The Composition of Insect-Protected Cottonseed Is Equivalent to That of Conventional Cottonseed

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Cotton plants have been developed that control the major lepidopteran insect pests of cotton by the stable introduction of a gene encoding an insecticidal protein from *Bacillus thuringiensis* subsp. *kurstaki*. These plants provide season-long protection against cotton bollworm, tobacco budworm, and pink bollworm. An important component of the safety and product assessment of these lines was the comparison of the nutrient and antinutrient levels in the seed both to the parental variety and to published values for other commercial cotton varieties. Compositional equivalence confirms the appropriateness of these cotton lines for use in food and feed products. The insect-protected lines and the parental control were shown to contain levels of nutrients comparable to those of other commercial varieties. Nutrients included protein, fat, carbohydrate, moisture, ash, amino acids, and fatty acids. The levels of the antinutrients gossypol, cyclopropenoid fatty acids, and aflatoxin in the seed from the insect-protected lines were similar to or lower than the levels present in the parental variety and reported for other commercial varieties. These analyses demonstrate that seed from the insect-protected cotton lines is compositionally equivalent to and as nutritious as seed from the parental and other commercial cotton varieties.

Keywords: *Insect-protected cotton; cottonseed; composition; nutrients; antinutrients; B.t.k. gene*

INTRODUCTION

Numerous new crop varieties protected against insect, fungal, and viral diseases and having improved quality attributes or offering more environmentally compatible means of weed control are under development and nearing market introduction (Fuchs et al., 1993). One of these products is cotton protected season-long against damage by the most devastating insect pests of cotton, the cotton bollworm, the tobacco budworm, and the pink bollworm (Perlak et al., 1991). Approximately 80% of the 11−13 million acres that are planted to cotton annually are infested with one or more of these insect pests, which currently cost the cotton grower in excess of $100 million annually to control with chemical insecticides (Williams, 1994; Head, 1991, 1992, 1993).

As an effective and environmentally superior approach to control these insect pests, a gene initially derived from a naturally occurring bacterium, *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*), has been stably inserted into the chromosome of cotton, enabling production of a protein that is active against these insect pests. Producing this protein within the cotton plant itself provides effective and season-long control of these insect pests (Wilson et al., 1994). *B.t.k.* has been used safely for over 30 years in microbial formulations (Lüthy et al., 1982). This organism and the insecticidal proteins produced have been shown to be very specific to the targeted insect pests and to cause no deleterious effects to nontarget organisms such as beneficial insects, birds, fish, and mammals, including humans (EPA, 1988).

Cottonseed provides an important source of oil for human consumption and cottonseed and processed cottonseed meal for animal feed (*Cottonseed and Its Products*, 1989). Therefore, the composition of the cottonseed has been extensively evaluated to confirm that these products derived from the insect-protected cotton varieties are equivalent to those of the parental variety as well as conventional cotton lines. For insect-protected cotton, these analyses have focused on three independent lines that contain the same *B.t.k.* gene but were derived by independent transformation events. Data presented in this paper demonstrate that the levels of nutrients (protein, fat, fiber, ash, carbohydrates, calories, amino acids, and fatty acids) in the insect-protected cottonseed and selected processed products are comparable to those of the parental variety and other commercial cotton varieties. In addition, the levels of antinutrients (gossypol, cyclopropenoid fatty acids, and aflatoxin) in cotton seed and selected processed products were also measured and shown to be similar or lower. These results confirm that the cottonseed produced from these three insect-protected cotton lines is compositionally equivalent to that from the parental variety as well as the other commercial varieties.

MATERIALS AND METHODS

Insect-Protected Cotton Lines. Compositional analyses were performed on three independent insect-protected cotton lines (lines 531, 757, and 1076) as well as the parental control (Coker 312). A gene that produces a protein that is 99.4% identical to the native *B.t.k.* protein produced by *B.t.k.* strain HD-73 (Adang et al., 1985) was introduced into cotton plants to confer insect protection (Perlak et al., 1990). This gene encoding the *B.t.k.* protein was modified as described by Perlak et al. (1991) for enhanced expression in plants. Two different

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promoters, derived from cauliflower and figwort mosaic viruses, were used that enabled expression throughout the cotton plant to provide effective insect control.

Agrobacterium-mediated transformation of cotton hypocotyl sections of Coker 312 was performed with modifications as described by Umbeck et al. (1987). Plants were regenerated with modifications as described by Trolinder and Goodin (1987). Insect-protected cotton plants were identified and evaluated as described by Perlak et al. (1991). Insecticidally efficacious plants were repeatedly self-pollinated to provide homozygous seed for subsequent field testing to select superior lines in terms of agronomics and insect efficacy. Insect-protected cotton lines 531, 757, and 1076 were selected for commercialization on the basis of these assessments. The genes from these lines were introduced into a variety of commercial cotton germplasms by traditional backcrossing to generate seed for further testing and commercialization.

Cottonseed Production. Cottonseed for the 1993 field tests with the insect-protected lines was produced from the R_4 generation of line 531 and the R_3 generation of lines 757 and 1076. Coker 312 was obtained from Seedco, Inc. (Lubbock, TX). These lines were grown at four or five of the following field sites: Starkville, MS; Bossier City, LA; West Sinton, TX; Tifton, GA; Maricopa, AZ; and Loxley, AL.

Samples of seed cotton from individual field sites were harvested, composited for the individual sites, ginned, acid-delinted, and analyzed by site. The remainder of the seed cotton from each of the sites was pooled to produce a composite sample for processing into refined oil and processed cottonseed meal for subsequent analyses. At the Alabama site in 1993, a planting error led to an inadvertent compositing of seed from lines 531 and 757. This led to one-third of the seed from the other line being included for the subsequent processing studies. Since both lines contain the same gene and encoded proteins and are compositionally equivalent (see data below), this error did not impact the validity of the conclusions of the processing portion of this study.

Cottonseed Processing. The composited seed cotton samples pooled across field sites were used as a source of cottonseed for processing. Cottonseed was processed into refined oil and processed cottonseed meal at the Engineering and Biosciences Research Center at Texas A&M University (College Station, TX) under Good Laboratory Practices (GLP) using a solvent extraction method (Cherry and Leffler, 1984). The processing procedure was a small-scale version of the commercial procedure.

Proximate Analysis. Proximate analysis (protein, fat, ash, carbohydrates, and moisture) on ginned, acid-delinted cottonseed was performed under GLP at Hazleton Laboratories, Inc. (Madison, WI), on cottonseed from each field site.

Ash content was measured according to Association of Official Analytical Chemists (AOAC) methods (AOAC, 1990a). Volatile organic matter was driven off when the sample was ignited at 550 °C in a muffle or electric furnace. The residue was quantitated gravimetrically and calculated to determine percent ash.

Protein content was estimated by determining the total nitrogen according to the Kjeldahl method, as previously described (Bradstreet, 1965; Kalthoff and Sandell, 1948; AOAC, 1990b), and multiplied by 6.25 to calculate the total protein.

Fat content was estimated by using the Soxhlet extraction method (AOAC, 1990c). The sample of seed tissue was dried to remove excess moisture, extracted with pentane, dried to remove pentane, and weighed to determine the amount of fat removed.

Moisture content was determined by loss on drying at 100 °C to constant weight as described (AOAC, 1990d).

Carbohydrate was estimated by difference using the fresh weight-derived data and the following equation (USDA, 1975a):

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

Calories were calculated using the Atwater factors with the fresh weight-derived data and the following equation (USDA, 1975b):

$$\text{calories (kcal/100 g)} + (4 \times \%\ \text{protein}) + (9 \times \%\ \text{fat}) + (4 \times \%\ \text{carbohydrates})$$

Amino Acid Composition. Acid-delinted, ground cottonseed samples were hydrolyzed with hydrochloric acid, adjusted to pH 2.2. The individual amino acids were quantitated using an automated amino acid analyzer. This assay was based on previously published references (AOAC, 1990e) and was performed under GLP at Hazleton Laboratories. The reference substances used for these analyses were K18 (Beckman, lot A304008), L-tryptophan (Sigma Chemical Co., lot 60H0635), cysteic acid monohydrate (Sigma, lot 50H2616), methionine sulfone (Sigma, lot 49F0113).

α-Tocopherol. Refined oil samples were saponified to release the tocopherols, which were then extracted with organic solvent, followed by quantitation on a high-performance liquid chromatography (HPLC) silica column using fluorescence detection (Cort et al., 1983; Speek et al., 1985; McMurray et al., 1980). The reference substance for this method, conducted under GLP at Hazleton Laboratories, was USP α-tocopherol, lot K.

Aflatoxin. The levels of aflatoxins B_1, B_2, G_1, and G_2 were determined on ground, acid-delinted cottonseed samples under GLP at Hazleton Laboratories. The sample was wetted with dilute hydrochloric acid and extracted with chloroform. A portion of the extract was applied to a silica gel column. Aflatoxins were eluted with methylene chloride/acetone and concentrated with a rotary evaporator. The extracts were then separated by HPLC and compared to a known standard (Pons et al., 1970; JAOAC, 1988a−c). The reference substance for this method was Aflatoxin Mix-M (Supelco, lot LA39657).

Preparation of Seed and Kernel Material for Gossypol and Fatty Acid Analyses. Cottonseeds were dehulled with a Bauer attrition mill and the kernels separated from the hulls by hand. The kernels and processed meal were ground using a stainless steel Wiley mill and then passed through a 20-mesh screen. Due to the high oil content, dry ice was added to the whole seed to facilitate grinding.

Moisture Determination for Gossypol and Fatty Acid Analyses. Percent moisture in cottonseed was determined by weight difference before and after lyophilization. Samples were lyophilized in tared flasks to remove all water and obtain a true dry weight to the nearest 0.1 mg.

Percent Lipid Determination and Fatty Acid Analyses. Lipids were extracted using a double Bligh and Dyer procedure (Bligh and Dyer, 1959), as described by Wood (1991). Analyses were performed under GLP at Texas A&M University (College Station, TX).

The dry weight of the sample and weight of the extracted lipid were used to calculate the total percentage lipid in the sample. Total lipids were extracted from the samples with chloroform/methanol. Approximately 2 mg of total lipid was saponified to obtain free fatty acids according to a mild alkaline hydrolysis procedure (Wood, 1986a). The free fatty acids were converted quantitatively to phenacyl derivatives according to the procedure of Wood and Lee (1983).

The phenacyl derivatives were analyzed by HPLC according to the method of Wood (1986a,b). Development of the HPLC method included a determination of retention time and elution order of individual fatty acid reference standards and a mixture of fatty acids. For the data presented, the fatty acid identity for each peak was determined by peak elution order and peak shape, which was monitored by a strip chart recorder. The absorption data for each peak were collected directly from the UV monitor and were integrated for percent of total peak area using an IBM Model 900 laboratory computer. The peak area for each fatty acid was directly proportional to the percent of each fatty acid contained in total lipid. The reference substance for the fatty acid analyses was commercial cottonseed oil.

Measurement of Free and Total Gossypol Levels. Free and total gossypol levels were measured in the cottonseed raw

Table 1. Summary of Proximate Analysis of Cottonseed from Insect-Protected and Coker 312 Cotton Lines

	mean (range)[b]				
characteristic[a]	Coker 312[c]	531[d]	757[d]	1076[d]	literature range
protein (%)	27.00 (23.3−28.4)	27.56 (22.8−31.0)	27.60 (23.4−30.5)	26.57 (23.5−28.9)	12−32[e]
fat (%)	22.96 (19.6−25.1)	23.23 (22.2−25.8)	22.95 (21.9−25.6)	20.80[f] (16.6−22.8)	16.1−26.7[g]
ash (%)	4.63 (4.3−5.0)	4.53 (3.9−4.7)	4.45 (3.8−4.8)	4.45 (4.1−4.7)	4.1−4.9[h]
carbohydrate (%)	45.40 (42.8−47.6)	44.68 (42.0−46.7)	44.99 (41.9−46.5)	48.17[f] (46.8−51.0)	
calories/100 g	496.32 (479−508)	498.11 (495−511)	496.91 (495−510)	486.03[f] (464−496)	
moisture (%)	12.36 (9.6−15.9)	13.43 (11.2−14.7)	13.18 (8.0−16.4)	10.60 (9.4−12.6)	5.4−10.1[i]

[a] Protein, fat, ash, carbohydrate, and calories reported as percent dry weight of sample. [b] Range denotes the lowest and highest individual values across sites for each line. [c] C312 is the parental control cotton line. Value reported is least squares mean of five samples. [d] Value reported is least squares mean of four samples. [e] Turner et al. (1976); Cherry et al. (1978a); Kohel et al. (1985). [f] Statistically significant from Coker 312 control line at the 5% level (paired *t*-test). [g] Cherry and Leffler (1984); Cherry et al. (1978a,b). [h] Cherry et al. (1978b); Belyea et al. (1989). [i] Cherry et al. (1978a).

meal (prior to processing), toasted cottonseed meal (processed), and refined cottonseed oil under GLP at the USDA-ARS Southern Crop Research Laboratory (College Station, TX). Prior to gossypol analysis, the processed (toasted) meal samples were placed in a vacuum desiccator to reduce moisture percentage so that it would be similar to that of the ground cottonseed samples. Evaluation of free gossypol levels was completed using HPLC according to the procedure described by Stipanovic et al. (1988) and the AOCS (1989a). Total gossypol levels (corrected for moisture) were measured spectrophotometrically using aniline as a complexing agent (Pons et al., 1958; AOCS, 1989b). The reference standard for these analyses was gossypol acetic acid obtained from Sigma, lot 102H4038.

Data Reduction and Statistical Analysis. All data reduction and statistical analyses were performed using the SAS statistical program (SAS Institute, 1990). Statistical analysis was completed for composition of cottonseed samples (proximate analyses, total gossypol, percent total lipids, and fatty acid levels) collected from individual field sites, because these data were generated on replicated samples. The means and standard errors for each line were computed using a least-squares mean procedure since the numbers of samples for the insect-protected and control lines were unequal. The data were statistically analyzed using a paired *t*-test. No statistical analysis was performed on components measured on the composited, processed samples, since there was only one sample per line.

RESULTS

Prior to designing a food and feed safety assessment program for a genetically engineered crop, one must understand the food and feed uses of the crop. There are three major cottonseed commodity products: seed cotton, cottonseed oil, and cottonseed meal. The primary use of seed cotton (nondelinted cotton seed) is for cattle feed (*Cottonseed and Its Products*, 1989). The defatted cottonseed meal is used almost exclusively for animal feed, primarily for cattle but also for swine, chickens, and fish (*Cottonseed and Its Products*, 1989). There is no reported food use of protein-containing products of cottonseed, due to the presence of the antinutrients gossypol and cyclopropenoid fatty acids (Morgan, 1980; *Cottonseed Oil*, 1990). Refined cottonseed oil and highly processed cottonseed linters (the fiber remaining after ginning seedcotton) are the only cotton products used for food. Since the fiber (linters) is essentially completely comprised of cellulose (>99.9%), the composition of the fiber has not been analyzed for the insect-protected cotton lines. The composition of components important for feed and food uses was assessed for the insect-protected cotton lines and compared to that of the parental control (Coker 312) as well as to the reported values for other commercial cotton varieties. All three insect-protected cotton lines were shown to be compositionally equivalent to Coker 312 and consistent with the reported values for other commercial cotton varieties.

Proximate Analysis of Cottonseed and Raw, Ground Cottonseed. The levels of the major components of cottonseed (protein, fat, carbohydrate, moisture, and ash) were determined for cottonseed from each of the field test sites. There were no statistically significant differences for any of the components for lines 531 and 757 compared to the Coker 312 control (Table 1). Cottonseed from line 1076 showed minor, but statistically significant, differences in percent fat, carbohydrate, and calories when compared to seed from the control line. Although the differences are statistically significant, they do not represent a meaningful difference in nutritional value of the seed. The ranges for percent fat and calories in cottonseed from line 1076 overlap with the levels for cottonseed from Coker 312. In addition, the level of fat in cottonseed from line 1076 falls well within reported ranges for cottonseed from commercial cultivars (Table 1). No published data were available for carbohydrate and calorie content of cottonseed, so comparisons to literature values could not be made. These parameters are not standard for assessing the composition of cottonseed.

Amino Acid Composition of Cottonseed. The amino acid composition of the seed from the insect-protected cotton lines was similar to the composition of the seed from the control line and similar to the reported ranges for amino acids measured in cottonseed (Table 2). Several amino acids in cottonseed from lines 531 and 757 showed statistically significant differences when compared to the measured amino acid in cottonseed from the control line. In most cases, the values fell within or very near the reported ranges for the amino acid levels in commercial cottonseed (Table 2).

Total Lipid Content and Fatty Acid Profile. Percent lipid and fatty acid profiles were evaluated in cottonseed and refined oil. Percent total lipids and the fatty acid profile for cottonseed from the insect-protected cotton lines was equivalent to cottonseed from the Coker 312 control (Table 3). No differences of biological importance were detected. Two minor statistically significant differences were detected for line 1076; however, the values for line 1076 were within reported values for the respective fatty acids in refined oil from commercial cottonseed varieties (Table 4).

The fatty acid composition of refined oil was assessed from processing of the composited seed sample for each line. Therefore, the data were not subjected to statistical analyses. A summary of the fatty acid profiles (including cyclopropenoid fatty acids) for the refined oil samples showed that the values for lines 531, 757, and 1076 and the Coker 312 control were consistent with

Table 2. Amino Acid Composition of Cottonseed from Insect-Protected and Coker 312 Cotton Lines[a]

amino acid	literature max[b]	literature min[b]	Coker 312[c]	531[d]	757[d]	1076[d]
aspartic acid	9.5	8.8	9.72	9.49	9.74	9.90
threonine	3.2	2.8	3.40	3.42	3.45	3.50
serine	4.4	3.9	4.62	4.67	4.72	4.69
glutamic acid	22.4	20.5	19.56	18.21[e]	18.36[e]	19.56
Proline	4.0	3.1	4.22	4.03	4.06	4.32
glycine	4.5	3.8	4.32	4.18	4.28	4.33
alanine	4.2	3.6	4.12	4.03	4.10	4.17
cysteine	3.4	2.3	1.60	1.68	1.73	1.60
valine	4.7	4.3	4.50	4.09[e]	4.26[e]	4.39
methionine	1.8	1.3	1.48	1.94[e]	1.92[e]	1.49
isoleucine	3.4	3	3.26	3.02[e]	3.14[e]	3.22
leucine	6.1	5.5	5.98	5.93	6.09	5.98
tyrosine	3.3	2.8	2.92	3.08[e]	3.05[e]	2.96
phenylalanine	5.6	5	5.32	5.28	5.35	5.26
lysine	4.1	3.9	4.50	4.73[e]	4.80[e]	4.57
histidine	2.8	2.6	2.72	2.94[e]	2.99[e]	2.74
arginine	12.3	10.9	11.20	11.68	12.13[e]	11.68
tryptophan	1.4	1	1.04	1.00	1.00	1.02

[a] Amino acids are reported in mg/kg of dry weight of protein in the cottonseed. [b] Lawhon (1977). [c] Value reported is least-squares mean of five samples. [d] Value reported is least-squares mean of four samples. [e] Significantly different from the Coker 312 control line at the 5% level (paired *t*-test).

literature ranges for fatty acid levels of cottonseed oil from commercial varieties (Table 4).

The cyclopropenoid fatty acids, sterculic and malvalic acid, are unique fatty acids common in cotton. Malvalic and sterculic acids are 17 and 18 carbons long, respectively, and contain a double bond at the cylcopropene ring. The levels of cyclopropene acids must be minimized due to undesirable effects in food and feed products (Cherry and Leffler, 1984; Phelps et al., 1965). The cyclopropenoid fatty acids inhibit the desaturation of stearic to oleic acid, which alters membrane permeability and increases the melting point of oils. The levels of cyclopropenoid fatty acids are greatly decreased during processing, with the greatest point of deactivation during the deodorization of the refined oil (*Cottonseed Oil*, 1993). The levels of the cyclopropene fatty acids, as well as dihydrosterculic acid, were similar in cottonseed and refined oil from Coker 312 and the insect-protected lines (Tables 3 and 4).

Gossypol Analyses. Gossypol is a polyphenolic pigment present in cotton that can cause discoloration and toxicity problems in food and feed products of cottonseed (Berardi and Goldblatt, 1980). Gossypol is typically measured in two forms, free and total. Free gossypol is the physiologically active form. The levels of free gossypol decrease during heat processing due to the binding of gossypol with proteins, thus making it unavailable and essentially biologically inactive (Cherry and Leffler, 1984; Berardi and Goldblatt, 1980).

Total gossypol levels were evaluated in cottonseed, raw meal, toasted (processed) meal, and refined oil. Free gossypol was measured in raw meal, toasted (processed) meal, and refined oil. Gossypol values were reported as percent dry weight of sample.

Levels of total gossypol were measured in cottonseed collected across field test locations. Gossypol levels in seed from the insect-protected lines were not significantly different (lines 531 and 757) from or were lower (line 1076) than the level measured in control seed (Table 5). These levels were well within the range of 0.39−1.7% total gossypol reported for cotton varieties grown under various field conditions (Berardi and Goldblatt, 1980; Abou-Donia, 1976).

Free gossypol and total gossypol were measured in the raw meal, toasted meal, and refined oil fractions resulting from processing of a single composite seed sample for each line. These data were not subjected to statistical analyses since only one sample was analyzed per line. For the raw meal fractions, total gossypol was similar to the values obtained for cottonseed (Table 5). A similar level of total gossypol would be expected since the raw meal fraction was simply dehulled, ground seed used for processing. Free gossypol made up approximately two-thirds of the total gossypol measured in the raw meal. Total gossypol and free gossypol were not detected in the refined oil fractions except in the oil produced from the Coker 312 line, which contained a very low level of total gossypol (0.09%). The level of gossypol was not significantly higher in cottonseed or raw meal material from the control line; therefore, scaleup of the process procedure is the most likely cause for a slightly higher total gossypol content in refined oil from the Coker 312 control.

As a result of processing, more than 98% of the total gossypol was converted to the bound form in all samples. Free gossypol was not detected in the toasted meal produced from lines 757 and 1076; levels in the toasted meal produced from line 531 and the Coker 312 control were within accepted levels for free gossypol when toasted cottonseed meal is used as a component of

Table 3. Lipid and Fatty Acid Composition of Cottonseed from Insect-Protected and Coker 312 Cotton Lines

component	mean (range)[a] Coker 312[b,c]	531[b,c]	757[b,c]	1076[b,c]
lipid	33.5 (30.9−35.5)	33.5 (30.8−35.9)	33.6 (32.1−36.9)	33.7 (31.4−36.3)
myristic (14:0)	0.94 (0.67−1.07)	0.88 (0.75−0.98)	0.97 (0.79−1.10)	1.02[d] (0.86−1.18)
pentadecanoic (15:0)	0.40 (0.32−0.60)	0.62 (0.32−0.90)	0.75 (0.24−1.22)	0.43 (0.29−0.77)
palmitic (16:0)	26.5 (24.8−27.8)	26.3 (25.1−27.2)	26.8 (23.8−28.1)	26.8 (24.4−28.1)
palmitoleic (16:1)	0.64 (0.48−0.71)	0.61 (0.54−0.64)	0.63 (0.58−0.66)	0.73[d] (0.66−0.78)
margaric (17:0)	0.16 (0.13−0.20)	0.18 (0.14−0.27)	0.17 (0.13−0.22)	0.19 (0.17−0.22)
stearic (18:0)	2.63 (2.32−3.26)	2.90 (2.71−3.26)	2.90 (2.74−3.19)	2.54 (2.46−2.63)
oleic (18:1)	15.3 (14.8−16.0)	16.8 (14.8−19.1)	15.7 (13.4−17.2)	15.2 (13.5−16.7)
linoleic (18:2)	47.8 (46.4−49.9)	45.6 (41.6−49.0)	46.0 (43.3−49.2)	47.7 (45.1−50.5)
linolenic (18:3)	0.20 (0.13−0.29)	0.14 (0.13−0.18)	0.17 (0.13−0.24)	0.17 (0.11−0.29)
arachidic (20:0)	0.29 (0.26−0.31)	0.28 (0.22−0.33)	0.26 (0.21−0.31)	0.29 (0.25−0.33)
behenic (22:0)	0.15 (0.12−0.17)	0.14 (0.13−0.15)	0.14 (0.11−0.16)	0.14 (0.11−0.15)
malvalic (C_{17})	0.37 (0.22−0.45)	0.38 (0.23−0.47)	0.42 (0.23−0.62)	0.28 (0.26−0.37)
sterculic (C_{18})	0.59 (0.48−0.70)	0.62 (0.54−0.69)	0.68 (0.47−0.86)	0.63 (0.48−0.78)
dihydrosterculic (C_{19})	0.36 (0.29−0.50)	0.49 (0.24−0.84)	0.76 (0.28−1.41)	0.31 (0.15−0.75)

[a] Range denotes the lowest and highest individual value across sites for each line. [b] Value of lipid is percent of dry sample weight. Value of fatty acid is percent of total lipid. [c] Values presented are least-squares mean and ranges (five samples for Coker 312 and four samples for lines 531, 757, 1076). [d] Significantly different from the Coker 312 control at the 5% level (paired *t*-test).

Table 4. Oil Composition from Insect-Protected Cotton Lines and Coker 312 Control

component	literature range	refined oil from line			
		Coker 312	531	757	1076
fatty acids[a]					
myristic (14:0)	0.5−2.5[b] 0.68−1.16[c]	0.98	0.77	0.86	0.88
palmitic (16:0)	17−29[b] 21.63−26.18[c]	25.42	25.08	24.96	25.94
palmitoleic (16:1)	0.5−1.5[b] 0.56−0.82[c]	0.64	0.58	0.60	0.63
margaric (17:0)	not available	0.19	0.10	0.11	0.11
stearic (18:0)	1.0−4.0[b] 2.27−2.88[c]	2.53	2.67	2.62	2.38
oleic (18:1)	13−44[b] 15.17−19.94[c]	14.92	15.89	15.49	13.64
linoleic (18:2)	33−58[b] 49.07−57.64[c]	50.27	50.88	50.09	50.80
linolenic (18:3)	0.1−2.1[b] 0.23[d]	0.16	0.17	0.15	0.15
arachidic (20:0)	<0.5,[b] 0.41[d]	0.21	0.30	0.26	0.27
behenic (22:0)	<0.5[b]	0.12	0.13	0.12	0.13
sterculic (C_{18})	0.08−0.56[e]	0.48	0.43	0.58	0.60
malvalic (C_{17})	0.22−1.44[e]	0.36	0.46	0.42	0.41
dihydrosterculic (C_{19})		0.22	0.16	0.35	0.26
antinutrients/vitamins[f]					
total gossypol	≤0.01% (1 ppm)[c]	0.09	ND[g]	ND	ND
free gossypol	≤0.01% (1 ppm)[c]	ND	ND	ND	ND
α-tocopherol		638	568	597	689

[a] Reported as percent of total lipids. One sample per line from a composite seed sample. [b] FAO/WHO Codex Alimentarius committee on fats and oils (*Cottonseed Oil*, 1993). [c] Cherry and Leffler (1984). [d] Cherry (1983). [e] Phelps et al. (1965). Values reported for crude cottonseed oil. [f] Free and total gossypol are reported as percent weight; tocopherol is reported as mg/kg. [g] ND, not dectected (limits of detection were 0.04% and 0.002% for measurement of total and free gossypol in oil, respectively).

Table 5. Gossypol Levels Determined in Seed, Raw Meal, and Toasted Meal from Insect-Protected and Coker 312 Cotton Lines

	% total gossypol		
	mean	range	% free gossypol
cottonseed			
Coker 312	1.16[a]	0.97−1.43[a]	NA[b]
531	1.10	0.86−1.29	NA
757	1.08	0.85−1.31	NA
1076	1.04[c]	0.85−1.22	NA
raw meal			
Coker 312	1.06[d]	NA	0.667
531	1.05	NA	0.687
757	1.09	NA	0.661
1076	0.83	NA	0.513
toasted meal			
Coker 312	1.11	NA	0.011
531	0.87	NA	0.008
757	0.81	NA	ND[e]
1076	0.72	NA	ND

[a] Values, expressed as percent dry weight, reported for seed samples are the least-squares mean (from statistical analyses); ranges represent the lowest and highest values from all samples. [b] NA, not applicable. Free gossypol not typically measured for raw cottonseed. Range for raw and toasted cottonseed meal is not applicable since only one composite sample analyzed. [c] Values are statistically significant compared to the Coker 312. [d] Values reported from raw meal and toasted meal samples are one value obtained from processing fractions generated from the composite of seed across field sites. [e] ND, not detectable (limit of detection for measurement of free gossypol in toasted meal = 0.007%).

animal feed. The Food and Drug Administration (FDA) guidelines allow a maximum of 0.06% free gossypol and 1.2% total gossypol for cottonseed meal used as chicken feed and no greater than 0.01% free gossypol when cottonseed meal is used as a protein supplement for swine (Beraradi and Goldblatt, 1980).

Aflatoxin Analyses. Aflatoxins are a group of mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* that may contaminate food and feed products (Jorgensen and Price, 1981). Cottonseed is one of the commodities most commonly contaminated by aflatoxins (Bagley, 1979). Cottonseed produced in regions where the pink bollworm is a significant insect pest typically have high levels of aflatoxin due to the boll damage caused by the pink bollworm larvae, allowing secondary infection by *A. flavus* or *A. parasiticus* (McMeans et al., 1976; Ashworth et al., 1971). Often the levels of aflatoxin are sufficiently high such that the seed cannot be used for animal feed. The maximum action level allowed by the FDA is 20 μg/kg (20 ppb) (Jorgensen and Price, 1981). The levels of the four primary aflatoxins (B_1, B_2, G_1, and G_2) in the cottonseed for the insect-protected lines and for the control line at all field 1993 sites were shown to be undetectable at a sensitivity of 1 ppb.

α-Tocopherol Analysis. Tocopherols are naturally present in cottonseed oil and serve as antioxidants, enhancing food storage properties. α-Tocopherols in particular have vitamin E potency. The levels of tocopherols vary in nature and are affected by processing. They are lost primarily during the steps of refining and deodorizing (*Cottonseed Oil*, 1993). α-Tocopherol levels, measured in refined oil prepared from lines 757, 1076, and 531 and the Coker 312 line were 689, 597, 568, and 638 mg/kg, respectively (Table 4). The levels were similar in cottonseed oil from the insect-protected and control cotton lines and similar to levels (136−660 mg/kg) previously reported in the literature (Rossel, 1991; Dicks, 1965).

DISCUSSION

The FDA provides guidance for determining whether a new plant variety is as safe and nutritious as its parental variety in the FDA Food Policy (FDA, 1992). The Food Policy document provides the basis for establishing that a new plant variety produced through genetic modification is equivalent to its traditional counterpart. The compositional data presented herein

follow the FDA guidance and lead to the conclusion that the cottonseed and cotton products derived from all three insect-protected cotton lines are substantially equivalent to the parental variety as well as to other commercial cotton varieties.

The levels of the nutrients (protein, fat, ash, carbohydrate, calories, amino acids, fatty acids, and α-tocopherol) for all three lines were comparable to those of the parental Coker 312 control as well as to the values reported for other commercial cotton varieties. Most importantly, from a safety perspective, the levels of the endogenous antinutrients (gossypol and cyclopropenoid fatty acids) in the insect-protected lines were also comparable to or lower than those of the Coker 312 control and similar to the reported values for other cotton varieties.

The level of aflatoxin in the cottonseed samples from the 1993 field trials showed undetectable levels for both the insect-protected lines and Coker 312 control. However, in cottonseed produced by line 531 and the Coker 312 control in one of the 1992 field trials, the level of aflatoxin B_1 in the Coker 312 was high (92.7 ppb), significantly exceeding the FDA allowable level of 20 ppb. The high aflatoxin level was observed in cottonseed produced for Coker 312 at the field site in Arizona, the only site in which the pink bollworm was the primary insect pest. The high aflatoxin level is attributable to the high degree of pink bollworm damage (McMeans et al., 1976; Ashworth et al., 1971). Pink bollworm is effectively controlled by the insect-protected line 531 (Wilson et al., 1994), and the level of aflatoxins was undetectable (<1 ppb) in seed from all of the 1992 field sites. This reduction in aflatoxin contamination in the insect-protected cotton plants provides an important benefit to the cotton grower in areas infested with pink bollworm and will enable the production of cottonseed with enhanced safety for use as animal feed.

On the basis of the data collected and the heterogeneity reported for different cotton varieties, it is concluded that the insertion of the genes into these cotton lines that confer season-long control of the cotton bollworm, tobacco budworm, and pink bollworm produced cottonseed that is compositionally equivalent and is as nutritious as the seed produced by cotton varieties currently on the market.

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