was stored at ambient temperature. Forage samples were shipped frozen on dry ice, and grain samples were shipped at ambient temperature. At Monsanto Company (St. Louis, MO), forage and grain samples were homogenized by grinding with dry ice to a fine powder and stored frozen at approximately −20 °C until compositional analysis.

Event-specific polymerase chain reaction analysis[22] was conducted to confirm the genetic identity of the harvested grain. Despite precautions to prevent cross-contamination between samples, all SmartStax, two control samples, and all samples from one reference hybrid grown at one field site contained the adventitious presence of unintended traits and were excluded from analyses. As a result, SmartStax and XE6001 samples were only used from four locations and 14 reference samples were available from five locations. Compositional analysis was conducted on all three replicates of SmartStax and XE6001 from each location and on one replicate of the reference hybrids from each location.

Compositional Analysis Methods. Compositional evaluations were performed using the guidelines outlined in the Organization for Economic Cooperation and Development (OECD) consensus document for maize composition.[16] These guidelines are globally accepted and are consistent with country-specific guidelines in the U.S., Canada, and other countries and regions. Forage samples were analyzed for proximates (ash, fat, moisture, and protein), acid detergent fiber (ADF), neutral detergent fiber (NDF), and minerals (calcium and phosphorus).[3] Grain samples were analyzed for proximates, ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids (FA), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), secondary metabolites [ferulic acid, 2-furaldehyde (furfural), and *p*-coumaric acid], antinutrients (phytic acid and raffinose), and vitamins (folic acid, β-carotene, niacin, vitamin B_1, vitamin B_2, vitamin B_6, and vitamin E). Total carbohydrates in forage and grain were calculated by difference, and moisture was determined for the re-expression of fresh weight values on a dry weight basis. Analytical methods used for the analysis of forage and grain components have been previously described by Drury et al.[3] Compositional analyses were conducted at Covance Laboratories Inc. in Madison, WI. Samples were analyzed in a randomized order to minimize assay bias.

Statistical Analysis of Compositional Data. Statistical analysis of the data involved a head-to-head comparison of SmartStax to XE6001 for every analyte across all sites to determine significant differences at $p < 0.05$. To complete a statistical analysis for a component in this study, at least 50% of the values for an analyte had to be greater than the assay limit of quantitation (LOQ).[23] The following 16 analytes, which naturally occur at low levels in corn, had >50% of observations less than the LOQ and were excluded from statistical analysis: caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, pentadecenoic acid, palmitoleic acid, heptadecanoic acid, heptadecenoic acid, γ-linolenic acid, eicosadienoic acid, eicosatrienoic acid, arachidonic acid, sodium, and

Table 1. Nutrient Composition of Forage from MON 89034 × TC1507 × MON 88017 × DAS-59122-7

component	SmartStax mean (range)[a]	XE6001 conventional control mean (range)[a]	commercial conventional hybrids (range)[b] [99% TI][c]	literature range[d]
		Proximate (% dwt[e])		
ash	3.85	4.12	(2.80−6.54)	1.527−9.638
	(3.08−4.68)	(2.98−6.01)	[0.16, 8.68]	
carbohydrate	86.52	86.82	(83.38−88.33)	76.4−92.1
	(84.00−88.57)	(84.12−89.13)	[80.33, 92.03]	
protein	7.69	7.20	(6.27−8.80)	3.14−11.57
	(6.80−8.60)	(5.39−8.32)	[5.01, 10.55]	
total fat	1.94	1.86	(0.91−2.72)	0.296−4.570
	(0.16−3.19)	(0.46−2.97)	[0, 3.67]	
		Fiber (% dwt)		
ADF	30.26	29.90	(25.84−39.37)	16.13−47.39
	(24.19−39.07)	(24.33−36.05)	[16.73, 47.63]	
NDF	41.68	43.56	(36.09−65.15)	20.29−63.71
	(31.57−51.88)	(36.34−47.76)	[13.81, 78.53]	
		Minerals (mg/kg dwt)		
calcium	0.19	0.20	(0.15−0.31)	0.0714−0.5768
	(0.11−0.34)	(0.13−0.31)	[0.0028, 0.41]	
phosphorus	0.20	0.18	(0.13−0.24)	0.0936−0.3704
	(0.13−0.24)	(0.11−0.23)	[0.067, 0.33]	

[a]The mean and range of 12 values (three replicates from each of four field sites). [b]The range of 14 values for commercial hybrids grown concurrently (three hybrids from each of four field sites and two hybrids from one field site). [c]TI = tolerance interval, specified to contain 99% of the commercial hybrid population with 95% confidence; negative limits set to zero. [d]From ref 24. [e]dwt = dry weight.

Table 2. FA Composition of Grain from MON 89034 × TC1507 × MON 88017 × DAS-59122-7

component (% total FA[a])	SmartStax mean (range)[b]	XE6001 conventional control mean (range)[b]	commercial conventional hybrids (range)[c] [99% TI][d]	literature range[e]
palmitic acid	10.64	10.56	(8.96−12.73)	7.94−20.71
	(10.18−11.05)	(10.09−11.14)	[6.60, 15.00]	
stearic acid	2.05	1.93[f]	(1.39−2.38)	1.02−3.40
	(1.96−2.28)	(1.90−1.98)	[0.58, 2.89]	
oleic acid	30.40	31.24[f]	(21.00−34.20)	17.4−40.2
	(29.60−31.71)	(29.85−32.92)	[10.72, 42.79]	
linoleic acid	55.09	54.53	(51.11−63.09)	36.2−66.5
	(53.39−56.03)	(52.23−56.02)	[44.51, 73.33]	
linolenic acid	1.00	0.96[f]	(0.86−1.31)	0.57−2.25
	(0.95−1.05)	(0.90−1.00)	[0.53, 1.54]	
arachidic acid	0.42	0.40[f]	(0.30−0.43)	0.279−0.965
	(0.40−0.46)	(0.37−0.42)	[0.23, 0.53]	
eicosenoic acid	0.26	0.27[f]	(0.20−0.30)	0.170−1.917
	(0.24−0.28)	(0.25−0.28)	[0.13, 0.34]	
behenic acid	0.14	0.12	(0.060−0.24)	0.110−0.349
	(0.067−0.22)	(0.064−0.22)	[0, 0.39]	

[a]FA = fatty acid. [b]The mean and range of 12 values (three replicates from each of four field sites). [c]The range of 14 values for commercial hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site). [d]TI = tolerance interval, specified to contain 99% of the commercial conventional hybrid population with 95% confidence; negative limits set to zero. [e]From ref 24. [f]Significant difference between SmartStax and control ($p < 0.05$).

furfural. For individual measurements less than the LOQ and with more than 50% of all of the values greater than the LOQ, a value equal to one-half of the LOQ was assigned prior to statistical analyses. Assigned individual values included 2 values for total fat in forage, 1 value for raffinose in grain, 15 values for 22:0 behenic acid in grain, and 11 values for p-coumaric acid in grain.

A studentized PRESS residuals test was applied to the adjusted data set to identify outliers. Extreme data points that are outside of the ±6 studentized PRESS residual range were considered for exclusion, as outliers, from the final statistical analysis. One leucine value and one copper value were identified as outliers and were removed from the statistical analysis.

Statistical analysis of the data from samples collected from each field site was conducted at Certus International, Inc. (Chesterfield, MO).

Statistical analyses were conducted on forage and grain using a mixed model analysis of variance for compositional data from the combination of all sites (combined site) using model 1

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk} \tag{1}$$

where Y_{ijk} is the unique individual observation, U is the overall mean, T_i is the substance effect, L_j is the random location effect, $B(L)_{jk}$ is the random block within location effect, LT_{ij} is the random location by substance interaction effect, and e_{ijk} is the residual error. For each compositional component, the forage and grain from SmartStax were compared to the forage and grain from XE6001. Statistically significant differences between the test values and the control values were declared at $p < 0.05$.

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dx.doi.org/10.1021/jf304005n | J. Agric. Food Chem. 2013, 61, 1991−1998

Table 3. Vitamin Composition of Grain from MON 89034 × TC1507 × MON 88017 × DAS-59122-7

component (mg/kg dwt[a])	SmartStax mean (range)[b]	XE6001 conventional control mean (range)[b]	commercial conventional hybrids (range)[c] [99% TI][d]	literature range[e]
folic acid	0.39 (0.33–0.46)	0.36 (0.29–0.43)	(0.28–0.45) [0.15, 0.57]	0.147–01.464
niacin	24.02 (20.11–29.35)	23.77 (19.14–27.84)	(13.88–27.09) [6.69, 34.92]	9.36–4.290
β-carotene	1.05 (0.89–1.19)	1.02 (0.79–1.20)	(0.54–1.48) [0, 1.98]	0.19–46.81
vitamin B_1	2.33 (2.05–2.70)	2.63[f] (2.36–3.20)	(2.13–3.73) [1.24, 4.86]	1.26–40.00
vitamin B_2	1.91 (1.23–2.76)	2.30 (1.30–2.94)	(1.28–3.68) [0, 5.68]	0.50–2.36
vitamin B_6	5.80 (5.39–6.14)	5.79 (5.30–6.49)	(4.51–7.24) [2.23, 8.85]	3.68–11.32
vitamin E	8.42 (6.57–9.97)	7.72 (6.27–8.63)	(5.95–15.52) [0, 22.92]	1.537–68.672

[a]dwt = dry weight. [b]The mean and range of 12 values (three replicates from each of four field sites). [c]The range of 14 values for commercial conventional hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site). [d]TI = tolerance interval, specified to contain 99% of the commercial hybrid population with 95% confidence; negative limits set to zero. [e]From ref 24. [f]Significant difference between SmartStax and control ($p < 0.05$).

Table 4. Proximate, Fiber, and Mineral Composition of Grain from MON 89034 × TC1507 × MON 88017 × DAS-59122-7

component	SmartStax mean (range)[a]	XE6001 conventional control mean (range)[a]	commercial conventional hybrids (range)[b] [99% TI][c]	literature range[d]
		Proximate (% dwt[e])		
ash	1.24 (1.08–1.36)	1.22 (1.02–1.51)	(1.07–1.53) [0.77, 1.81]	0.616–6.282
carbohydrate	85.39 (84.45–85.96)	85.41 (84.60–86.53)	(82.35–86.70) [79.24, 90.01]	77.4–89.5
protein	9.85 (9.22–10.62)	9.78 (9.01–10.39)	(9.21–12.80) [6.20, 15.18]	6.15–17.26
total fat	3.52 (3.18–3.98)	3.60 (3.13–4.04)	(2.77–4.60) [1.35, 5.45]	1.742–5.900
		Fiber (% dwt)		
ADF	2.93 (2.32–4.56)	2.97 (2.02–4.22)	(2.55–3.92) [1.60, 4.68]	1.82–11.34
NDF	11.68 (10.29–14.85)	11.62 (9.77–14.43)	(8.62–12.88) [6.22, 15.51]	5.59–22.64
total dietary fiber	16.91 (13.74–21.83)	16.48 (12.33–21.89)	(12.78–20.65) [8.28, 24.21]	9.01–35.31
		Minerals (mg/kg dwt)		
calcium	37.67 (30.70–45.23)	39.67 (31.54–50.92)	(27.46–60.23) [5.86, 83.14]	12.7–208.4
copper	2.33 (1.63–4.21)	1.93 (1.34–3.95)	(1.51–3.42) [0, 4.96]	0.73–18.50
iron	21.11 (18.79–23.37)	21.86 (18.63–24.16)	(15.63–24.35) [11.51, 29.14]	10.42–49.07
magnesium	1159.84 (988.75–1300.90)	1170.40 (1023.97–1282.63)	(936.10–1346.80) [659.92, 1708.83]	594.0–1940.0
manganese	5.98 (5.14–6.46)	6.22 (5.22–7.41)	(5.50–7.15) [4.24, 8.21]	1.69–14.30
phosphorus	2990.96 (2440.94–3438.91)	2923.70 (2596.81–3234.96)	(2522.62–3697.86) [1776.54, 4654.30]	1470.0–5330.0
potassium	3185.16 (2800.90–3472.85)	3135.51 (2984.05–3442.07)	(2802.26–3887.01) [2003.91, 4604.37]	1810.0–6030.0
zinc	20.81 (17.44–24.44)	22.66 (18.86–27.03)	(18.64–34.20) [10.42, 37.84]	6.5–37.2

[a]The mean and range of 12 values (three replicates from each of four field sites). [b]The range of 14 values for commercial conventional hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site). [c]TI = tolerance interval, specified to contain 99% of the commercial hybrid population with 95% confidence; negative limits set to zero. [d]From ref 24. [e]dwt = dry weight.

Table 5. Amino Acid Composition of Grain from MON 89034 × TC1507 × MON 88017 × DAS-59122-7

component (% dwt[a])	SmartStax mean (range)[b]	XE6001 conventional control mean (range)[b]	commercial conventional hybrids (range)[c] [99% TI][d]	literature range[e]
alanine	0.71 (0.65−0.78)	0.72 (0.62−0.80)	(0.67−0.96) [0.44, 1.18]	0.44−1.39
arginine	0.42 (0.38−0.46)	0.40 (0.30−0.45)	(0.38−0.54) [0.25, 0.61]	0.12−0.64
aspartic acid	0.64 (0.58−0.69)	0.63 (0.51−0.70)	(0.60−0.82) [0.39, 0.97]	0.33−1.21
cystine/cysteine	0.20 (0.18−0.21)	0.19 (0.15−0.21)	(0.16−0.25) [0.11, 0.30]	0.13−0.51
glutamic acid	1.83 (1.67−1.99)	1.85 (1.60−2.03)	(1.68−2.54) [1.08, 3.07]	0.97−3.54
glycine	0.36 (0.33−0.38)	0.35 (0.29−0.37)	(0.34−0.45) [0.24, 0.50]	0.18−0.54
histidine	0.26 (0.24−0.28)	0.26 (0.22−0.28)	(0.25−0.35) [0.19, 0.38]	0.14−0.43
isoleucine	0.33 (0.30−0.36)	0.33 (0.28−0.38)	(0.31−0.45) [0.20, 0.53]	0.18−0.69
leucine	1.20 (1.09−1.31)	1.22 (1.06−1.37)	(1.11−1.73) [0.67, 2.12]	0.64−2.49
lysine	0.29 (0.26−0.32)	0.28 (0.21−0.30)	(0.26−0.36) [0.17, 0.40]	0.17−0.67
methionine	0.18 (0.17−0.19)	0.19 (0.17−0.20)	(0.17−0.26) [0.10, 0.30]	0.12−0.47
phenylalanine	0.49 (0.45−0.53)	0.49 (0.41−0.55)	(0.46−0.67) [0.28, 0.83]	0.24−0.93
proline	0.84 (0.77−0.93)	0.85 (0.72−0.96)	(0.75−1.17) [0.47, 1.41]	0.46−1.63
serine	0.48 (0.43−0.51)	0.48 (0.43−0.52)	(0.43−0.66) [0.26, 0.80]	0.24−0.77
threonine	0.33 (0.30−0.35)	0.33 (0.26−0.36)	(0.31−0.44) [0.20, 0.51]	0.22−0.67
tryptophan	0.065 (0.050−0.077)	0.063 (0.054−0.075)	(0.051−0.084) [0.032, 0.10]	0.027−0.22
tyrosine	0.31 (0.24−0.34)	0.30 (0.18−0.35)	(0.19−0.42) [0.11, 0.56]	0.10−0.64
valine	0.45 (041−0.48)	0.45 (0.37−0.49)	(0.43−0.59) [0.30, 0.68]	0.27−0.86

[a]dwt = dry weight. [b]The mean and range of 12 values (three replicates from each of four field sites). [c]The range of 14 values for commercial hybrids grown concurrently (three references from each of four field sites and two references from one field site). [d]TI = tolerance interval, specified to contain 99% of the commercial conventional hybrid population with 95% confidence; negative limits set to zero. [e]From ref 24.

Table 6. Anti-nutrient and Secondary Metabolite Composition of Grain from MON 89034 × TC1507 × MON 8017 × DAS-59122-7

component	SmartStax mean (range)[a]	XE6001 conventional control mean (range)[a]	conventional commercial references (range)[b] [99% TI][c]	literature range[d]
		Antinutrient (% dwt[e])		
phytic acid	0.73 (0.53−0.87)	0.71 (0.57−0.80)	(0.53−0.90) [0.25, 1.25]	0.111−1.570
raffinose	0.095 (0.074−0.12)	0.088 (0.028−0.12)	(0.089−0.18) [0.026, 0.23]	0.020−0.320
		Secondary Metabolite (mg/kg dwt)		
ferulic acid	1614.21 (956.82−1974.89)	1477.59 (930.26−1874.30)	(1422.12−2085.20) [858.39, 2495.12]	291.9−3885.8
p-coumaric acid	54.49 (28.12−82.91)	45.94 (28.12−86.95)	(91.06−219.73) [0, 281.45]	53.4−576.2

[a]The mean and range of 12 values (three replicates from each of four field sites). [b]The range of 14 values for commercial conventional hybrids grown concurrently (three replicates from each of four field sites and two replicates from one field site). [c]TI = tolerance interval, specified to contain 99% of the commercial hybrid population with 95% confidence; negative limits set to zero. [d]From ref 24. [e]dwt = dry weight.

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dx.doi.org/10.1021/jf304005n | J. Agric. Food Chem. 2013, 61, 1991−1998

The conventional reference hybrids were used to estimate natural compositional variation that can generally be attributed to differences in the germplasm and environment. Thus, a range of values was determined for each component analyzed, and population tolerance intervals (TIs) were developed. TIs were expected to contain, with 95% confidence, 99% of the values expressed in the population of conventional corn hybrids.

■ RESULTS AND DISCUSSION

Least-squares means and the range of observed values for SmartStax and the control, XE6001, are presented in Tables 1−6. The range of observed values, 99% TI for the reference corn hybrids, and the range of values reported in the International Life Science Institute Crop Composition Database (ILSI-CCDB) for conventional corn[24] are also presented. The few statistically significant differences ($p < 0.05$) between SmartStax and XE6001 reflect small mean differences and can be contrasted to the wide ranges of values that characterize natural variability in corn. The data presented in Tables 1−6 thus highlight the similarity in values and provide a basis for how significant differences can be further assessed for biological relevance.

Forage Composition. There were no significant differences ($p < 0.05$) between SmartStax and the control for the eight components analyzed in forage (Table 1). Comparative values reflected small mean differences and overlapping ranges. In comparison to the 99% TIs estimated from the reference substances and conventional values found in the ILSI-CCDB,[24] the observed values for forage nutrients in SmartStax and control were representative of the values typically observed in the natural population of conventional corn.

Grain Composition. There were no significant differences ($p < 0.05$) between SmartStax and the control for 46 of the 52 components measured in grain (Tables 2−6). Significant differences ($p < 0.05$) were observed in grain between SmartStax and the control for five FA (stearic acid, oleic acid, linolenic acid, arachidic acid, and eicosenoic acid) and for vitamin B_1 (Tables 2 and 3). These differences were further evaluated in context of the natural variability of nutrient components in corn.

FA levels in corn can vary extensively based on genetics and environment.[25,26] In this study, significant differences were observed between SmartStax and the control for stearic acid, oleic acid, linolenic acid, arachidic acid, and eicosenoic acid (Table 2). The differences in mean values between SmartStax and the control were small (<6.5%). Considering the variability in individual values and the overlapping ranges of values between SmartStax and the control, the observed differences reflect the sensitivity of the study design to detect small changes. For instance, least-squares mean values for oleic acid were 30.40 and 31.24% total FA for SmartStax and control corn, a relative difference of −2.7%. The ranges of values for oleic acid overlap extensively between SmartStax and the control and are characteristic of the variability observed in the natural population of corn. Similar results are observed for the lower abundant FA (stearic acid, linolenic acid, arachidic acid, and eicosenoic acid). Relative differences were small (0.1−6.2% of the control); ranges of values overlapped extensively between SmartStax and the control; and individual values are characteristic of the variability observed in the natural population of conventional corn, as represented by the 99% TIs and conventional values reported in the ILSI-CCDB.[24] These data, presented graphically in Figures 1−3, emphasize
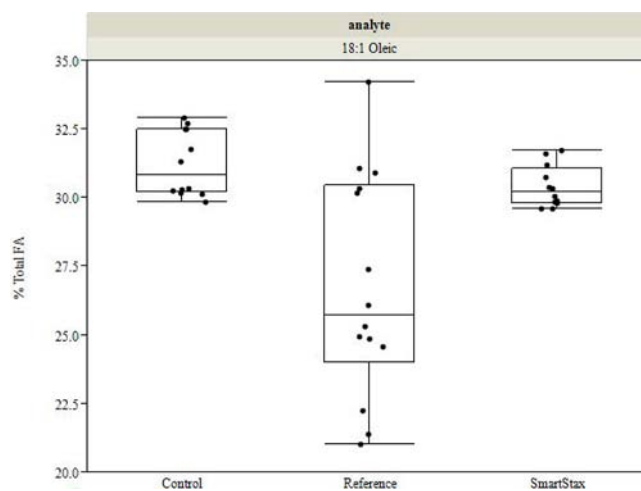

Figure 1. Boxplot of oleic acid values in SmartStax, conventional control, and conventional reference hybrids. Each box extends from the lower (25th) to the upper (75th) quartile, and the line in each box represents the median. The whiskers extend to extreme data points (minimum and maximum). See Table 2 for details.
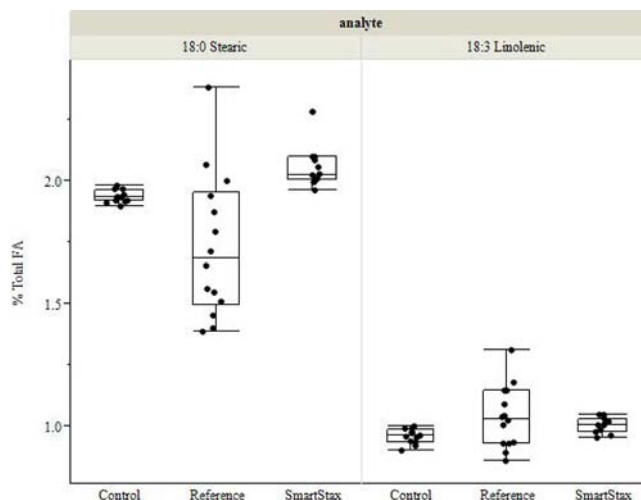

Figure 2. Boxplot of stearic acid and linolenic acid values in SmartStax, conventional control, and conventional reference hybrids. Each box extends from the lower (25th) to the upper (75th) quartile, and the line in each box represents the median. The whiskers extend to extreme data points (minimum and maximum), unless points are farther away from the quartiles than 1.5 times the box length, in which case the whiskers only cover the remaining points. See Table 2 for details.

the similarities in values between SmartStax and the control and illustrate the wide range of variability in the individual values for SmartStax, control, and reference hybrids as a result of genetics and growing locations. The FA data presented in Figure 4 and Table 2 demonstrate that the overall FA profile of SmartStax is not altered from conventional corn.

A total of seven vitamin components were evaluated in this study, and there were no differences between SmartStax and the control, with the exception of vitamin B_1 (thiamin) (Table 3). The significant difference in vitamin B_1 reflected a small difference in mean values between SmartStax and the conventional control. The relative magnitude of the difference was a decrease of 11.41% in vitamin B_1 levels, from 2.63 mg/kg dwt in the control to 2.33 mg/kg dwt in SmartStax, a difference
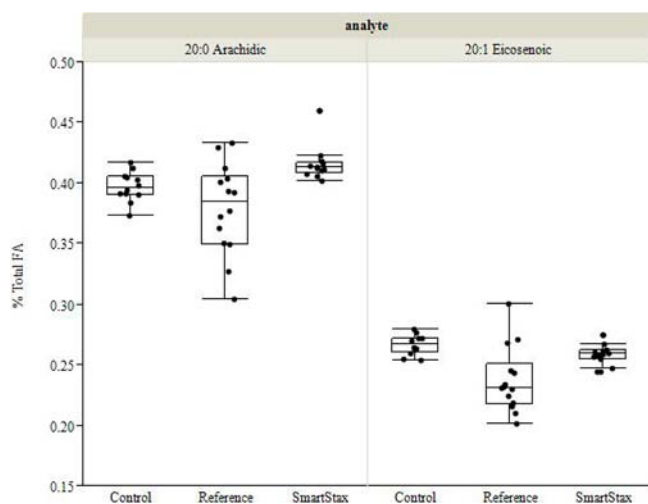
Figure 3. Boxplot of arachidic acid and eicosenoic acid values in SmartStax, conventional control, and conventional reference hybrids. Each box extends from the lower (25th) to the upper (75th) quartile, and the line in each box represents the median. The whiskers extend to extreme data points (minimum and maximum), unless points are farther away from the quartiles than 1.5 times the box length, in which case the points are shown separately. See Table 2 for details.
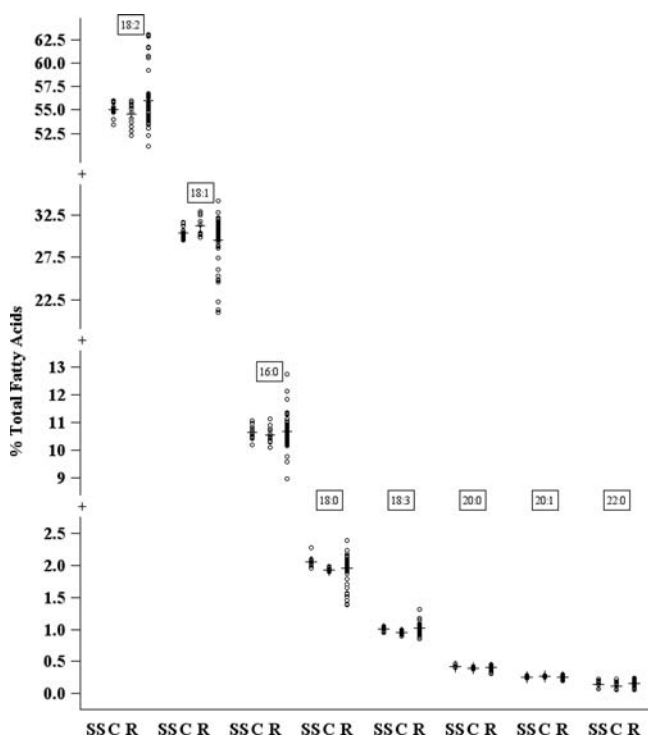

Figure 5. Boxplot of vitamin B_1 values in SmartStax, conventional control, and conventional reference hybrids. Each box extends from the lower (25th) to the upper (75th) quartile, and the line in each box represents the median. The whiskers extend to extreme data points (minimum and maximum). See Table 3 for details.

Figure 4. FA profile in grain of SmartStax (SS), control (C), and conventional reference (R) hybrids. FA are labeled by the number of carbons and double bonds (16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:0, arachidic acid; 20:1, eicosenoic acid; and 22:0, behenic acid). Mean values are indicated by a cross bar (−).

of −0.30 mg/kg dwt. The vitamin B_1 data presented graphically in Figure 5 emphasize the similarities in values between SmartStax and the control and illustrate the considerable variability in individual values across sites for SmartStax, control, and references hybrids because of genetics and growing locations. It is unlikely that the small difference for vitamin B_1 is
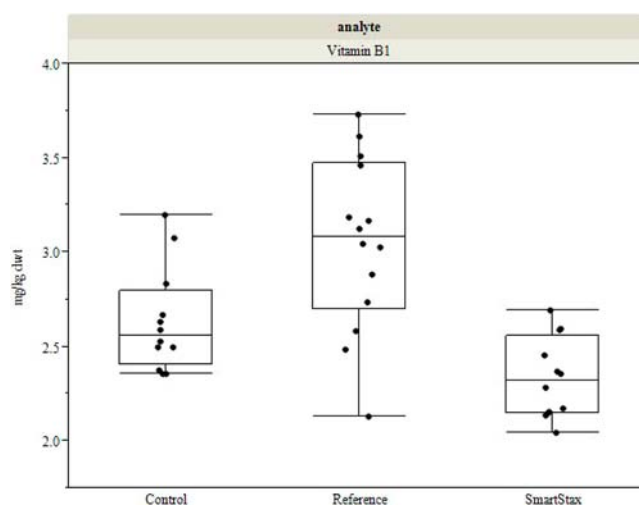
due to the introduction of herbicide-tolerance and insect-resistance traits into SmartStax, because there was no impact on the overall vitamin profile.

Overall, there were few significant differences between compositional components in SmartStax and control corn, and those were characterized by small magnitudes of difference. The observation of small differences also applied to the seed and forage components that were not different between SmartStax and the control and highlighted the overwhelming similarity between SmartStax and control corn. Levels of protein can be used to illustrate the lack of impact of incorporating multiple traits into a single product. The mean value for seed protein in SmartStax was 9.85% dwt, whereas the control value was 9.78% dwt. The relative magnitude of difference, with respect to the control, was less than 1%. Tables 2−6 provide further examples of compositional parity characterized by small differences between SmartStax and the control. Overall, analysis of the forage and grain composition confirms the lack of impact of incorporating multiple traits into a single product.

In summary, these compositional analyses show that levels of key nutrients, antinutrients, and secondary metabolites in forage and grain of SmartStax are comparable to the conventional control. It was therefore concluded that stacking of four events containing eight biotech genes into SmartStax did not affect the composition of forage and grain. Results support the conclusion that the compositional equivalence observed for the single-event products extends to the combined-trait product, SmartStax.

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Notes

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