The Composition of Glyphosate-Tolerant Soybean Seeds Is Equivalent to That of Conventional Soybeans[1,2]

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ABSTRACT One important aspect of the safety assessment of genetically engineered crops destined for food and feed uses is the characterization of the consumed portion of the crop. One crop currently under development, glyphosate-tolerant soybeans (GTS), was modified by the addition of a glyphosate-tolerance gene to a commercial soybean cultivar. The composition of seeds and selected processing fractions from two GTS lines, designated 40-3-2 and 61-67-1, was compared with that of the parental soybean cultivar, A5403. Nutrients measured in the soybean seeds included macronutrients by proximate analyses (protein, fat, fiber, ash, carbohydrates), amino acids and fatty acids. Antinutrients measured in either the seed or toasted meal were trypsin inhibitor, lectins, isoflavones, stachyose, raffinose and phytate. Proximate analyses were also performed on batches of defatted toasted meal, defatted nontoasted meal, protein isolate, and protein concentrate prepared from GTS and control soybean seeds. In addition, refined, bleached, deodorized oil was made, along with crude soybean lecithin, from GTS and control soybeans. The analytical results demonstrated that the GTS lines are equivalent to the parental, conventional soybean cultivar. J. Nutr. 126: 702–716, 1996.

INDEXING KEY WORDS:

- *glyphosate-tolerant soybeans* • *soybeans*
- *composition* • *nutrient* • *antinutrient*

Genetic modification techniques hold much promise for the development of improved crops. New crop varieties that resist pests and diseases or that have improved quality characteristics are currently in development (Gasser 1989). Other crops in development are tolerant to nonselective herbicides. One such crop, glyphosate-tolerant soybeans (GTS)[4,5] has been engineered for selectivity to foliar application of the herbicide glyphosate (Delannay et al. 1995, Padgette et al. 1995 and 1996). The sensitivity of crop plants such as soybeans to glyphosate has prevented the use of this herbicide during the growing season on crops. To offer farmers the advantages of glyphosate for effective control of weeds and to take advantage of this herbicide's environmental and safety characteristics, we have introduced a single gene that confers high level glyphosate tolerance to a commercial cultivar of soybeans (Padgette et al. 1995 and 1996). This gene encodes a glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). 5-Enolpyruvylshikimate-3-phosphate synthase is present in plants, bacteria and fungi, but not animals, as a component of the shikimate pathway of aromatic amino acid biosynthesis (Haslam 1993). Animals do not make their own aromatic amino acids but rather receive them from plant, microbial or animal-derived foods.

Two GTS lines, denoted 40-3-2 and 61-67-1, were extensively studied with respect to their environmental, food and feed safety. The glyphosate tolerance locus associated with GTS line 40-3-2 has been transferred

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[4] Abbreviations used: BAPNA, benzoyl-D-arginine-*p*-nitroanilide; CP4 EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4; GTS, glyphosate-tolerant soybeans; HU, hemagglutinating units; RBDO, refined, bleached, deodorized oil; TI, trypsin inhibitor; TIU, trypsin inhibitor units.

[5] Glyphosate is the active ingredient of the broad-spectrum, nonselective herbicide Roundup®, and GTS are also denoted as Roundup Ready® soybeans (Monsanto Company, St. Louis, MO).

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to other soybean cultivars through traditional breeding methods. The glyphosate tolerance gene behaves as a stable, simple dominant trait (Padgette et al. 1995).

Regulation of GTS in the United States is divided among three government agencies. First, a determination is required from the U.S. Department of Agriculture (USDA) that GTS line 40-3-2 and all progenies derived from crosses between 40-3-2 and traditional soybean cultivars pose no plant pest risk and should no longer be considered a regulated article. This determination was made by the USDA and published (USDA 1994). Second, an amendment must be obtained to the glyphosate herbicide application label from the Environmental Protection Agency (EPA) to allow for in-season application of glyphosate on GTS. This approval was granted in May 1995. Finally, the food and feed safety of GTS is under the jurisdiction of the Food and Drug Administration (FDA). A consultation process with the FDA was completed in 1994 following the guidance outlined in FDA's policy *Foods Derived From New Plant Varieties* (U.S. FDA 1992). That policy addresses the safety and regulatory status of foods derived from new plant cultivars, including plants developed by genetic modification.

The food and feed safety assessment performed on GTS consisted of three main components: *1)* study of the composition of the seed and selected processing fractions; *2)* study of the introduced protein, CP4 EPSPS (Harrison et al. 1996); and 3) animal studies with GTS (Hammond et al. 1996). The composition study, which is the topic of this article, demonstrated that the levels of nutrients (protein, oil, fiber, ash, carbohydrates, energy, amino acids and fatty acids) in GTS line 40-3-2 are comparable to those of the parental cultivar and are consistent with established ranges for soybeans. In addition, natural soybean antinutrients (trypsin inhibitor, lectins, isoflavones, stachyose, raffinose and phytate) were also measured in GTS, and comparisons with the parental control again indicated equivalence. These results thus confirm that GTS is equivalent in composition to soybeans currently in commerce. The accompanying articles present the protein safety data (Harrison et al. 1996) and the animal feeding study data (Hammond et al. 1996).

MATERIALS AND METHODS

General. All field studies, processing and analytical assays were performed according to Good Laboratory Practice guidelines (CFR 1992).

Soybean seed utilized. The GTS lines 40-3-2 and 61-67-1 were obtained by particle gun bombardment of the Asgrow (Kalamazoo, MI) cultivar A5403 with vectors PV-GMGT04 and PV-GMGT05, respectively, which contain the CP4 EPSPS gene. The development, identification and characterization of the GTS lines (focusing on line 40-3-2), as well as multi-year yield tests with glyphosate-treated GTS, have recently been described (Delannay et al. 1995, Padgette et al. 1995 and 1996). Expression of CP4 EPSPS confers glyphosate tolerance to the GTS lines. In addition to the CP4 EPSPS gene, a gene encoding the Escherichia coli β-glucuronidase protein (Jefferson et al. 1986) is also present in line 61-67-1 but not line 40-3-2. R_1 progenies (first generation) from the 40-3 and 61-67 R_1 seed were grown in the greenhouse during the winter of 1990–1991 and evaluated for glyphosate tolerance by spray test (Padgette et al. 1995a). R_2 seeds (second generation) from individual R_1 plants were planted in the field during the summer of 1991. The 40-3-2 and 61-67-1 R_2 progenies were selected based on high levels of glyphosate tolerance (little or no damage after application of 1.68 $kg \cdot ha^{-1}$ of glyphosate) (Padgette et al. 1995). Progenies of these GTS lines (R_3 seeds) were planted in two successive generations for seed increase in Puerto Rico during the winter of 1991–1992. R_4 seeds harvested from the first generation test for lines 40-3-2 and 61-67-1 were planted for the 1992 Puerto Rico field experiment to supply seeds (R_5 generation) for compositional analysis and processing. The GTS seeds planted for a 1992 field trial in the United States (nine sites) were of the R_4 and R_5 generations, and the harvested seed (R_5 and R_6 generations) was utilized for compositional analysis and processing, as well as animal feeding studies (Hammond et al. 1996). The GTS R_6 generation seeds from the 1992 U.S. field trials were then planted at four 1993 U.S. field locations to supply additional seeds (R_7 generation) for compositional analysis. The A5403 control soybean seed samples were grown at the same time and in the same field locations as the test materials, from commercially available Asgrow A5403 seeds (Maturity Group V). The field sites utilized in the 1992 study were Macon, MO, Washington, LA, Martinsville, IN, Greenville, MS, Newport, AR, Proctor, AR, Winterville, GA, Seven Springs, NC, and Marion, AR. The field sites utilized in the 1993 study were Gordon, AL, Salisbury, MD, Steele, MO, and Marion, AR. This field program has been described elsewhere (Padgette et al. 1995).

Processing experiments. Three separate processing experiments were performed by Texas A&M University, Engineering and Biosciences Research Center (College Station, TX), using soybean seed from three different sources. The seeds utilized and the fractions produced are shown in **Table 1.** Processing was performed to mimic commercial procedures as closely as possible, although the scale was much smaller. Briefly, the soybean samples were dried and cleaned by aspiration and screening. After the hulls were mechanically cracked, aspiration was used to separate the hull and kernel fractions. The kernel fractions were heat conditioned, flaked, expanded into collets, and solvent extracted with hexane to remove the crude oil. The spent collets with the residual hexane were toasted for pro-

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TABLE 1

Glyphosate-tolerant soybean processing experiments

Experiment	Seed source	Weight of soybeans used	Processing fractions produced
		kg	
1	Puerto Rico	39–42	Defatted toasted meal; refined, bleached, deodorized oil
2	U.S. small-scale multi-site	45–51	Defatted toasted meal; refined, bleached, deodorized oil; crude lecithin; protein isolate; protein concentrate; defatted non-toasted meal
3	U.S. large-scale seed increase	722–731	Defatted toasted meal

duction of defatted toasted meal. Toasting temperature was 105–114°C for 20–30 min, after direct steam injection. Due to the small seed sample size, the samples were processed by batch, instead of continuously as in a commercial operation, for both the Puerto Rico seed (Table 1, Experiment 1) and the small-scale experiment using U.S.-produced seed (Table 1, Experiment 2). For the small-scale experiment using U.S.-produced seed (Table 1, Experiment 2), the samples used for the processing experiments consisted of a composite sample of soybean seeds from eight of the field sites (the Marion, AR, samples were omitted). For the large-scale experiment using U.S.-produced seed (Table 1, Experiment 3), seed was obtained from the Marion, AR, site and processed to defatted toasted meal as described above, except that a continuous extractor and desolventizer-toaster were used instead of batch units.

Defatted nontoasted meal was also produced from the small-scale U.S.-produced seed (Table 1, Experiment 2). This served as the starting material for protein concentrate and protein isolate production. For protein concentrate production, the nontoasted defatted meal was ground into flour, aqueous ethanol was added, the solution was centrifuged, and the solid protein curd was suspended in water and acidified with 3.0 mol/L HCl to pH 4.5. The centrifuged solids were resuspended in water to form a slurry, adjusted to pH 6.8 with 6.25 mol/L NaOH and spray dried, resulting in the protein concentrate fraction. Protein isolate was produced by grinding the nontoasted defatted meal into flour, adding water to form a dispersion, heating the solution to 55°C, and adjusting the pH to 8.5 with 6.25 mol/L NaOH. After mixing, the solution was centrifuged, the soluble protein solution was acidified to pH 4.5 with 3.0 mol/L HCl, and the resulting solution was centrifuged. The recovered solid protein fraction was suspended in water, centrifuged again, and resuspended in water. After neutralization of this suspension to pH 6.8 with 6.1 mol/L NaOH, the slurry was spray dried, resulting in the protein isolate fraction.

Refined, bleached, deodorized oil (RBDO) was produced from the crude oil after hexane removal (Table 1, Experiments 1 and 2). Briefly, the crude oil was degummed by adding water, heating, cooling, decanting the oil, and filtering. The fraction that settled after cooling was the crude lecithin. The degummed oil was refined by adding NaOH to neutralize the free fatty acids, heating, refrigerating, decanting, and filtering. The decanted, filtered, refined oil was then bleached by heating in the presence of activated bleaching earth under vacuum with agitation, deodorized by steam heating under vacuum (220–230°C, 30 min), cooling to 140–150°C, and adding 2.60×10^{-4} mol/L citric acid at a rate of 1 mL per 100 g of oil.

Analytical assays. Soybean compositional assays were performed at Ralston Analytical Laboratories (St. Louis, MO) except for the protein-in-oil assays, which were performed at Monsanto. Specified reagents were obtained from Sigma Chemical (St. Louis, MO) unless otherwise noted. Seed samples for compositional analyses were shipped and stored at ambient temperature. Brief descriptions of the procedures utilized are given below.

Acid-stable amino acids. Samples were hydrolyzed in vacuo with hydrochloric acid and then neutralized. Amino acids were separated on an anion exchange column followed by ninhydrin detection. This assay is based on previously published methods (Blackburn 1978, Moore and Stein 1954 and 1963).

Sulfur amino acids. Cystine and cysteine in samples were oxidized to cysteic acid and methionine was oxidized to methionine sulfone by treatment with performic acid solution for 16 h at approximately 0°C. After acid hydrolysis, the sample was separated on an anion exchange column and detected by ninhydrin reaction. This assay has been previously described (Method 985.28 of AOAC 1990, Moore 1963, Schram et al. 1954).

Tryptophan-alkaline hydrolysis. Tryptophan was determined by hydrolysis of the sample, in vacuo, with sodium hydroxide solution. The hydrolyzate was analyzed by reverse-phase HPLC using a C18 column followed by detection at 280 nm (Method 988.15, AOAC 1990).

Ash. The sample was charred and ashed to a constant weight at 550°C in a muffle furnace. The residue was quantified and the percentage of ash determined (Methods 945.38, 945.18, 923.03, 926.04, 922.066 and 930.30, AOAC 1990).

Carbohydrates. Carbohydrates were calculated by difference using the fresh weight–derived data according to the following equation: g/100 g carbohydrates = 100 g/100 g minus (g/100 g protein + g/100 g fat + g/100 g ash + g/100 g moisture).

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Fat by ether extraction. Ether-soluble material (primarily "free" fats and oils) was extracted from the sample with petroleum ether in a Soxhlet extractor. The ether was volatilized and the dried residue quantified gravimetrically and calculated as the percentage of fat. The method is based on previously published methods (Method 920.39 of AOAC 1990, Bhatly 1985, Foster and Gonzales 1992).

Fatty acid profile. Lipids were extracted from soybeans and soybean products (except oil) with chloroform-methanol. Extracted lipid or oil was saponified with alcoholic potassium hydroxide. Fatty acids were partitioned into hexane, washed with water, and dried with sodium sulfate. The fatty acids were then esterified with methanol using boron trifluoride as a catalyst, partitioned into heptane, and injected on a gas chromatograph with a flame ionization detector. The percentages of individual fatty acid methyl esters were calculated relative to the total amount of fatty acid methyl esters present. This assay has been previously described (Methods 983.23 and 969.33 of AOAC 1990, Hammarstand 1966).

Crude fiber. The sample was dried, if necessary, to remove excessive moisture, ground to pass a 1.0-mm screen, and extracted with petroleum ether using a Goldfisch extractor to remove fat. It was then digested in refluxing 0.22 mol/L H_2SO_4, filtered, digested in refluxing 0.30 mol/L NaOH, and filtered. The residue was washed, dried, weighed, ignited and reweighed. Crude fiber was calculated from the loss on ignition of the residue (Method 962.09, AOAC 1990).

Free and bound isoflavones. Daidzein, genistein, coumestrol and biochanin A were determined by HPLC using a C18 reverse-phase column and UV detection at 260 nm. Free and bound isoflavones were extracted at ambient temperature with a mixture of ethanol and dilute HCl. Free isoflavones were determined from the extract following cleanup using a C18 solid-phase extraction cartridge. Total isoflavones were determined after refluxing a portion of the extract with an equal volume of 4 mol/L HCl. Bound isoflavones were calculated by subtracting the free from the total isoflavones for a given sample. This assay was based on previously published methods (Pettersson and Kiessling 1984, Seo and Morr 1984). For coumestrol, values less than 10 μg/g were considered unreliable due to poor peak shape.

Lecithin analysis. The main components of lecithin (phosphatidyl ethanolamine, phostidic acid, phosphatidyl inositol and phosphatidyl choline) were analyzed by HPLC separation on a deactivated silica column followed by UV detection at 206 nm (Method Ja 7b-91, American Oil Chemists' Society 1993).

Lectins. Lectins were determined according to a modification of literature procedures by measuring the agglutinating properties of soybean sample extract on rabbit red blood cells (Klurfeld and Kritchevski 1987, Liener 1955). Method development utilized soybean seed and toasted meal.

Moisture. Moisture was determined by loss on drying at 133°C in a forced draft oven, (Method 44-154, American Association of Cereal Chemists 1983 and Method 930-15, AOAC 1990).

Total Kjeldahl nitrogen and protein. Total nitrogen was determined by the Kjeldahl method (Method 945.38, AOAC 1990). Protein was calculated from total nitrogen using N $\times$ 6.25.

Nitrogen solubility analysis. The nitrogen solubility index, defined as [(g/100 g water-soluble protein)/(g/100 g total protein)] $\times$ 100, was determined using a previously published procedure (Method Ba 11-65, American Oil Chemists' Society 1989) (percentage values reported as g/100 g).

Phytic acid. Phytic acid was extracted with dilute hydrochloric acid and separated from inorganic phosphates on an anion exchange column. Phytate was eluted with a sodium chloride solution. The eluate was digested with sulfuric-nitric acid, freeing phosphorus, which was reacted with ammonium molybdate and sulfonic acid solutions to form a blue color complex that was measured spectrophotometrically. Values were converted to phytic acid based on molecular weight equivalence. The method is based on published procedures (Method 986-11 of AOAC 1990, Ellis and Morris 1983, Harland and Oberleas 1977).

Protein or amino acids in oil. To investigate whether any appreciable amount of protein (or amino acids) is found in soybean oil, 0.5-mL samples of oil were hydrolyzed with equal volumes of trifluoroacetic acid-HCl-propionic acid (50:25:25) for approximately 24 h at approximately 145°C. After cooling, the released amino acids were extracted from the oil phase with 2 $\times$ 0.5 mL of 0.1 mol/L HCl containing 300 mL/L methanol. The combined extracts were evaporated, reconstituted in 200 μL of Na-S citrate buffer, and analyzed on an amino acid analyzer (model 6300, Beckman, Fullerton, CA).

Sugar analysis. Sugars were measured by HPLC using a Waters (Milford, MA) μ-Bond-a-pak carbohydrate column, or equivalent, as previously described (Dunmire and Otto 1979), except that no miniprep column was utilized and the mobile phase was acetonitrile-water (70:30) for stachyose and raffinose analyses.

Trypsin inhibition. Components that inhibit trypsin activity were extracted at a pH of 9.5 to 9.8 using a sodium hydroxide solution. An aliquot of the sample suspension was mixed with a known volume of trypsin solution and incubated several minutes to allow the trypsin-inhibiting factors to react with the added trypsin. An aliquot of benzoyl-D-arginine-*p*-nitroanilide (BAPNA) was added to the suspension. Uninhibited trypsin catalyzes the hydrolysis of BAPNA, forming yellow *p*-nitroanaline. After 10 min of reaction, the hydrolysis was halted by lowering the solution pH with acetic acid, thereby denaturing the enzyme. The solutions were evaluated spectrophotometrically, and trypsin inhibition was evaluated from the difference in the degree

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of BAPNA hydrolysis between the sample solution and the uninhibited trypsin solution. One trypsin unit is defined as an increase equal to 0.01 absorbance units at 410 nm after 10 min of reaction per 10 mL of final reaction volume, read in 1.27-cm tubes. This method is based on published procedures (Method 71-10 of American Association of Cereal Chemists 1983, Kakade et al. 1974). Note that mg TI (trypsin inhibitor)/g = (TIU [trypsin inhibitor units]/mg)/1.9 (Anderson et al. 1979).

Urease. Urease (EC 3.5.1.5) was assayed using a method (Method Ba 9-58, American Oil Chemists' Society 1989) based on treatment of the sample with phosphate buffer containing urea. Urea reacts with any urease present, liberating ammonia, which raises the pH of the solution.

Statistical analysis. The compositional results of the soybean seeds were processed by the SAS statistical program, Version 6 (1989, SAS Institute, Cary, NC). Analysis of each characteristic from the 1992 seed samples consisted of nine plot values (one per site) for each of the three soybean lines: two GTS lines (40-3-2 and 61-67-1) and one control line (A5403). The individual plot values were converted from a fresh weight to a dry weight basis using the percent moisture values (Value, dry weight = [Value, fresh weight]/[(1 − moisture)/100]). This adjustment was performed on all of the characteristics except moisture, fatty acids, urease and lectin. The data had a randomized complete block design structure with line as a treatment effect and location as a blocking effect. To determine whether block effects were significant, each characteristic was analyzed using a two-way variance with line and location as main effects. All compositional characteristics with the exception of the fatty acid 17:0 had significant block effects at the 5% level. To maintain consistency in the analyses, 17:0 was analyzed using a blocked design despite its nonsignificant blocking effect. Means and SE were calculated, and differences between the A5403 control line and the GTS lines were determined using protected pairwise t tests, which is equivalent to using Fisher's protected least significant difference procedure (Steel and Torrie 1980). This procedure involves first analyzing all the characteristics for line differences using a two-way ANOVA. Then, those lines for which the F test for lines was significant were compared with the control using the standard pairwise t test multiple comparison procedure at the 5% level. The SE listed were computed using the mean square error and not the individual line variances. The statistical analysis for the 1993 U.S. compositional data was for proximate values only, because these were the only analyses performed. The method of analysis was the same as described above, except that the data consisted of four plot values for one GTS line (40-3-2) and one control line (A5403).

RESULTS

Although the majority of the compositional and processing studies were performed on two GTS lines, line 40-3-2 and line 61-67-1, only GTS line 40-3-2 and its progeny will be introduced into commerce. Data for line 61-67-1 were utilized in the statistical analyses in addition to the line 40-3-2 data, and are included herein, to obtain a more precise estimate of error in the experiment. Data for soybean seeds are reported as means of the measurements from nine (1992 season) or four (1993 season) samples from the different field sites for each soybean line, along with the ranges of values determined for each sample set. For the data presented below, literature ranges for most of the compositional variables are provided, where available. Although this information is useful to frame the experimental results obtained in this study, the literature data may not be directly comparable, due to differences in analytical methodology or sample preparation. In addition, much of the literature data is relatively old and may not completely encompass the compositional variables of modern soybean varieties.

Compositional data on seed from a single site in Puerto Rico (see Materials and Methods), which were similar to those obtained in the U.S. studies, will not be discussed because the Puerto Rico study was a statistically separate study at one site, whereas the U.S. study covered seed derived from nine sites. The results using material from the U.S. sites were therefore more representative of the wide geographical area in which soybeans are grown. The Puerto Rico GTS and control seed compositional data are available through an auxiliary publication, as is data on the processing studies performed (Table 1, Experiment 1).[6]

Proximate analysis of seed. Proximate analyses (protein, oil, fiber, ash, carbohydrates, moisture and energy) were performed on the soybean seeds from the 1992 and 1993 U.S. field trials (**Table 2**). For the 1992 nine-site experiment, several minor differences between GTS and control soybeans were determined to be statistically significant ($P < 0.05$). These differences included ash (5.24 vs. 5.04 g/100 g for the control), fat (16.28 vs. 15.52 g/100 g) and carbohydrate (37.1 vs. 38.1 g/100 g) for line 40-3-2, and ash (5.17 vs. 5.04 g/100 g) for line 61-67-1. These differences were small and were considered biologically unimportant. Equivalence in the ash, fat and carbohydrate levels is further supported by data from the four-site 1993 study (Table 2), in which no statistically significant differences in line 40-3-2 proximate analysis results vs. the control were ob-

[6] Supplementary information has been deposited with American Society for Information Science, National Auxiliary Publication Service (NAPS). See NAPS document no. 04949 for four pages of supplementary material. Order from NAPS, c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163-3513. Remit with your order, not under separate cover, \$7.75 (U.S. funds on a U.S. Bank only) for photocopies or \$4.00 for microfiche. Outside the U.S. and some parts of Canada, add postage of \$4.50 for photocopies, \$1.75 for microfiche. Institutions and organizations may order by purchase order; however, there is a billing and handling charge for this service of \$15, plus any applicable postage.

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TABLE 2

***Summary of proximate analyses of control and glyphosate-tolerant soybean (GTS) seeds from 1992–1993 U.S. field trial*[1]**

Characteristic	Seed line: A5403 (control) Mean	Range	SEM	GTS 40-3-2 Mean	Range	SEM	GTS 61-67-1 Mean	Range	SEM	Literature range
	g/100 g dry weight (unless noted)									
1992 (9 sites)										
Protein	41.6	37.5–44.6	0.095	41.4	37.0–45.0	0.095	41.3	36.8–45.0	0.095	36.9–46.4[2]
Ash	5.04	4.29–5.34	0.039	5.24*	4.75–5.57	0.039	5.17*	4.75–5.46	0.039	4.61–5.37[2]
Moisture, *g/100 g fresh wt*	8.12	7.55–8.73	0.054	8.12	7.74–8.85	0.054	8.20	7.72–8.54	0.054	7–11[2,3]
Fat	15.52	14.10–18.63	0.201	16.28*	14.04–19.53	0.201	16.09	14.05–18.86	0.201	13.2–22.5[2,4]
Fiber	7.13	5.91–7.89	0.107	6.87	5.50–7.43	0.107	7.08	5.63–8.06	0.107	4.7–6.48[2,5]
Carbohydrates	38.1	33.9–41.3	0.247	37.1*	32.1–40.0	0.247	37.5	33.3–40.4	0.247	30.9–34.0[2,6]
1993 (4 sites)										
Protein	41.5	39.7–43.35	0.025	41.4	39.6–43.2	0.025	NT			
Ash	5.36	4.99–5.88	0.036	5.43	5.21–5.87	0.036	NT			
Moisture, *g/100 g fresh wt*	6.12	5.30–6.49	0.255	6.34	6.10–6.59	0.255	NT			
Fat	20.11	18.46–21.42	0.378	20.42	18.37–23.31	0.378	NT			
Fiber	6.71	5.74–7.37	0.099	6.63	5.45–7.37	0.099	NT			
Carbohydrates	33.0	29.3–34.8	0.354	32.7	27.6–35.0	0.354	NT			

[1] Means reported are from single assays on single samples from nine sites in 1992 and four sites in 1993. * Significantly different from the control line at the 5% level (protected least significant difference procedure); data from each year were analyzed separately. NT = not tested.
[2] Smith and Circle (1972).
[3] Perkins (1995).
[4] Wilcox (1985).
[5] Mounts et al. (1987).
[6] Orthoefer (1978).

served. Note that line 61-67-1 was not included in the 1993 study due to a discontinuation of commercial development for the line.

Amino acid analysis of seed. No significant differences in the levels of any of the 18 amino acids measured, including aromatic amino acids, were found between the GTS seeds and the control soybean seeds (**Table 3**).

Fatty acid analysis of seed. The fatty acid compositions of the soybean seeds from the 1992 U.S. field trials were also measured (**Table 4**). The only significant difference in seed fatty acid composition was found in line 40-3-2, which had 0.53% 22:0 compared with 0.50% 22:0 for the A5403 control. We consider this small difference to be inconsequential.

Trypsin inhibitor and urease activity of seed. Analysis indicated that there were no significant differences in trypsin inhibitor content between GTS and the control soybeans (**Table 5**). Urease, used as a marker enzyme to establish the extent of processing (Herkelman et al. 1991) (see sections below on toasted meal), was also measured in seeds to allow comparison to post-processing meal urease levels. There was no significant difference in urease level between the GTS lines and the control line (Table 5).

Lectin analysis of seed. There were no significant differences in lectin activity between GTS and control soybeans (Table 5). Levels of lectin activity were found to be very low in the soybean seeds [less than 7 hemagglutinating units (HU)/mg extracted protein], lower than previously reported for other soybean lines (60–426 HU/mg protein) (Kakade et al. 1972). A positive control of purified soybean lectin yielded readings of 461–541 HU/mg total protein.

Isoflavone analysis of seed. The levels of the total and free forms of the isoflavones genistein and daidzein and bound coumestrol and biochanin A were determined. However, only minute quantities of biochanin A were detected, and the bound coumestrol was lower than the confidence limit of the assay (10 μg/g). No significant differences were detected in any of the isoflavones measured (free, bound, total) in GTS soybean seeds relative to controls (**Table 6**).

Composition of toasted meal. **Table 7** presents data for the defatted toasted meal fractions from the small-scale (Table 1, Experiment 2) and large-scale (Table 1, Experiment 3) processing experiments. The toasted meal from the large-scale experiment was utilized in diet formulations for the rat, catfish and chicken growth studies described in an accompanying report (Hammond et al. 1996). The proximate analyses of the GTS toasted meal batches were comparable to those of the A5403 toasted meal (Table 7).

The phytate concentrations in the GTS toasted meal

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TABLE 3

Summary of amino acid analyses of soybean seeds from the nine-site 1992 U.S. field trial[1]

Amino acid	A5403 (control) Mean	A5403 (control) Range	A5403 (control) SEM	GTS 40-3-2[2] Mean	GTS 40-3-2[2] Range	GTS 40-3-2[2] SEM	GTS 61-67-1[2] Mean	GTS 61-67-1[2] Range	GTS 61-67-1[2] SEM	Literature range[3]
	g/100 g dry wt									
Aspartic acid	4.53	4.18–4.99	0.065	4.42	3.85–5.03	0.065	4.48	3.87–4.84	0.065	3.87–4.98
Threonine	1.60	1.51–1.73	0.021	1.56	1.41–1.74	0.021	1.58	1.41–1.69	0.021	1.33–1.79
Serine	2.10	1.96–2.28	0.029	2.04	1.80–2.29	0.029	2.07	1.82–2.21	0.029	1.81–2.32
Glutamic acid	7.34	6.64–8.16	0.111	7.10	6.00–8.19	0.111	7.26	6.12–7.91	0.111	6.10–8.72
Proline	2.03	1.86–2.23	0.029	1.98	1.76–2.25	0.029	2.02	1.69–2.17	0.029	1.88–2.61
Glycine	1.72	1.60–1.87	0.022	1.67	1.48–1.88	0.022	1.69	1.48–1.82	0.022	1.88–2.02
Alanine	1.71	1.60–1.86	0.022	1.67	1.48–1.87	0.022	1.69	1.51–1.81	0.022	1.49–1.87
Valine	1.85	1.71–2.02	0.025	1.80	1.58–2.02	0.025	1.83	1.60–1.95	0.025	1.52–2.24
Isoleucine	1.78	1.65–1.95	0.025	1.73	1.51–1.95	0.025	1.76	1.54–1.89	0.025	1.46–2.12
Leucine	3.05	2.81–3.37	0.041	2.97	2.60–3.37	0.041	3.03	2.62–3.25	0.041	2.71–3.20
Tyrosine	1.45	1.35–1.59	0.019	1.40	1.23–1.55	0.019	1.43	1.26–1.54	0.019	1.12–1.62
Phenylalanine	1.97	1.78–2.19	0.028	1.90	1.64–2.20	0.028	1.95	1.66–2.13	0.028	1.70–2.08
Histidine	1.06	0.98–1.16	0.015	1.03	0.91–1.18	0.015	1.04	0.92–1.11	0.015	0.89–1.08
Lysine	2.61	2.47–2.84	0.036	2.56	2.30–2.85	0.036	2.58	2.29–2.75	0.036	2.35–2.86
Arginine	2.94	2.56–3.46	0.055	2.85	2.20–3.57	0.055	2.90	2.36–3.34	0.055	2.45–3.49
Cysteine	0.60	0.54–0.66	0.008	0.62	0.56–0.67	0.008	0.60	0.58–0.64	0.008	0.56–0.66
Methionine	0.55	0.51–0.59	0.006	0.55	0.50–0.60	0.006	0.54	0.53–0.56	0.006	0.49–0.66
Tryptophan	0.59	0.56–0.63	0.004	0.59	0.55–0.64	0.004	0.58	0.54–0.63	0.004	0.53–0.54

[1] Means are from single assays on single samples from nine sites in 1992. [GTS = glyphosate-tolerant soybeans.]
[2] No significant differences from the control line were observed at the 5% level (protected least significant difference procedure).
[3] Han et al. (1991), Orthoefer (1978).

TABLE 4

Summary of fatty acid analyses of soybean seeds from the nine-site 1992 U.S. field trial[1,2]

Fatty acid	A5403 (control) Mean	A5403 (control) Range	A5403 (control) SEM	GTS 40-3-2 Mean	GTS 40-3-2 Range	GTS 40-3-2 SEM	GTS 61-67-1 Mean	GTS 61-67-1 Range	GTS 61-67-1 SEM	Literature range[3]
	g/100 g									
6:0[4]	0.11	0.10–0.13	0.002	0.11	0.10–0.15	0.002	0.11	0.10–0.15	0.002	
16:0	11.19	10.63–11.69	0.029	11.21	10.63–11.68	0.029	11.14	10.65–11.62	0.029	7–12
17:0	0.13	0.11–0.14	0.004	0.13	0.11–0.17	0.004	0.13	0.12–0.14	0.004	
18:0	4.09	3.85–4.55	0.037	4.14	4.01–4.29	0.037	4.05	3.78–4.40	0.037	2–5.5
18:1 *cis*	19.72	15.02–31.19	0.173	19.74	15.56–32.52	0.173	19.81	15.22–31.41	0.173	20–50
18:2	52.52	44.03–54.96	0.208	52.31	42.41–54.48	0.208	52.48	43.35–55.07	0.208	35–60
18:3	8.02	5.08–10.26	0.062	8.23	4.99–10.37	0.062	8.12	5.10–10.27	0.062	2–13
20:0	0.36	0.31–0.43	0.005	0.37	0.30–0.47	0.005	0.35	0.30–0.44	0.005	
20:1	0.17	0.14–0.26	0.003	0.17	0.14–0.28	0.003	0.17	0.14–0.27	0.003	
22:0	0.50	0.46–0.59	0.006	0.53*	0.49–0.62	0.006	0.49	0.45–0.63	0.006	
24:0	0.18	0.13–0.24	0.005	0.19	0.15–0.27	0.005	0.18	0.16–0.25	0.005	
Unknowns	2.63	2.36–2.80	0.062	2.48	2.06–2.94	0.062	2.59	2.23–2.91	0.062	

[1] Means are from single assays on single samples from nine sites in 1992. Fatty acids 7:0, 8:0, 9:0, 10:0, 11:0, 12:0, 13:0, 14:1, 15:0, 16:1, 17:1, 18:1 *trans*, 19:0, 20:2, 20:3, 20:4, 20:5, 22:2, 22:6 and 24:1 were <0.1% for all three replicates of all three lines. These values were not statistically analyzed. * Significantly different from the control line at the 5% level (protected least significant difference procedure). [GTS = glyphosate-tolerant soybean.]

[2] For 14:0, 23 of 27 values were designated "<0.1 g/100 g." Therefore statistical analysis was determined to be not meaningful and was not performed.

[3] Pryde (1990).

[4] The value "0.10 g/100 g" was substituted for the samples within the set that had "<0.1 g/100 g" of the fatty acid.

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TABLE 5

***Summary of lectin, trypsin inhibitor and urease analyses of soybean seeds from the nine-site 1992 U.S. field trials*[1]**

	Seed line									
	A5403 (control)			GTS 40-3-2[2]			GTS 61-67-1[2]			Literature range
Characteristic	Mean	Range	SEM	Mean	Range	SEM	Mean	Range	SEM	
Lectin analysis[3]										
HU/mg extracted protein	6.3	4.5–12.8	0.530	5.6	3.3–8.9	0.530	6.6	2.7–8.5	0.530	
HU/mg total protein	3.0	1.9–6.0	0.259	2.6	1.5–4.2	0.259	3.2	1.2–4.5	0.259	
HU/mg sample	1.2	0.8–2.4	0.099	1.0	0.6–1.6	0.099	1.2	0.5–1.8	0.099	
Trypsin inhibitor analysis[4]										
TIU/mg sample dry wt	43.0	33.2–54.5	0.731	45.0	35.5–59.5	0.731	42.9	35.9–53.1	0.731	26.4–93.2[5]
mg TI/g dry wt	22.6	17.5–28.7	0.385	23.7	18.7–31.3	0.385	22.6	18.9–28.0	0.385	16.7–27.2[6]
Urease analysis[7]										
Urease, *Δ pH*	2.18	2.12–2.24	0.009	2.17	2.03–2.26	0.009	2.17	2.10–2.24	0.009	

1 Means are from single assays on single samples from nine sites in 1992. GTS = glyphosate-tolerant soybean.
2 No significant differences from the control line were observed at the 5% level (protected least significant difference procedure).
3 Fresh weight basis. HU = hemagglutinating unit.
4 TI = trypsin inhibitor, TIU = trypsin inhibitor units.
5 Kakade et al. (1972).
6 Anderson and Wolf (1995).
7 Fresh weight basis.

samples were similar to those in the control samples (Table 7). Levels ranged from 1.76 to 1.93 g/100 g dry wt for the samples tested.

As also shown in Table 7, the stachyose concentrations of the toasted meal batches ranged from 5.52 to 6.65 g/100 g dry wt across all three soybean lines, and the raffinose concentrations of the toasted meal batches ranged from 0.90 to 1.11 g/100 g dry wt across all three soybean lines. The levels of stachyose and raffinose in toasted meal from the GTS lines were comparable to the levels in the A5403 toasted meal batches.

The TI values for the two batches of toasted meal of all of the soybean lines tested ranged from 2.1 to 3.4 TIU/mg sample dry wt (Table 7). The urease activity ranged from 0.01 ΔpH to 0.17 ΔpH in the toasted meal batches. These results can be compared with the range of the means for TI in the seeds of the three soybean lines (43–45 TIU/mg sample dry wt) and the range of the means for seed urease (2.17–2.18 ΔpH) (Table 5). The TI levels in the toasted meal lots analyzed in this study are all comparable to, or lower than, literature values (Table 7). The nitrogen solubility index for the six toasted meal batches ranged from 10.6 to 13.9 g/

TABLE 6

***Summary of isoflavone analyses of soybean seeds from the nine-site 1992 U.S. field trials*[1]**

	Seed line									
	A5403 (control)			GTS 40-3-2[2]			GTS 61-67-1[2]			Literature range[3]
Isoflavone	Mean	Range	SEM	Mean	Range	SEM	Mean	Range	SEM	
	μg/g dry wt									
Total genistein	833	286–1380	39.126	830	287–1243	39.126	857	257–1260	39.126	416.1–1000
Free genistein	7.8	0.9–21.6	2.636	9.5	0.9–30.6	2.636	14.7	0.8–58.1	2.636	
Bound genistein	825	279–1360	40.489	820	278–1240	40.489	842	250–1257	40.489	
Total daidzein	734	219–1190	34.732	721	219–1260	34.732	748	203–1300	34.732	330.6–706
Free daidzein	33.8	12.4–52.2	1.232	37.3	13.5–56.5	1.232	37.8	11.8–58.4	1.232	
Bound daidzein	700	206–1140	34.606	683	206–1220	34.606	711	192–1260	34.606	
Total coumestrol[4]	4.6	0.0–14.0	0.575	5.4	0.0–11.9	0.575	5.4	0.0–12.9	0.575	
Total biochanin	1.6	0.0–9.3	0.138	1.9	0.0–11.5	0.138	1.6	0.0–9.5		

1 Means are from single assays on single samples from nine sites in 1992. GTS = glyphosate-tolerant soybean.
2 No significant differences from the control line were observed at the 5% level (protected least significant different procedure).
3 Pettersson and Kiessling (1984), Wang et al. (1990).
4 Coumestrol levels lower than 10 μg/g should be considered not valid, due to poor HPLC peak shape.

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TABLE 7

Analysis of defatted toasted soybean meal from the nine-site 1992 U.S. processing studies[1]

	Seed line						
	Small scale			Large scale			
Component	A5403	40-3-2	61-67-1	A5403	40-3-2	61-67-1	Literature range
	g/100 g dry wt (unless noted)						
Protein	54.4	54.4	53.8	54.0	52.2	53.8	44–61.4[2]
Ash	6.58	6.93	6.69	6.83[3]	6.69	6.74	5.5–6.5[4]
Moisture, *g/100 g fresh wt*	11.70	8.51	9.90	9.37	5.58[3]	9.36[3]	
Fat	2.30	0.87	1.95	0.93	1.10	0.72	0.50–2.40[5]
Fiber	4.53	3.90	3.67	4.51	4.68	4.88	3.50–6.50[6]
Carbohydrates	36.8	37.8	37.5	38.3	40.0	38.7	32.0–38.0[7]
Phytate[3]	1.76	1.93	1.81	1.91	1.82	1.92	1.3–4.1[8]
Stacchyose	5.67	5.61[3]	6.00	6.00	5.52	6.65	4.0–5.3[9]
Raffinose	0.96	1.02[3]	1.11	0.90	0.94	1.10	1.0–2.0[10]
Urease, Δ *pH*	0.03	0.04	0.01	0.17	0.09	0.01	0.05–0.20[11]
Trypsin inhib.,[12] *TIU/mg dry wt*	3.4	3.3	3.4	2.6	2.6	2.1	3.8–17.9[13]
Nitrogen solubility,[3] *g/100 g*	12.0	11.4	13.9	10.6	13.1	11.7	
Lectin,[14] *HU/mg protein extracted*	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	
Lectin, *HU/mg total protein*	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	
Lectin, *HU/mg sample*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	

1 Values are from single assays on single samples, except as noted. A5403 is the control line; 40-3-2 and 61-67-1 are glyphosate-tolerant soybean lines.
2 Orthoefer (1978), Smith and Circle (1972).
3 Values are means from duplicate assays on single samples.
4 Fulmer (1988), Orthoefer (1978).
5 Han et al. (1991), Orthoefer (1978).
6 Mounts et al. (1987), Orthoefer (1978).
7 Waggle and Kolar (1979).
8 Anderson and Wolf (1995), Mohamed et al. (1991).
9 Coon et al. (1988), Kuo et al. (1988), Rackis (1974).
10 Rackis (1974).
11 Lee and Garlich (1992).
12 TIU = trypsin inhibitor units.
13 Anderson and Wolf (1995), Rackis (1974).
14 HU = hemagluttinating units.

100 g, which indicates low protein solubility, as expected for complete processing (heating) of the toasted meal (Table 7).

The levels of lectins in the toasted meal samples were below the detectable limits (<0.05 HU/mg total protein) (Table 7), relative to the mean seed lectin values of 2.6–3.2 HU/mg total protein (Table 5).

The isoflavones measured for soybean seeds were also measured in the toasted soybean meal batches (**Table 8**). The GTS toasted meal batches were all comparable to the A5403 toasted meal batch, as expected from the absence of differences found in the whole seeds (Table 6).

Proximate analysis of additional protein processing fractions. The levels of macronutrients (protein, ash, fat, fiber and carbohydrate) in defatted nontoasted meal, protein isolate and protein concentrate made from GTS were comparable to the levels in the fractions made from the parental soybean control cultivar (**Table 9**).

Fatty acid analysis of refined, bleached, deodorized oil and crude lecithin analysis. The fatty acid compositions of GTS and control RBDO were comparable (**Table 10**). In addition, crude wet lecithin from each of the GTS lines and the A5403 control line was analyzed for phosphatidyl ethanolamine, phosphatidic acid, phosphatidyl inositol and phosphatidyl choline (**Table 11**). The chemical composition of the GTS crude lecithin was comparable to that of the crude lecithin derived from the A5403 control line.

Determination of protein or amino acid levels found in refined, bleached, deodorized oil. To confirm the previous literature results indicating little or no protein in soybean oil, we initially attempted, unsuccessfully, to reproduce and obtain higher recoveries using the phosphate-buffer extraction procedure, then used the amino acid analysis procedure described in Materials and Methods. Microgram amounts of protein or amino acids present in the oil were then calculated from the sum of the amino acids present in the re-

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TABLE 8

Isoflavone analysis of toasted soybean meal from the 1992 U.S. processing studies[1]

Component	Seed line: Small scale A5403	Small scale 40-3-2	Small scale 61-67-1	Large scale A5403	Large scale 40-3-2	Large scale 61-67-1	Literature range[2]
	$\mu g/g$ dry wt						
Genistein total	938	976	989	1290	1350	1340	753–1601
Genistein free	18.6	23.7	23.9	43.4	47.8	46.1	
Genistein bound	919	952	965	1250	1300	1300	
Daizdein total	837	856	850	1250	1310	1300	200–625.4
Daizdein free	343	511	350	896	847	701	
Daizdein bound	494	345	500	354	466	598	
Coumestrol total[3]	0	0	0	1.7	0	0	
Biochanin A total	0	0	0	1.1	0	0	

[1] Values are from single assays on single samples. A5403 is the control line; 40-3-2 and 61-67-1 are glyphosate-tolerant soybean lines.
[2] Murphy (1982), Pettersson and Kiessling (1984), Wang et al. (1990).
[3] Coumestrol concentrations lower than 1 $\mu g/g$ should be considered not valid, due to poor HPLC peak shape.

sulting extract. The phrase "protein or amino acids" is used here because, although the experiment was designed to measure any protein found in the oil, free amino acids would also be detected and would contribute to the calculated results. We used an acid blank (no added oil or protein) as a negative control; therefore any protein or amino acid contamination in the reagents or introduced during processing would show up as a background level in the acid blank. In addition, three different concentrations of soybean seed protein, extracted from A5403 seeds, were analyzed as positive controls. As a control for hydrolysis efficiency in oil, two A5403 seed protein levels were spiked into A5403 RBDO samples, which were then analyzed for protein content. Finally, the three soybean oil batches (A5403, 40-3-2 and 61-67-1) were analyzed for protein. These data are summarized in **Table 12.** Mean values for protein or amino acids detected in the samples were 0.22, 0.26 and 0.29 μg for lines A5403, 40-3-2 and 61-67-1, respectively, using 0.5-mL samples. Using 0.92 as the density of soybean oil (Merck and Co. 1983), the weight of a 0.5-mL aliquot is approximately 0.46 g, allowing calculation of results (in $\mu g/g$) to yield 0.48, 0.57 and 0.63 $\mu g/g$ for lines A5403, 40-3-2 and 61-67-1, respectively. These results are slightly lower than the 0.72–0.96 $\mu g/g$ literature values discussed above. However, the results obtained for the acid blanks (mean of 0.33 μg) seriously call into question whether any protein at all is actually present in any of the soybean oil samples.

DISCUSSION

The design of a food and feed safety assessment program for a genetically engineered crop requires a good understanding of the uses of the crop in animal and human nutrition. There are three major soybean commodity products: beans, oil and meal. A 27.3-kg bushel of soybeans yields approximately 21.8 kg of protein-rich meal and 5 kg of oil (American Soybean Association 1992). The primary use of the defatted toasted soybean meal is in animal feed (approximately 97% of total) (Horan 1974). The various soybean protein fractions used for human consumption are mainly derived from the processing of nontoasted defatted soybean flakes. Food products containing soybean protein include baked goods, confections, meat products, textured foods and nutritional supplements (American Soybean Association 1992, Waggle and Kolar 1979). Soybean oil is also extensively used in the food industry in products such as cooking oil and salad dressings. In fact, soybean oil is currently the major edible oil used in the United States (Mounts 1988).

The compositional portion of the GTS food and feed safety assessment focused on analysis of the soybean seed under the hypothesis that if the GTS seed is equivalent to control soybean seed, then processed products derived from the seed will also be equivalent. The manufacture and analysis of several important commercial soybean protein products were undertaken to test this hypothesis (and because virtually all soybeans are processed prior to human or animal consumption). Defatted toasted meal was chosen because it is the main soybean protein product used in animal feed. Defatted nontoasted meal (flour) was prepared because this is the starting material for a large number of soybean protein products used in food. Protein isolate and protein concentrate were manufactured from the defatted meal because of the food use of these two fractions. In addition, crude lecithin and RBDO were manufactured. The soybeans from which the tested and analyzed seed was derived were not treated with glyphosate in order to

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TABLE 9

Proximate analysis of defatted non-toasted soybean meal, isolate, and concentrate from the 1992 U.S. processing study[1]

Component	Seed line A5403	40-3-2	61-67-1	Literature range
	g/100 g dry wt (unless noted)			
Defatted meal (non-toasted)				
Protein	53.2	53.6	52.8	40.0–59.0[2]
Ash	6.53	6.89	6.65	6.0–6.4[3]
Moisture, *g/100 g fresh wt*	6.55	11.90	4.17	
Fat	2.30	0.73	2.13	0.9–1.0[4]
Fiber	4.52	4.23	3.71	2.5–4.5[5]
Carbohydrates	38.0	38.8	38.4	34.0–38.0[6]
Urease, Δ *pH*	2.30	2.45	2.19	
Trypsin inhib.,[7] *TIU/mg dry wt*	65.9	83.5	73.6	
Protein isolate				
Protein	84.6	82.2	87.8	85.2–92.0[8]
Ash	3.30	3.89	4.20	2.3–7.6[9]
Moisture, *g/100 g fresh wt*	4.77	5.26	4.87	
Fat	1.06	1.68	0.57	0.1–2.5[10]
Fiber	<0.2	<0.2	<0.2	0.1–0.4[11]
Carbohydrates	11.0	12.2	7.5	0.3–0.6[12]
Protein concentrate				
Protein	65.1	67.2	71.4	66.2–78.1[13]
Ash	4.91	5.91	5.84	4.7–6.5[13]
Moisture, *g/100 g fresh wt*	9.36	5.31	11.80	
Fat	5.27	4.47	1.43	0.9–2.0[14]
Fiber	4.60	2.80	3.19	2.8–5.0[15]
Carbohydrates	24.7	22.5	21.3	17.1–25.0[16]

[1] Values are from single assays on single samples. A5403 is the control line; 40-3-2 and 61-67-1 are glyphosate-tolerant soybean lines.
[2] Smith and Circle (1972), Wolf (1983).
[3] Fulmer (1988), Smith and Circle (1972).
[4] Horan (1974), Smith and Circle (1972).
[5] Fulmer (1988), Sipos (1988).
[6] Sipos (1988), Waggle and Kolar (1979).
[7] TIU = trypsin inhibitor units.
[8] Torun (1979), Waggle and Kolar (1979).
[9] Smith and Circle (1972), Wolf (1983).
[10] Horan (1974), Wolf (1983).
[11] Smith and Circle (1972), Wolf (1983).
[12] Waggle and Kolar (1979), Wolf (1983).
[13] Bookwalter (1978), Smith and Circle (1972).
[14] O'Dell (1979), Wolf (1983).
[15] Mattil (1974), Wolf (1983).
[16] Mattil (1974), Smith and Circle (1972).
[17] Rackis (1974), Wolf (1983).

focus the analysis on any effects of the introduced gene and protein.

Some explanation is warranted regarding which compounds were chosen for measurement. Typically, although new soybean varieties are extensively tested for agronomic and yield variables, compositional analysis is limited in many cases to protein and oil. We also measured ash, fiber and moisture to complete the proximate analysis of macronutrients (carbohydrate was calculated), and the data demonstrated that GTS seeds are comparable in proximate composition to control seeds. Because soybean is used extensively as a protein source, the amino acid composition of the GTS seed was measured and found to be equivalent to that of the control seeds. The lack of alteration of aromatic amino acid levels in GTS is in accordance with our expectation that 5-enolpyruvylshikimate-3-phosphate synthase is not the rate-limiting step in aromatic amino acid biosynthesis in bacteria and plants (Herrmann 1983). Because soybean oil is used extensively in food, the fatty acid composition of the GTS seeds was also determined and found to be equivalent to that of the control seeds.

TABLE 10

Fatty acid analysis of refined, bleached, deodorized soybean oil from the 1992 U.S. processing study[1]

Fatty acid	Seed line A5403	40-3-2	61-67-1	Literature values[2]
	g/100 g			
6:0 (caproic)	0.16	0.20	0.13	
7:0 (hepatonoic)	0.39	0.46	0.38	
16:0 (palmitic)	10.46	10.50	10.38	7–12, 10.7
17:0 (margaric)	0.12	0.14	0.14	
18:0 (stearic)	4.09	4.19	4.08	2–5.5, 3.9
18:1 *cis* (oleic)	21.13	21.41	21.47	20–50, 22.8
18:2 (linoleic)	52.20	51.71	52.01	35–60, 50.8
18:3 (linolenic)	7.41	7.51	7.56	2–13, 6.8
19:0 (nonadecanoic)	0.13	<0.10	<0.10	
20:0 (arachidic)	0.13	0.27	0.25	0.2, <1.0
20:1 (eicosenoic)	0.17	0.17	0.17	<1.0
22:0 (behenic)	0.55	0.52	0.53	<0.5
24:0 (lignoceric)	0.15	0.16	0.18	
Unknowns	2.68	2.47	2.46	

[1] Values are from single assays on a single sample. Fatty acids 8:0, 9:0, 10:0, 11:0, 12:0, 13:0, 14:1, 15:0, 16:1, 17:1, 18:1 trans, 20:2, 20:3, 20:4, 20:5, 22:1, 22:2, 22:6, and 24:1 were <0.1 g/100 g for all three lines. A5403 is the control line; 40-3-2 and 61-67-1 are glyphosate-tolerant soybean lines.
[2] Pryde (1990).

TABLE 11

Analysis of crude soybean lecithin from the 1992 U.S. processing study[1]

Component	Seed line A5403	40-3-2	61-67-1
	g/100 g		
Phosphatidyl ethanolamine	5.54	5.85	6.29
Phostidic acid	18.41	18.10	19.73
Phosphatidyl inositol	6.45	5.72	6.66
Phosphatidyl choline	8.57	6.12	8.85

[1] Values are from single assays on a single sample. A5403 is the control line; 40-3-2 and 61-67-1 are glyphosate-tolerant soybean lines.

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TABLE 12

Summary of protein/amino acid content of refined, bleached, deodorized soybean oil from the 1992 U.S. processing study

Sample[1]	n	Mean	SD	Median
			µg protein	
Acid blank (no oil or protein)	3	0.331	0.082	0.289
0.5 µg soybean seed protein (no oil)	3	1.053	0.452	1.23
1.5 µg soybean seed protein (no oil)	3	1.41	0.325	1.28
A5403 oil + 0.5 µg soybean seed protein	3	0.507	0.042	0.496
A5403 oil + 1.5 µg soybean seed protein	3	1.066	0.091	1.03
A5403 oil	3	0.218	0.133	0.164
40-3-2 oil	3	0.263	0.005	0.265
61-67-1 oil	3	0.286	0.078	0.288

[1] Oil, when present, was added at a volume of 0.5 mL. A5403 is the control line; 40-3-2 and 61-67-1 are glyphosate-tolerant soybean lines.

Compilation of a list of antinutrients to be measured to ensure that enhanced levels were not present in GTS was more challenging because, in most cases, the literature does not provide clear consensus as to which compounds in soybeans contribute to antinutritive properties. It was of interest to determine whether GTS has TI activity comparable to that of the control soybeans because of the widely reported contribution of TI to the antinutritive activity of unheated soybean products (Anderson et al. 1979, Rackis 1974, Rackis et al. 1986). These analyses indicated comparable TI levels in the GTS and control soybean lines. Because processing soybean protein significantly inactivates TI, the level of TI in the toasted soybean meal made from GTS and control soybeans was measured. On the basis of the TI results, both of the toasted meal batches for all three lines were fully processed. We note that an initial defatted toasted meal processing experiment using seeds growth in Puerto Rico (Table 1, Experiment 1) exhibited incomplete processing, as reflected by higher than expected trypsin inhibitor and urease values.[6] These results were attributed to the processing procedure and not the soybean lines, based on additional laboratory experiments (data not shown) and the expected results obtained in the repeat two-experiment data set shown in Table 7.

Another antinutrient measured was lectin. An early report (Liener 1953) indicated that soybean hemagglutinin, sometimes called soybean lectin, accounts for part of the growth inhibition seen in rats fed raw soybean meal, although later reports question the significance of lectin as an antinutrient (Leiner 1980, Orthoefer 1978, Pusztai 1989, Rackis 1974). Analyses performed on the soybean seeds indicated comparable lectin activity in GTS and control soybean seeds. Because of the difference between literature lectin values and the values we found for our control, it was difficult to compare our seed results to literature values for hemagglutinating proteins in soybeans. The low lectin activity obtained may be due to the variability in red blood cell lots used in the lectin assay. Lectin analysis of the toasted meal samples demonstrated that toasting significantly inactivates lectin activity in both the GTS and control lines. This result is parallel to literature reports that lectin activity is substantially reduced upon moist heating (Rackis 1972).

The isoflavones genistein, daidzein and coumestrol are naturally present in soybeans and have been reported to possess a number of biochemical activities in mammalian species, including estrogenic and hypocholesterolemic activities (Murphy 1982, Wang et al. 1990). It has been postulated that these compounds may contribute to deleterious health effects in animals fed diets containing large amounts of soybean meal (Setchell et al. 1987). However, the absence of reports on estrogenic responses in humans consuming soybean protein products suggests that there is no appreciable increase in estrogen activity in humans when practical levels of soybeans are ingested (Wolf 1983). Isoflavones have recently attracted considerable attention due to their potential as anticarcinogens (Wang and Murphy 1994, and references therein). No significant differences in isoflavone levels were found between the GTS and control soybean seeds. An extremely large range of experimental values was obtained for genistein and daidzein. This large site-to-site variability is likely attributable to the effect of environmental influences on the formation of these compounds. It has been previously noted (Wolf 1983) that isoflavones remain active in commercially processed soybean meal and thus are stable to moist heat. The results obtained for the toasted meal samples support this observation.

The compositions of the processed fractions (including protein, fat, fiber, ash and carbohydrates in the protein fractions, fatty acid analysis of oil, and crude lecithin composition) were measured. In some cases, the values reported for certain components from the manufactured fractions did not fall within ranges cited in the literature for soybean products. These results are not surprising, considering that the processing was conducted on an experimental scale (40–730 kg), rather than on a commercial scale [>818,000 kg/d for toasted soybean meal (Mounts et al. 1987)]. Therefore, for these small-scale GTS processing fractions, the most important comparison is to the fractions from the parental soybean controls.

Phytic acid, stachyose and raffinose were also measured in the toasted meal. Phytic acid (phytate) is the hexaphosphoric acid derivative of inositol and exists mainly in soybeans as an insoluble calcium-magnesium-potassium complex that is not nutritionally available (Mohamed et al. 1991, Orthoefer 1978). Because phytate is involved in mineral availability, phy-

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tate levels in the GTS meal were measured and found to be comparable with that of the A5403 control line meal. The gas production following ingestion of soybeans by laboratory animals depends mainly on the low-molecular-weight carbohydrates, which exist primarily as sucrose, raffinose and stachyose (Rackis 1972 and 1974). The flatus activity of stachyose in rats is much greater than that of raffinose (Rackis 1974). The analyses reported herein demonstrated that GTS meal and control soybean meal had comparable levels of stachyose and raffinose.

To address whether soybean oil may be a route of exposure to proteins from GTS, we determined the protein content of the RBDO. In one previous study demonstrating that soybean oil is not allergenic to soybean-sensitive individuals, the authors stated that "soybean allergens appear to be proteinaceous, and soybean oil is also devoid of protein" (Bush et al. 1985). In another study, processed soybean oil was stated to have 720 μg protein/kg, using a phosphate-buffered saline extraction and dye-binding assay, with only 10% recovery and possible interferences with the protein assay (Klurfeld and Kritchevski 1987). Tattrie and Yaguchi (1973) concluded that only trace nitrogen was present in refined and bleached soybean oil, but 0.96 μg protein/g was detected using amino acid analysis. These authors also concluded that "no proteins could be detected in peanut, coconut, soybean, and herring oils which could conceivably cause an allergic reaction" (Tattrie and Yaguchi 1973). Therefore all literature sources obtained regarding the level of protein in soybean oil indicated that little or no detectable protein is present in refined soybean oil. Our results support two conclusions: *1*) if there is any protein present in soybean oil, it is present at extremely low levels, probably below the level of confidence of the assay utilized; and *2*) there is no meaningful difference between the GTS lines and the control. These results provide strong support for the contention that, for all practical purposes, soybean oil does not represent a source of exposure for proteins present in soybean seeds, including GTS.

The results of all of the analyses reported here demonstrate that the compositions of GTS seeds and the processed fractions (toasted meal, defatted meal, protein isolate, protein concentrate, RBDO and lecithin) are comparable to those of the control soybean seeds and fractions. Processing (to toasted meal) inactivated TI and lectin in GTS, as expected. On the basis of these evaluations, we conclude that, except for the tolerance to glyphosate, the GTS lines are substantially equivalent to the parental cultivar and other soybean cultivars now being marketed.

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