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Chemical Composition of Glyphosate-Tolerant Soybean 40-3-2 Grown in Europe Remains Equivalent with That of Conventional Soybean (*Glycine max* L.)

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The composition of glyphosate-tolerant (Roundup Ready) soybean 40-3-2 was compared with that of conventional soybean grown in Romania in 2005 as part of a comparative safety assessment program. Samples were collected from replicated field trials, and compositional analyses were performed to measure proximates (moisture, fat, ash, protein, and carbohydrates by calculation), fiber, amino acids, fatty acids, isoflavones, raffinose, stachyose, phytic acid, trypsin inhibitor, and lectin in grain as well as proximates and fiber in forage. The mean values for all biochemical components assessed for Roundup Ready soybean 40-30-2 were similar to those of the conventional control and were within the published range observed for commercial soybean. The compositional profile of Roundup Ready soybean 40-3-2 was also compared to that of conventional soybean varieties grown in Romania by calculating a 99% tolerance interval to describe compositional variability in the population of traditional soybean varieties already on the marketplace. These comparisons, together with the history of the safe use of soybean as a common component of animal feed and human food, lead to the conclusion that Roundup Ready soybean 40-3-2 is compositionally equivalent to and as safe and nutritious as conventional soybean varieties grown commercially.

KEYWORDS: Soybean (*Glycine max* L.); Roundup Ready; composition; nutritional profile

INTRODUCTION

Glyphosate, the active ingredient in the Roundup family of agricultural herbicides (Roundup, Roundup Ultra, and Roundup Ready are registered trademarks of Monsanto Technology LLC), is one of the most broadly applied herbicides in the world. It is remarkably effective against the majority of annual and perennial grasses and broad-leaf weeds and has superior environmental and toxicological characteristics with extremely low toxicity to mammals, birds, and fish (*1*).

Glyphosate acts by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme that catalyzes an essential step in aromatic acid biosynthesis in plants and microorganisms (*2*, *3*). The CP4 EPSPS protein produced by *Agrobacterium* sp. strain CP4 is structurally and functionally similar to plant EPSPS enzymes but has a much reduced affinity for glyphosate (*4*). In plants, EPSPS is localized in the chloroplasts or plastids (*5*). Expression of the *Agrobacterium*-based CP4 EPSPS fused to a chloroplast transit peptide confers glyphosate tolerance to a given transformant plant while maintaining aromatic amino acid biosynthesis. Since 1996, this approach has allowed the development and commercialization of a range of Roundup Ready crops including soybean (*Glycine max*) (*6*−*8*), canola (*Brassica napus*), cotton (*Gossypium hirsutum*) (*9*), and corn (*Zea mays*) (*10*). This paper reports on the compositional analysis of Roundup Ready soybean 40-3-2 produced by the stable insertion of a gene cassette that expresses the CP4 EPSPS protein.

The comparative safety assessment process (*11*−*17*) considers two potential sources of health consequences of foods or feeds derived from genetically modified crops. First, effects potentially attributable to the activity and presence of the introduced trait must be addressed; a comprehensive safety assessment of the CP4 EPSPS protein has been extensively reviewed (*18*). Second, effects potentially attributable to the novel crop plant, based upon plant characteristics and composition, must be considered (*19*). Thus, the comparative safety assessment process requires evaluation of the chemical composition of Roundup Ready

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soybean 40-3-2 relative to that of a conventional variety to determine if significant compositional changes are induced by the insertion of the *cp4 epsps* genes into the soybean genome or by the heterologous expression of CP4 EPSPS. In consultation with government agencies, the Organization for Economic Cooperation and Development (OECD) has promoted a list of well-defined metabolic constituents for assessment in compositional studies of new biotechnology crops (*15*−*17*). The OECD consensus documents emphasize measurements of essential nutrients and known antinutrients and toxicants. This is predicated on the premise that such targeted analyses would most effectively discern any compositional changes that imply potential safety and antinutritional concerns. The purpose of this assessment was to evaluate the chemical composition, on the basis of the OECD consensus recommendations, of Roundup Ready soybean 40-3-2 relative to that of a conventional soybean with a similar genetic background as well as with those of commercially available soybean varieties. Roundup Ready soybean has now been grown in eight countries with 102 million acres cultivated in 2003 (*20*). Over 50% of the soybeans grown and consumed globally are derived from Roundup Ready soybean event 40-3-2. Romania is the only country in Europe that has approved cultivation of Roundup Ready soybean 40-3-2.

MATERIALS AND METHODS

Soybean Samples for Compositional Analysis. Samples were collected from field trials conducted in 2005. Roundup Ready soybean 40-3-2 and a conventional control (Dekabig) were grown at five replicated trials in Romania [Calasari (coded R1); Bucharest (coded R2); Timisoara (coded R3); Iasi (coded R4); and Brailia (coded R5)]. Roundup Ready soybean 40-3-2 and Dekabig, along with three to four commercially available soybean varieties, were planted in a randomized complete block design composed of three blocks or replications. The commercial lines were Denny, Fukui, Osaka, and Taira (R1); Danubian, Nikko, Sakai, and Sapporo (R2); Triump, Denny, Zen, and Fukui (R3); Columna, Danubian, Denny, and Nikko (R4); and Nikko, Sakai, Taira, and Osaka (R5). The Roundup Ready soybean 40-3-2 plots were treated with three applications of Roundup Ultra herbicide: at pre-emergence, at early post-emergence (V4−V6 stage), and at late post-emergence (V8 or 30 in. tall, whichever came first). The forage was collected from plants at the R6 growth stage, and seed was collected at the R8 growth stage when the pods were fully mature. Forage and harvested seed samples were ground to a fine powder in the presence of dry ice and maintained frozen until required for compositional analysis. The identity of forage samples was based on sample-handling records. The identity of the harvested seed samples was based on sample-handling records and PCR analyses of genomic DNA isolated from the soybean seed.

Compositional Analyses. Compositional analyses were conducted to measure proximates (moisture, fat, ash, protein, and carbohydrate by calculation), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, amino acids, fatty acids, isoflavones, raffinose, stachyose, phytic acid, trypsin inhibitor, and lectin contents in harvested seed. Proximates, ADF, and NDF contents were measured in forage. All compositional analyses were performed at Covance Laboratories Inc. (Madison, WI). A single analytical measurement was made for each replicate sample. Duplicate analyses for each component were run on a predetermined set of samples as an analytical quality control measure.

Protein Determination. Protein levels were estimated by determining the total nitrogen content using the Kjeldahl method, as previously described (*21*, *22*). Protein was calculated from total nitrogen using the formula N × 6.25. Fat content of the harvested seed was estimated by use of the Soxhlet extraction method (*23*). Fat content of forage was determined by fat-acid hydrolysis, followed by extraction with ether and hexane (*24*, *25*).

Ash, Moisture, and Carbohydrate Determination. Ash content was estimated by ignition of a sample in an electric furnace and quantitation of the ash by gravimetric analysis (*26*). Moisture content was determined by loss of weight upon drying in a vacuum oven at 100 °C to a constant weight (*27*, *28*). Carbohydrate levels were estimated by using the fresh weight-derived data and the following equation (*29*):

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

Fiber Analysis. ADF was estimated by treating the sample with an acidic boiling detergent solution to dissolve the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected and determined gravimetrically (*30*). The NDF was estimated by treating the sample with a neutral boiling detergent solution to dissolve the protein, enzymes, carbohydrate, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected and determined gravimetrically (*30*, *31*).

Amino Acid Composition. Three procedures described in the literature (*32*) were used to estimate the values for 18 amino acids in harvested seed. The procedure for tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid before hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct hydrolysis with hydrochloric acid. The individual amino acids were then quantitated using an automated amino acid analyzer.

Fatty Acid Composition. The lipid in the harvested seed was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride/methanol. The resulting methyl esters were extracted with heptane containing tridecanoic methyl ester as an internal standard. The methyl esters of the fatty acids were analyzed on an Agilent 6890 gas chromatograph fitted with a 0.25 mm i.d. × 30 m long, 0.25 μm film thickness polyethylene glycol capillary column (J&W Scientific DB-Wax) and a flame ionization detector; external standards were used for quantitation (*33*).

Isoflavones. Samples were extracted using a solution of hydrochloric acid and reagent alcohol heated on hot plates and refluxed for at least 4 h. The extract was brought to volume, diluted, shaken, and centrifuged at approximately 1200 rpm for approximately 10 min. An aliquot of the supernatant was placed onto a C18 solid-phase extraction column. Unwanted components of the matrix were rinsed off with 20% methanol and then the isoflavones were eluted with 80% methanol. Samples were then analyzed on a Perkin-Elmer series 200 high-performance liquid chromatography system (*34*, *35*) with 260 nm ultraviolet detection and a 200 mm × 1.2 mm, 5 μm C18 column (Thermo Electron Hypersil ODS). Samples were quantitated by comparison to an external standard curve containing daidzein, genistein, and glycitein.

Raffinose and Stachyose Determination. The raffinose and stachyose assay was based on two methods (*36*, *37*) in which samples were extracted with deionized water, and the extracts were treated with a solution of hydroxylamine hydrochloride in pyridine containing phenyl-α-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoroacetic acid and analyzed using an Agilent 6890 gas chromatograph fitted with a 0.32 mm i.d. × 30 m long, 0.25 μm film thickness capillary column (Alltech AT-50) and a flame ionization detection.

Phytic Acid Determination. Phytic acid was quantitated in harvested seed following extraction using ultrasonication with 0.5 M hydrochloric acid and subsequent centrifugation for approximately 15 min at approximately 1500 rpm (*38*, *39*). The extract was purified by placing it on a silica-based anion exchange (SAX) column, washing with 0.05 M hydrochloric acid, and eluting with 2.0 M hydrochloric acid. The eluant was concentrated by drying under nitrogen and brought to volume with 25% tetrabutylammonium hydroxide in methanol. Samples were quantitated on a Perkin-Elmer Series 200 high-performance liquid

Table 1. Proximate and Fiber Composition of Forage from Glyphosate-Tolerant Soybean 40-3-2

component[a]	40-3-2 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	lit.[f] (range)[d]	ILSI[g] (range)[d]
moisture	70.56 (66.30–75.50)	70.50 (64.50–75.90)	(51.70–75.00) [49.14, 85.29]	74–79	73.5–81.6
protein	18.84 (14.74–31.37)	18.50 (15.88–23.65)	(12.72–22.73) [14.16, 23.63]	11.2–17.3	14.38–24.71
fat	5.39 (1.98–6.91)	5.04 (2.68–6.64)	(2.91–8.67) [2.30, 9.95]	3.1–5.1	1.30–5.13
ash	6.64 (5.34–7.56)	6.71 (5.72–8.26)	(4.68–9.24) [3.61, 9.34]	8.8–10.5	6.72–10.78
carbohydrates	69.13 (60.09–75.63)	69.74 (64.89–73.09)	(62.95–74.67) [60.96, 76.06]	na[i]	59.8–74.7
ADF[h]	31.93[j] (26.38–35.71)	30.26 (26.88–33.82)	(22.72–37.92) [22.52, 39.00]	32–38	na[i]
NDF[h]	38.62 (31.16–64.89)	34.94 (29.44–39.26)	(27.65–52.22) [20.61, 52.66]	34–40	na[i]

[a] Percent dry weight of sample, except moisture. [b] Nontransgenic control. [c] Commercial nontransgenic varieties planted at each site. [d] Range denotes the lowest and highest individual values across all sites. [e] Tolerance interval is specified to contain 99% of the commercial soybean population where negative limits are set to zero. [f] OECD, 2001 (*44*). [g] International Life Sciences Institute crop composition database, Ridley et al. (*45*). [h] ADF, acid detergent fiber; NDF, neutral detergent fiber. [i] na, not available. [j] Statistically different from the control at the 5% level ($p < 0.05$).

Table 2. Proximate and Fiber Composition of Harvested Seed from Glyphosate-Tolerant Soybean 40-3-2

component[a]	40-3-2 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	lit. (range)[d]	ILSI[j] (range)[d]
moisture	5.38 (4.90–5.81)	5.44 (4.97–5.71)	(4.71–5.76) [4.64, 6.18]	5.3–8.73[f], 5.18–14.3[g]	4.7–34.4
protein	37.73 (32.86–40.83)	38.48 (33.41–41.24)	(32.54–42.50) [31.19, 45.73]	329–436[h] g/kg of dw 360–484[i] g/kg of dw	33.19–45.48
fat	17.28 (15.79–19.04)	17.39 (15.80–20.10)	(15.16–20.28) [14.29, 21.82]	198–267[h] g/kg of dw 160–231[i] g/kg of dw	8.10–23.56
ash	5.49 (4.86–6.27)	5.54 (5.06–6.44)	(4.53–6.23) [4.59, 6.15]	4.61–5.94[g]; 4.29–5.88[f]	3.88–6.99
carbohydrates	39.50 (34.98–45.11)	38.58 (35.56–44.35)	(33.84–42.39) [31.67, 44.56]	29.3–41.3[f]	29.6–50.2
ADF[k]	16.85 (13.29–19.19)	16.94 (13.78–19.36)	(11.85–21.84) [10.78, 22.71]	na[l]	7.81–18.61
NDF[k]	18.57 (13.40–22.14)	18.54 (13.46–22.96)	(12.77–23.30) [9.55, 25.96]	na[l]	8.53–21.25
crude fiber	12.87 (9.74–14.43)	12.76 (9.82–14.29)	(7.32–16.33) [8.39, 16.70]	na[l]	4.12–13.87

[a] Percent dry weight of sample, except moisture. [b] Nontransgenic control. [c] Commercial nontransgenic varieties planted at each site. [d] Range denotes the lowest and highest individual values across all sites. [e] Tolerance interval is specified to contain 99% of the commercial soybean population where negative limits are set to zero. [f] Padgette et al. (*6*). [g] Taylor et al. (*7*). [h] Maestri et al. (*46*). [i] Hartwig and Kilen (*47*). [j] International Life Sciences Institute crop composition database, Ridley et al. (*45*). [k] ADF, acid detergent fiber; NDF, neutral detergent fiber. [l] na, not available.

chromatography system using a 5 μm, 150 mm $\times$ 4.1 mm polymer HPLC column (Hamilton PRP-1) and fitted with a refractive index detector. Sample concentrations were determined by comparing the signal of the unknowns against calibration standards prepared from phytic acid, dodecasodium salt hydrate, obtained from Sigma-Aldrich (St. Louis, MO).

Table 3. Amino Acid Composition of Harvested Seed from Glyphosate-Tolerant Soybean 40-3-2

component[a]	40-3-2 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	lit.[f] (range)[d]	ILSI[a,g] (range)[d]
alanine	1.71 (1.56–1.82)	1.73 (1.61–1.81)	(1.56–1.88) [1.51, 1.96]	1.60–1.86	15.13–21.04
arginine	2.76 (2.46–3.06)	2.83 (2.55–3.15)	(2.42–3.36) [2.20, 3.60]	2.56–3.46	22.85–34.00
aspartic acid	4.35 (3.98–4.76)	4.41 (4.07–4.77)	(3.90–4.82) [3.75, 5.15]	4.18–4.99	38.08–51.22
cystine	0.60 (0.57–0.67)	0.59 (0.55–0.66)	(0.50–0.66) [0.46, 0.70]	0.54–0.66	3.70–8.08
glutamic acid	6.84 (6.10–7.43)	6.95 (6.33–7.52)	(5.97–7.87) [5.66, 8.43]	6.64–8.16	58.43–82.01
glycine	1.64 (1.50–1.79)	1.65 (1.52–1.78)	(1.45–1.81) [1.41, 1.93]	1.60–1.87	14.58–19.97
histidine	1.01 (0.93–1.10)	1.02 (0.95–1.10)	(0.92–1.12) [0.89, 1.17]	0.98–1.16	8.78–11.75
isoleucine	1.69[h] (1.54–1.85)	1.73 (1.56–1.85)	(1.51–1.89) [1.43, 2.04]	1.65–1.95	15.39–20.77
leucine	2.93 (2.70–3.16)	2.98 (2.76–3.21)	(2.62–3.27) [2.55, 3.44]	2.81–3.37	25.90–36.22
lysine	2.46 (2.31–2.67)	2.48 (2.33–2.64)	(2.26–2.69) [2.19, 2.83]	2.47–2.84	22.85–28.39
methionine	0.56 (0.49–0.62)	0.55 (0.50–0.61)	(0.49–0.61) [0.46, 0.64]	0.51–0.59	4.31–6.81
phenylalanine	1.95 (1.73–2.07)	1.99 (1.84–2.11)	(1.75–2.23) [1.68, 2.35]	1.78–2.19	16.32–23.46
proline	1.91 (1.72–2.07)	1.95 (1.80–2.10)	(1.70–2.19) [1.63, 2.29]	1.86–2.23	16.87–22.84
serine	2.03 (1.86–2.17)	2.04 (1.92–2.19)	(1.83–2.25) [1.80, 2.34]	1.96–2.28	11.06–24.84
threonine	1.54 (1.45–1.68)	1.54 (1.46–1.68)	(1.41–1.67) [1.37, 1.71]	1.51–1.73	11.39–18.62
tryptophan	0.45 (0.34–0.52)	0.47 (0.41–0.53)	(0.36–0.55) [0.33, 0.58]	0.56–0.63	3.56–5.02
tyrosine	1.31 (1.19–1.43)	1.33 (1.24–1.44)	(1.12–1.48) [1.14, 1.54]	1.35–1.59	10.16–16.13
valine	1.80[h] (1.65–1.96)	1.84 (1.67–1.96)	(1.61–1.99) [1.53, 2.16]	1.71–2.02	15.97–22.04

[a] Percent dry weight of sample except for ILSI column, where data are reported as mg/g dry weight; conversion formula % DW = [mg/g] × 0.1. [b] Nontransgenic control. [c] Commercial nontransgenic varieties planted at each site. [d] Range denotes the lowest and highest individual values across all sites. [e] Tolerance interval is specified to contain 99% of the commercial soybean population where negative limits set to zero. [f] Padgette et al. (*6*). [g] International Life Sciences Institute crop composition catabase, Ridley et al. (*45*). [h] Statistically different from the control at the 5% level ($p < 0.05$).

Trypsin Inhibitor Determination. Trypsin inhibitor activity in harvested seed was determined using AOCS method Ba 12-75 (*40*). The ground, defatted sample was suspended in 0.01 N sodium hydroxide, an appropriate dilution of the suspension was made, and a series of aliquots resulting in increased levels of the diluted suspension was mixed with 0.02 mg/mL trypsin solution and 0.4 mg/mL of the synthetic substrate, benzoyl-DL-arginine-*p*-nitroanilide. After 10 min, the action of trypsin was stopped by the addition of 5.2 M acetic acid, the mixture was centrifuged or filtered, and the absorbance of the supernatant or filtrate was measured at 410 nm. Trypsin inhibitor

Table 4. Fatty Acid Composition of Harvested Seed from Glyphosate-Tolerant Soybean 40-3-2

fatty acid[a]	40-3-2 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	lit.[f,g] (range)[d]	ILSI[h,i] (range)[d]
16:0 palmitic	1.79 (1.60–2.01)	1.82 (1.62–2.06)	(1.40–2.16) [1.08, 2.49]		
	10.80 (*10.54–11.06*)	*10.93* (*10.65–11.15*)	(*9.16–12.07*)	*10.63–11.69*	*9.55–15.77*
18:0 stearic	0.84 (0.58–1.11)	0.84 (0.60–1.10)	(0.50–1.16) [0.33, 1.31]		
	5.05 (*3.64–6.37*)	*5.06* (*3.78–6.13*)	(*3.30–6.58*)	*3.85–4.55*	*2.70–5.88*
18:1 oleic	3.51 (2.80–4.20)	3.49 (2.80–4.27)	(2.60–4.75) [1.68, 5.62]		
	21.10 (*17.39–23.32*)	*20.91* (*17.51–23.11*)	(*15.80–25.93*)	*15.02–31.19*	*14.3–32.2*
18:2 linoleic	8.80 (7.91–9.91)	8.80 (8.04–9.79)	(7.58–10.59) [6.77, 11.85]		
	53.10 (*50.67–56.90*)	*52.99* (*50.72–56.60*)	(*48.40–59.49*)	*44.03–54.96*	*48.2–58.8*
18:3 linolenic	1.49 (1.31–1.81)	1.52 (1.32–1.76)	(1.27–1.98) [0.93, 2.19]		
	9.04 (*7.97–10.42*)	*9.19* (*8.02–10.69*)	(*7.47–11.24*)	*5.08–10.26*	*3.00–12.52*
20:0 arachidic	0.063 (0.045–0.083)	0.063 (0.046–0.080)	(0.038–0.080) [0.029, 0.093]		
	0.37 (*0.28–0.46*)	*0.38* (*0.29–0.45*)	(*0.25–0.47*)	*0.31–0.43*	*0.16–0.48*
20:1 eicosenoic	0.030 (0.025–0.037)	0.031 (0.026–0.039)	(0.024–0.045) [0.015, 0.053]		
	0.18 (*0.16–0.21*)	*0.19* (*0.16–0.21*)	(*0.16–0.24*)	*0.14–0.26*	*0.14–0.35*
22:0 behenic	0.058 (0.047–0.072)	0.059 (0.049–0.069)	(0.043–0.072) [0.040, 0.078]		
	0.35 (*0.30–0.39*)	*0.36* (*0.31–0.40*)	(*0.28–0.42*)	*0.46–0.59*	*0.28–0.59*

[a] Values in italics are percentage dry weight; values of fatty acids expressed as percent of total fatty acid are presented in regular font. Statistical analyses were performed on data expressed as percent dry weight, whereas the percent total fatty acid data are presented to facilitate comparisons with literature and ILSI data. The analytical method included the measurement of the following fatty acids that were not detected in the majority of samples analyzed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), γ-linolenic (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4). [b] Nontransgenic control. [c] Commercial nontransgenic varieties planted at each site. [d] Range denotes the lowest and highest individual values across all sites. [e] Tolerance interval is specified to contain 99% of the commercial soybean population where negative limits set to zero. [f] Padgette et al. (*6*). [g] Values expressed as percent of total fat except for palmitic acid (16:1) that is expressed as percent of triglyceride fatty acids. [h] International Life Sciences Institute crop composition database, Ridley et al. (*45*). [i] Values expressed as percent of total fat.

activity was calculated from the change in absorbance versus aliquot volume and expressed in trypsin inhibitor units (TIU) per milligram of fresh weight of sample.

Lectin Determination. Samples were suspended in phosphate-buffered saline (PBS), shaken, and filtered. An aliquot of the resulting extract was serially diluted in 10 cuvettes containing PBS. A 10% hematocrit of lyophilized rabbit blood in PBS was added to each dilution. After 2.5 h, the absorbance of each dilution of the sample and lectin control was measured by a spectrophotometer at 620 nm, using PBS to zero the instrument. One hemagglutinating unit (HU) was defined as the level that caused 50% of the standard cell suspension to form a sediment in 2.5 h (*41*).

Statistical Analysis of Composition Data. All substances were grown in single plots randomly assigned within each of three replication blocks. All soybean compositional analysis components were statistically analyzed using a mixed-model analysis of variance. The combined-site analyses used the model

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

where Y_{ijk} = unique individual observation, U = overall mean, T_i = substance effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by substance interaction effect, and e_{ijk} = residual error. For each compositional component, the forage and harvested seed from Roundup Ready soybean 40-3-2 were compared to the conventional control.

A range of observed values from the commercially available soybean varieties (reference substances) was determined for each analytical component. Additionally, the reference substance data were used to develop population tolerance intervals. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional analyte, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of

Table 5. Isoflavone Composition of Harvested Seed from Glyphosate-Tolerant Soybean 40-3-2

isoflavone[a]	40-3-2 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] (99% tolerance interval][e]	lit.[f] (range)[d]	ILSI[g] (range)[d]
daidzein	1161.82 (755.26–1663.86)	1171.77 (713.00–1702.08)	(567.39–2094.57) [0, 2822.32]	9.88–124.2	60.0–2453.5
genistein	1641.80[h] (988.70–2379.95)	1717.00 (911.88–2600.70)	(613.79–2367.61) [0, 3354.84]	13–150.1	144.3–2837.2
glycitein	86.80 (46.00–151.26)	90.55 (65.95–132.21)	(46.16–349.19) [0, 281.87]	4.22–20.4	15.3–310.4

[a] Units expressed as μg/g of dry weight. [b] Nontransgenic control. [c] Commercial nontransgenic varieties planted at each site. [d] Range denotes the lowest and highest individual values across all sites. [e] Tolerance interval is specified to contain 99% of the commercial soybean population where negative limits are set to zero. [f] USDA-ISU Isoflavone Database (*48*) with units expressed as mg/100 g of fw. [g] International Life Sciences Institute crop composition database, Ridley et al. (*45*). [h] Statistically different from the control at the 5% level ($p < 0.05$).

Table 6. Raffinose, Stachyose, Phytic Acid, Trypsin Inhibitor, and Lectin Composition of Harvested Seed from Glyphosate-Tolerant Soybean 40-3-2

component[a]	40-3-2 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	lit.[f,g] (range)[d]	ILSI[h] (range)[d]
raffinose	0.33 (0.24–0.43)	0.35 (0.25–0.46)	(0.22–0.63) [0.032, 0.75]		0.21–0.66
stachyose	2.25 (1.43–2.81)	2.43 (1.76–3.37)	(1.52–3.28) [1.07, 3.64]		1.21–3.50
trypsin inhibitor (TIU/mg of DW)	32.10 (23.64–43.45)	32.63 (25.77–57.77)	(21.41–66.00) [1.00, 62.87]	33.2–54.5[f]	19.59–118.68
lectin (HU/mg of FW)	1.20 (0.62–2.39)	1.40 (0.68–1.99)	(0.26–4.53) [0, 3.29]	0.8–2.4[f] 37–323[g] HU/mg of protein	0.09–8.46
phytic acid	1.17 (0.77–1.78)	1.13 (0.67–1.53)	(0.56–1.93) [0.16, 2.09]		0.634–1.960

[a] Units expressed as percent dry weight except trypsin inhibitos and lectins that are expressed as noted. [b] Nontransgenic control. [c] Commercial nontransgenic varieties planted at each site. [d] Range denotes the lowest and highest individual values across all sites. [e] Tolerance interval is specified to contain 99% of the commercial soybean population where negative limits are set to zero. [f] Padgette et al. (*6*). [g] Kakade et al. (*49*). [h] International Life Sciences Institute crop composition database, Ridley et al. (*45*).

conventional references. Each tolerance interval estimate was based upon one summary value for each unique reference substance. As multiple observations existed for some reference varieties, that is, Nikko, data were first summarized by substance within site and then across sites. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero. SAS software (*42*) was used by Certus International Inc., Chesterfield, MO, to generate all summary statistics and perform all analyses.

The following 14 analytes with >50% of the observations at or below the limit of quantitation of the assay were excluded from statistical analysis: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 γ-linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. These analytes are known to occur at low or nondetectable levels in soybean oil (*43*).

A total of 49 different components were therefore evaluated (7 in forage and 42 in seed). The 42 components in harvested seed resulted from the difference between the initial 56 components analyzed minus the 14 fatty acids having levels below the limit of quantitation. Except for moisture and lectins, all component values were converted from a fresh weight to a dry weight basis (**Tables 1**−**5**).

RESULTS AND DISCUSSION

Safety assessments of genetically enhanced crops typically rely on a comparative approach focusing on selected nutritional and antinutritional components in food and feed. This paper describes the nutritional composition of Roundup Ready soybean 40-3-2 grown in field trials in Romania relative to that of a conventional control with a similar genetic background. The comparative assessment was conducted using a mixed-model analysis of variance with statistical significance assigned at the $p < 0.05$ level. In addition, the compositional profile of Roundup Ready soybean 40-3-2 was compared to those of conventional soybean varieties grown in the same field trials by calculating a 99% tolerance interval to address compositional variability in commercially available conventional soybean. Composition values for Roundup Ready soybean 40-3-2 were also compared with values derived from the published literature or values described in previous studies.

Proximate and Fiber Composition. Compositional analysis results for soybean seed and forage are presented in **Tables 1** and **2**, respectively. These results demonstrate that the levels

of proximate components (moisture, protein, ash, and carbohydrate) and fiber (ADF, NDF, and crude) in the harvested seed and forage of Roundup Ready soybean 40-3-2 were comparable to those in the harvested seed and forage of the conventional control. For forage, a statistically significant difference was observed for ADF. Individual values were within the tolerance interval determined for the commercial varieties and within published literature ranges (*44*). For harvested seed, the combined site analysis revealed no statistical differences. The tolerance interval results demonstrate that, with a confidence level of 95%, the levels of proximates and fiber for Roundup Ready soybean 40-3-2 were within the same population as those of conventional, commercially available soybean.

Amino Acid Composition. The content of the 18 amino acids measured in the harvested seed of Roundup Ready soybean 40-3-2 was comparable to that of the conventional control (**Table 3**). Combined site analysis revealed statistical differences in isoleucine and valine, with these values being numerically lower in Roundup Ready soybean 40-3-2. In both cases, mean differences were small (generally around 5%). Additionally, these values were within the 99% tolerance interval for commercial varieties, within published literature ranges, and within the range of historical conventional control values determined from previous studies (*45*).

Fatty Acid Composition. The content of the fatty acids in harvested seed of Roundup Ready soybean 40-3-2 was comparable to that observed in the harvested seed of the conventional control (**Table 4**). Combined site analysis revealed no statistical differences in fatty acid composition. These results demonstrate, with a confidence level of 95%, that the levels of these fatty acids were within the same population as those of conventional, commercially available soybean. Summary statistics and all analyses of fatty acid composition were conducted on values expressed as percentage dry weight. To facilitate comparison with data from previous studies **Table 4** also presents values expressed as percentage of total fatty acid.

Isoflavones. The content of the isoflavones in harvested seed of Roundup Ready soybean 40-3-2 was comparable to that observed in the harvested seed of the conventional control (**Table 5**). Combined site analysis indicated a statistically significant difference in genistein. Individual values were within the 99% tolerance interval for commercial varieties, within published literature ranges (*48*), and within the range of historical conventional control values determined from previous studies (*45*). These results demonstrate, with a confidence level of 95%, that the levels of these isoflavones were within the same population as those of conventional, commercially available soybean.

Antinutrients. The contents of stachyose, raffinose, phytic acid, trypsin inhibitor, and lectin in the harvested seed of Roundup Ready soybean 40-3-2 were comparable with those observed in the harvested seed of the conventional control (**Table 6**). Combined site analyses revealed no statistical differences in any of these antinutrients. These results demonstrate, with a confidence level of 95%, that the levels of these antinutrients were within the same population as those of conventional, commercially available soybean.

Phytic acid, the hexakis-*o*-phosphate of *myo*-inositol, is widely distributed in plants (*50*). Seeds can accumulate up to 90% of stored organic phosphate as phytic acid, which upon ingestion can act to limit the uptake of minerals such as calcium in higher animals. The trypsin inhibitors in soybeans have been well studied and are known to affect the nutritive value of raw soybeans (*51*).

Conclusions. The results of compositional analyses for Roundup Ready soybean 40-3-2 grown in Romania demonstrate that the harvested seed and forage of Roundup Ready soybean 40-3-2 are comparable to those of the conventional soybean control and the commercially available soybean varieties. The incorporation of reference varieties into field trials to establish a population-tolerance interval is important and relevant as the composition of any crop, including soybean, varies as a result of many factors, including variety type, growing conditions, storage, and handling. The values for nutritional and antinutritional components in Roundup Ready soybean 40-3-2 all fell within the range of natural variability found in conventional soybean varieties. These findings are consistent with earlier reports of the compositional equivalence of Roundup Ready soybean 40-3-2 and its conventional counterparts (*6*−*8*).

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