Compositional Analysis of Tubers from Insect and Virus Resistant Potato Plants

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Genetically modified potato plants that are resistant to the Colorado potato beetle, plus either the potato leaf roll virus or potato virus Y, have recently been commercialized. As part of the safety assessment for plants produced by modern biotechnology, the composition of the food/feed must be compared to that of the food/feed produced by an equivalent plant variety from a conventional source. The composition of important nutritional and antinutritional factors in tubers produced by virus- and insect-resistant potato plants were compared to tubers produced by conventional potato plants. Key nutritional, quality, and antinutritional components measured were total solids, vitamin C, dextrose, sucrose, soluble protein, and glycoalkaloids. Proximate analyses included fat, ash, calories, total protein, and crude fiber. Minor nutrients measured were vitamin B_6, niacin, copper, magnesium, potassium, and amino acids. The results from these analyses confirm that tubers produced by insect- and virus-protected varieties are substantially equivalent to tubers produced by conventional potato varieties.

Keywords: *Colorado potato beetle; composition; insect protection; nutritional components; potato; virus resistant; PLRV; PVY*

INTRODUCTION

Potatoes are one of the most important food crops throughout the world. Cost-effective control of potato pests is a key factor in determining the quality, yield, and profitability of a potato crop. Potato leaf roll virus (PLRV), potato virus Y (PVY), and the Colorado potato beetle (CPB) are three major potato pests. Severe infections with PLRV can cause yield losses of as much as 50% (van der Wilk, 1991). Nearly all commercial varieties of potato are susceptible to infection from PLRV with worldwide yield losses estimated at 10% (van der Wilk, 1991). In the Russet Burbank variety, plants that are infected with PLRV during the growing season can develop net necrosis, a condition in which phloem cells in the tubers become necrotic, leaving a network of coarse brown strands in the vascular ring of the flesh. This condition greatly reduces the value of the tubers for fresh or processing use (USDA, 1986). PVY is considered one of the most damaging potato viruses because it causes economically significant yield depression. Severe infestations with PVY can reduce yield by as much as 80% (Bemster and de Boks, 1987). The CPB is the most damaging insect pest of potato. If not controlled, CPB can lower yields by as much as 85% (Hare, 1980; Ferro, 1983; Shields and Wyman, 1984).

Principal methods to control PLRV and PVY are to plant certified seed that contains low levels of virus and the application of insecticides to kill the green peach aphid (GPA), the primary vector of these viral diseases. Control of CPB is almost entirely through the application of insecticides. The effectiveness of the insecticides varies due to pesticidal characteristics and limitations. For instance, pesticides that are used today to control aphids in potato either do not kill the aphids quickly enough to prevent the spread of the virus or do not last long enough to kill aphids in the later portion of the growing season. Many pesticides used for control of aphids and other insect pests of potato are nonselective, killing many naturally occurring beneficial insects that prey on aphids and CPB and help suppress harmful insect populations (van Emden et al., 1969). In addition, more stringent pesticide regulations have resulted in fewer options for chemical insect control in recent years (Food Quality Protection Act, 1996). Some of the most effective and persistent insecticides have been withdrawn from the market, making it more difficult to control virus levels in seed and commercial potatoes (Thomas et al., 1993). To maintain the yield and quality of tubers produced in North America, more effective new technologies are needed.

To provide control options for PLRV, CPB, and PVY, select clones of Russet Burbank and Shepody potato varieties were supplemented with genes that control these important insect and virus pests of potato. Genes were inserted into the genome of potato using an *Agrobacterium*-based plant transformation system. Six clones were selected for commercial development. Three clones of the Russet Burbank potato variety contained genes to control CPB and PLRV; one clone contained genes to control CPB and PVY. Two clones of the

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Shepody potato variety contained genes that provide resistance to CPB and PVY. Clones that were resistant to CPB and PVY were referred to as NewLeaf Y potatoes, whereas those that were resistant to CPB and PLRV were referred to as NewLeaf Plus potatoes.

All six of the clones were approved by Canadian and United States regulatory agencies and are currently in commerce in North America. Food and feed safety approvals of the tubers derived from NewLeaf Plus and Newleaf Y potato clones were obtained after voluntary consultations with the U.S. Food and Drug Administration and mandatory reviews by the Canadian Food Inspection Agency, Health Canada, the Animal Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA), and the U.S. Environmental Protection Agency (EPA).

Food and feed safety evaluations of the tubers produced by NewLeaf Plus and NewLeaf Y potato plants took into consideration the safety of the expressed proteins and assessment of the composition of the tubers produced by the transformed plants. Compositional data on NewLeaf Plus and NewLeaf Y potato varieties, the subject of this paper, were generated on the basis of scientific recommendations for establishing the substantial equivalence of transgenic crops compared to conventional varieties for the purpose of safety and nutritional assessment. Substantial equivalence is a concept used to identify similarities or differences of a food/feed derived from a transgenic crop in comparison to a food/feed derived from a conventional crop having a known history of safe use (FAO, 2000). International expert groups from the Organization for Economic Cooperation and Development (OECD), the Food and Agricultural Organization of the United Nations (FAO), the World Health Organization (WHO), Health Canada, and the FDA have concluded that compositional analysis of the new food source should be conducted and the results compared to an appropriate counterpart that has an accepted standard of safety (FAO/WHO, 1991; OECD, 1993; WHO, 1995; FAO, 1996; Health Canada, 1994; FDA, 1992). They have recommended that this compositional comparison be based on key nutrients and toxicants for the food source in question. Key nutrients are those that may have a substantial impact in the overall diet and may include major constituents such as fats, proteins, and carbohydrates or minor components such as certain minerals and vitamins. Key toxicants are those toxicologically significant components known to be present in the species for which toxic potency and level may be significant to animal or human health. The objective of this study was to compare the composition of tubers produced by NewLeaf Plus and NewLeaf Y potato clones to the composition of tubers produced by conventional potato varieties. This paper summarizes the comparisons done to confirm that tubers produced by insect- and virus-resistant clones are substantially equivalent to those produced by conventional potato varieties.

MATERIALS AND METHODS

Plant Production. Genes were inserted into the genome of potato cultivars Russet Burbank (RB) or Shepody (SE) using an *Agrobacterium*-based plant transformation system (Newell et al., 1991). Genes were inserted to control CPB, PLRV, and PVY. To control CPB and PLRV, the *cry3A* gene, which encodes for the CPB-active protein from *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*) (Perlak et al., 1993), and the *orf1/orf2* viral gene sequence found in the naturally occurring PLRV (Kaniewski et al., 1994; Lawson et al., 2000) were inserted into the genome of RB. To control CPB and PVY, the potato virus Y coat protein gene (*PVYcp*) (Lawson et al., 1990), which imparts resistance to the aphid-transmissible PVY, and the *cry3A* gene were inserted into the genome of RB and SE. In addition to the *orf1/orf2*, *cry3A*, and *PVYcp* genes, plants contain either one of two selectable marker genes, the *nptII* gene (Shaw et al., 1993) or the *CP4 EPSPS* gene from the *Agrobacterium* sp. strain *CP4* (Padgette et al., 1996b). These selectable marker genes were used during the plant transformation process to select for modified plant cells that contain the desired trait.

Field Trials. Plants were grown from second field generation seed potatoes at locations representing several of the major potato production areas within the United States and Canada. In the United States, sites were located near Homestead, FL; Aberdeen, ID; Wilder, ID; Island Falls, ME; Lakeview, MI; Stanton, MI; Echo, OR; Hermiston, OR; Ephrata, WA; Pasco, WA; and Coloma, WI. In Canada, sites were located near Lethbridge, AB; Spruce Grove, AB; Winkler, MB; Hartland, NB; New Denmark, NB; Summerside, PE; and Sainte-Foy, PQ. Plants were cultivated and tubers produced using traditional agronomic practices (including pesticide-based control methods). Agronomic practices and pest control measures used were recommended by regional potato extension specialists and typically used in commercial potato production in each respective area. Although all varieties were not grown at every location, a conventional control variety was grown and sampled at the same location as each NewLeaf Plus or NewLeaf Y variety. Trials were conducted using a randomized complete block design. Plots were replicated four times at the majority of the field sites. Tubers were harvested from each plot ~140 days after planting and stored at 6−9 °C at 70−90% relative humidity prior to analysis. A subsample of five tubers was selected from the tubers harvested from each plot for subsequent analyses.

Analytical Experiments. *Sample Processing.* Harvested tubers were washed and sliced (unpeeled) into ~4−5 mm slices or cubes (approximately 1 × 1 cm). Approximately 225 g of tuber tissue was pooled from each of the five tubers. The sliced tissue was flash frozen using dry ice or liquid nitrogen and then lyophilized to dryness. The dried pieces were ground into a powder with the aid of a Waring blender or Wiley mill equipped with a 40 mesh screen and used for subsequent analyses.

Immunological Detection of the Cry3A Protein. To confirm sample identity, all tubers were assayed for the presence of the Cry3A protein. The Cry3A protein was extracted from the tuber powder using an aqueous extraction buffer, and the extract was assayed for the presence of the Cry3A protein using either a highly sensitive time-resolved immunofluorescent sandwich assay (Joaquim et al., 1999; Bookout et al., 2000) or ELISA. The Cry3A ELISA was a double-antibody sandwich assay using rabbit polyclonal Cry3A antibody for antigen capture and peroxidase-labeled anti-Cry3A polyclonal rabbit antibody for detection of captured Cry3A. The assay procedure was similar to the procedure described by Rogan et al. (1992).

Analysis for Key Quality and Nutritional Parameters: Total Solids, Sugars, Glycoalkyloids, Vitamin C, and Soluble Protein. Total solids were estimated according to methods described in the literature (AOAC, 1995a,b). Subsamples (20−40 g) of diced potato tuber samples were prepared from 10 tubers as described previously. Typically, subsamples were weighed to the nearest 0.01 g prior to lyophilization and then reweighed after completion of lyophilization. Percent total solids were computed from the dry weight and fresh weight (dry weight ÷ fresh weight) × 100 and reported as the mean of two analyses.

Dextrose and sucrose measurements were carried out on freeze-dried tuber tissue using a published analytical method (AOAC, 1995c). Dextrose (J. T. Baker Inc., Phillipsburg, NJ) was used as the reference standard. Results were reported as the mean percent dextrose and percent sucrose (fresh weight basis) derived from the mean of two analyses.

Total glycoalkaloid analysis was carried out on lyophilized and ground tuber tissue. The procedure was based on methods described by Bergers (1980) and measured the total amount of solanines, chaconines, and other glycoalkaloids. Solanine (Sigma Chemical Co., St. Louis, MO) was used as the reference standard. A single analysis was done per sample. The glycoalkaloid level was reported as total milligrams of glycoalkaloids per 100 g of fresh tuber weight.

Total ascorbic acid (vitamin C) analysis was carried out on lyophilized potato tuber tissue according to a published method (AOAC, 1995d). L-Ascorbic acid (J. T. Baker) was used as the reference standard. A single analysis was performed per sample. The vitamin C level was reported as milligrams of ascorbic acid per 100 g of fresh tuber weight.

Soluble Protein. Soluble protein was determined on lyophilized and ground tuber tissue using the dye binding method described by Bradford (1976). Results were reported as a percentage of sample dry weight.

Proximate Analyses: Total Protein, Fat, Ash, Crude Fiber, and Carbohydrates. Lyophilized tuber samples were further dried prior to analyses to remove residual moisture. Except for calories, all values were reported as grams per 100 g of dry weight. Calories were reported as calories per 100 g of dry weight.

Total protein level was estimated via total nitrogen determination using the LECO FP-428 Nitrogen/Food Protein Determinator (LECO Corp., St. Joseph, MI) according to AOAC Methods 992.23 and 990.03. (AOAC, 1995e,f). In this analysis, the sample is combusted in a pure oxygen stream to form H_2O, CO_2, NO, and NO_2 (NO_x). Water and carbon dioxide are removed and NO_x compounds are converted to protein using the factor 6.25.

Total fat content was estimated according to the AOAC Method 920.39 (AOAC, 1995g). In this analysis, the sample was extracted in petroleum ether. The extract was evaporated, dried, and weighed. The reported value was the percent fat.

Ash content was determined according to AOAC Method 923.03 (AOAC, 1995h). In this analysis, all volatile organic matter is driven off when the sample is ignited at 550 °C in an electric furnace. The nonvolatile material remaining is determined gravimetrically and referred to as ash. Results were reported as the grams of ash per 100 g of dry weight.

Crude fiber content was assessed according to AOAC Method 962.09 (AOAC, 1995i). In this analysis, the sample is digested with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions. The residue is dried and ignited at 550 °C. The crude fiber was the weight lost upon ignition. Results were reported as grams of fiber per 100 g of dry weight.

Total carbohydrates were assessed according to a published method (USDA, 1975a). The total carbohydrate level was determined by calculating the difference between dry weight and the protein, moisture, ash, and fat content. Results were reported as grams of carbohydrates per 100 g of dry weight.

The total calories in the sample were assessed according to a published method (USDA, 1975b). Calories were calculated using the Atwater factors with the fresh weight-derived data and the following equation: calories (kcal/100 g) = (4 × % protein) + (9 × % fat) + (4 × % carbohydrates). Average values derived from these tests [4 cal/g (protein), 9 cal/g (fat), and 4 cal/g (carbohydrate)] were used to calculate the calories of each sample.

Minor Nutrient and Amino Acid Analyses: Copper, Magnesium, Potassium, Vitamin B_6, Niacin, and Amino Acid Profile. Analysis of copper, magnesium, and potassium was done using ICP emission spectrometry (ICPL) according to a method published in the literature (AOAC, 1995j). The sample was dried, precharred, and ashed overnight at 500 ± 50 °C. The ashed sample was treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured by the inductively coupled plasma, with the emission of the standard solutions.

The total amino acid profile was assessed for each sample according to a published method (AOAC, 1995k). The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the sample for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using a Beckman model 7300 automated amino acid analyzer. The amino acids measured are listed in Tables 7 and 8: aspartic acid included asparagine, glutamic acid included glutamine, and total cystine included cysteine.

Vitamin B_6 (B_6A) analysis was conducted according to AOAC Method 961.15 (AOAC, 1995l). The sample was hydrolyzed with dilute sulfuric acid in an autoclave, and the pH was adjusted to remove interfering substances. The amount of vitamin B_6 was estimated by comparing the growth response of the yeast *Saccharromyces carlsbergenesis* introduced into the sample with the growth response of yeast in a vitamin B_6 standard. The response was measured turbidimetrically.

Niacin levels were assessed in accordance with AOAC Method 944.13 (AOAC, 1995m). For this analysis, the sample was hydrolyzed with sulfuric acid, and the pH was adjusted to remove interferences. The amount of niacin was assessed by comparing the growth response of the bacteria *Lactobacillus plantarum* introduced into the sample with the growth response of bacteria in a niacin standard. The response was measured turbidimetrically.

Statistical Analyses. Comparisons of combined least-squares sample mean values for key quality, nutritional, and proximate parameters were done to determine if the means for the Newleaf Plus and NewLeaf Y varieties were statistically different from the mean of the conventional control. All statistical analyses were conducted using the SAS system (version 6.07, SAS Institute Inc., Cary, NC). Combined least-squares means were computed from a mixed model analysis of variance using the SAS Mixed procedure. The mixed model is a general linear model containing both fixed effects and random effects. The mixed model analysis uses the maximum likelihood technique to estimate the random effects and to perform F and or t tests on the means of the fixed effects. Replicates and, when appropriate, locations and/or years were treated as random effects in the mixed model. Standard errors for means contained variance components for all relevant sources of variation (e.g., plot, replicates, location, and years). Differences from the control were tested using a T statistic generated from the LSMEANS statement in the Mixed procedure. This was the mixed model analogue of the least significant difference (LSD) procedure. All statistical significance was determined at the 5% (i.e., $P \leq 0.05$) level.

Proximate composition values were adjusted for moisture content in the tuber powder and expressed on a dry weight basis using the following formula:

$$\text{adjusted value} = (\text{value} \times 100)/[100 - \text{moisture (\%)}]$$

RESULTS

All samples derived from genetically modified potato plants contained detectable levels of the Cry3A protein. As expected, the Cry3A protein was not detected in tubers derived from conventional varieties included as controls.

Key Quality and Nutritional Parameters. The majority of the nutrients, quality parameters, and glycoalkaloids measured for virus- and insect-resistant clones were not statistically significantly different from the values obtained from the conventional control and were consistent with previously published levels of key nutrients and glycoalkaloids in tubers. Table 1 presents the values for total solids, vitamin C, soluble protein, sugars, and total glycoalkaloids in NewLeaf Plus Russet Burbank and conventional Russet Burbank potatoes.

Table 1. Comparison of Nutritional and Quality Parameters of NewLeaf Plus Russet Burbank Clones and Conventional Russet Burbank[a]

	NewLeaf Plus RB clone no. [mean (SE)[b]]				
parameter	RBMT21-129	RBMT21-350	RBMT22-082	RB control	literature range[c]
total solids (% FW)	21.6 (0.42)	21.9 (0.43)	21.0 (0.42)	21.5 (0.42)	16.8−26.8[d]
% dextrose (% FW)	0.087 (0.0073)	0.094 (0.0076)	0.113 (0.0073)	0.099 (0.0073)	0.03−0.52
% sucrose (% FW)	0.182[e] (0.0185)	0.201 (0.0186)	0.177[e] (0.0185)	0.199 (0.0185)	0.05−0.88
vitamin C (mg/100 g of FW)	10.1 (0.66)	9.9 (0.66)	10.4 (0.66)	10.0 (0.66)	10.3−22.0
% soluble protein (% DW)	5.0 (0.11)	5.1 (0.11)	5.0 (0.11)	5.0 (0.11)	3.3−7.3
total glycoalkaloids (mg/100 g of FW)	5.4 (0.69)	4.8 (0.71)	5.1 (0.69)	4.3 (0.69)	2.5−16.1

[a] Samples were collected from tubers harvested in 1995 from three field locations in the United States (Echo, OR; Ephrate, WA; Pasco, WA). Statistical analyses were conducted as described in the text. [b] Numbers in parentheses are standard error of the mean. [c] Literature ranges were taken from Pavek et al. (1980−1992) and include values for the Russet Burbank, Atlantic, Gemchip, and Norchip varieties. [d] Literature range for total solids calculated from a conversion from specific gravity. [e] Underscored values are statistically different from the RB control ($P \leq 0.05$).

Table 2. Comparison of Nutritional and Quality Parameters of NewLeaf Shepody and Conventional Shepody[a]

	NewLeaf Y SE clone no. [mean (SE)[b]]			
parameter	SEMT15-02	SEMT15-15	SE control	literature range[c]
total solids (%FW)	22.3 (0.57)	22.6 (0.55)	22.7 (0.53)	16.8−26.8[d]
% dextrose (%FW)	0.22 (0.134)	0.21[e] (0.134)	0.23 (0.134)	0.03−0.52
% sucrose (%FW)	0.28 (0.080	0.31 (0.079)	0.29 (0.079)	0.05−0.88
vitamin C (mg/100 g of FW)	22.7 (1.21)	23.9 (1.19)	23.8 (1.15)	10.3−22.0
soluble protein (%DW)	6.6[e] (0.28)	6.4 (0.27)	6.3 (0.27)	3.3−7.3
total glycoalkaloids (mg/100 g of FW)	5.5 (1.10)	5.3 (1.09)	4.6 (1.07)	2.5−16.1

[a] Samples were collected from tubers harvested in 1995 and 1996 at six field locations in the United States (Homestead, FL; Echo, OR; Coloma, WI; Lakeview, MI; Wilder, ID; Island Falls, ME) and two locations in Canada (Lethbridge, AB; Sainte-Foy, PQ). Statistical analyses were conducted as described in the text. [b] Numbers in parentheses are standard error of the mean. [c] Literature ranges were taken from Pavek et al. (1980−1992) and include values for the Russet Burbank, Atlantic, Gemchip, and Norchip varieties. [d] Literature range for total solids calculated from a conversion from specific gravity. [e] Underscored values are statistically different from the SE control ($P \leq 0.05$).

Table 3. Comparison of Nutritional and Quality Parameters of NewLeaf Y Russet Burbank and Conventional Russet Burbank Potato Tubers[a]

parameter	NewLeaf Plus clone RBMT15-101 [mean (SE)[b]]	RB control	literature range[c]
total solids (%FW)	20.7 (0.81)	20.7 (0.81)	16.8−26.8[d]
% dextrose (%FW)	0.24[e] (0.098)	0.21 (0.098)	0.03−0.52
% sucrose (%FW)	0.18 (0.033)	0.18 (0.033)	0.05−0.88
vitamin C (mg/100 g of FW)	14.5[e] (2.84)	13.4 (2.84)	10.3−22.0
soluble protein (%DW)	5.1 (0.20)	5.4 (0.20)	3.3−7.3
total glycoalkaloids (mg/100 g of FW)	10.6 (2.54)	11.7 (2.54)	2.5−16.1

[a] Samples were collected from tubers harvested in 1995 and 1996 from three field trial locations in the United States (Coloma, WI; Stanton, MI; Aberdeen, ID) and two locations in Canada (New Denmark, NB; Lethbridge, AB). Statistical analyses were conducted as described in the text. [b] Numbers in parentheses are standard error of the mean. [c] Literature ranges were taken from Pavek et al. (1980−1992) and include values for the Russet Burbank, Atlantic, Gemchip, and Norchip varieties. [d] Literature range for total solids calculated from a conversion from specific gravity. [e] Underscored values are statistically different from the RB control ($P \leq 0.05$).

There were no statistically significant differences in the levels of any of the nutrients or glycoalkaloids measured with one exception: the amount of sucrose in Newleaf Plus Russet Burbank clones (line) RBMT21-129 and RBMT22-082 was statistically significantly lower ($P \leq 0.05$) than the control. The difference in sugar content observed between tubers derived from NewLeaf Plus Russet Burbank and conventional Russet Burbank was minor and did not decrease by more than 12%. The concentration of dextrose in the tubers derived from NewLeaf Plus clones is well within previously reported levels and likely has no nutritional impact on the quality of the tubers produced by NewLeaf Plus potato plants. For example Pavek et al. (1980−1992) reported a 9-fold range in sucrose levels (Table 1); others have reported total sugar content ranging from trace levels to as high as 10% of tuber dry weight (Kadam et al., 1991).

The levels of key nutrients and antinutrients for NewLeaf Y Shepody (lines SEMT15-02 and SEMT15-15) and NewLeaf Y Russet Burbank (line RBMT15-101) are presented in Tables 2 and 3. There were three parameters that were statistically significantly different ($P \leq 0.05$) from the control in these clones: the amount of dextrose in NewLeaf Y Shepody line SEMT15-15 was slightly lower than the level of dextrose in the conventional Shepody, the level of dextrose in NewLeaf Y Russet Burbank line RBMT15-101 was slightly greater than the dextrose level in conventional Russet Burbank, the amount of soluble protein was slightly greater in NewLeaf Y Shepody line SEMT15-02, and vitamin C levels in NewLeaf Y Russet Burbank line RBMT15-101 were greater than the level of vitamin C in conventional Russet Burbank. As described previously, sugar content is highly variable and influenced by a number of factors including tuber maturity at harvest, length of time in storage, and regional differences in sugar content within the tuber itself (Kadam et al., 1991). The differences in dextrose seen between the NewLeaf Y and conventional varieties were minor (9% lower for NewLeaf Y SEMT15-15 and 14% greater for NewLeaf Y RBMT15-101). The difference in soluble protein levels between NewLeaf Y Shepody clone SEMT15-02 and conventional Shepody was <5%. Furthermore, this difference was not noticed in total protein levels measured in tubers derived from

Table 4. Comparison of Proximate Values for NewLeaf Plus Russet Burbank and Conventional Russet Burbank Potato Tubers[a,b]

	NewLeaf Plus RB clone [mean (SE)[c]]				
parameter	RBMT21-129	RBMT21-350	RBMT22-082	RB control	literature range[d]
total protein	9.86 (1.041)	9.94 (1.028)	9.93 (1.028)	9.90 (1.028)	7.1−14.6
fat	0.20[e] (0.033)	0.19 (0.032)	0.20[e] (0.032)	0.16 (0.032)	0.1−0.8
ash	4.68 (0.218)	4.70 (0.200)	4.78 (0.200)	4.75 (0.200)	2.2−9.5
crude fiber	1.64 (0.093)	1.55 (0.080)	1.61 (0.080)	1.68 (0.080)	0.2−3.5
total carbohydrates	85.24 (0.095)	85.17 (0.960)	85.09 (0.960)	85.18 (0.960)	84.5 (av)
calories	382.3 (0.99)	382.1 (0.93)	381.9 (0.93)	381.8 (0.93)	350 (av)

[a] Samples were collected from tubers harvested in 1996 from three field trial locations in Canada (Spruce Grove, AB; Winkler, MB; New Denmark, NB). Statistical analyses were conducted as described in the text. [b] Except for calories, reported values are in g/100 g of dry weight (corrected for moisture content in the tuber powder). [c] Numbers in parentheses are standard error of the mean. [d] Literature ranges for total protein, fat, ash, total carbohydrates, and calories are from Sherz et al. (1989). Values for crude fiber are from Talburt and Smith (1967). [e] Underscored values are statistically different from the RB control ($P \leq 0.05$).

Table 5. Comparison of Proximate Values for NewLeaf Y Shepody and Conventional Shepody Potato Tubers[a,b]

	NewLeaf Y SE clone [mean (SE)[c]]			
parameter	SEMT15-02	SEMT15-15	SE control	literature range[d]
total protein	11.43 (1.023)	10.76 (1.023)	11.03 (0.991)	7.1−14.6
fat	0.17 (0.011)	0.19[e] (0.011)	0.14 (0.010)	0.1−0.8
ash	4.63 (0.473)	4.64 (0.472)	4.69 (0.471)	2.2−9.5
crude fiber	1.33 (0.097)	1.42 (0.097)	1.53 (0.086)	0.2−3.5
total carbohydrates	83.77 (1.334)	84.41 (1.334)	84.14 (1.309)	84.5 (av)
calories	382.3 (1.89)	382.4 (1.89)	381.9 (1.88)	350 (av)

[a] Samples were collected from tubers harvested in 1996 from two field locations in the United States (Coloma, WI; Island Falls, ME) and two in Canada (Lethbridge, AB; Sainte-Foy, PQ). Statistical analyses were conducted as described in the text. [b] Except for calories, reported values are in g/100 g of dry weight (corrected for moisture content in the tuber powder). Calories is reported in calories per 100 g of dry weight. [c] Numbers in parentheses are standard error of the mean. [d] Literature ranges for total protein, fat, ash, total carbohydrates, and calories are from Sherz et al. (1989). Values for crude fiber are from Talburt and Smith (1987). [e] Underscored values are statistically different from the SE control ($P \leq 0.05$).

this line. For instance, the amount of total protein in tubers derived from this clone was not different from the amount of total protein in tubers derived from the conventional Shepody variety (Table 5). The difference in vitamin C content between NewLeaf Y Russet Burbank clone RBMT15-101 and conventional Russet Burbank was less than a 9% increase and, although statistically significant, was within the range previously reported for these components in potato (Pavek et al., 1980−1992). In light of the large variation in the levels of these key nutrients and quality components for potatoes already accepted by the industry and in commerce, these small differences in values for vitamin C, sugar, or protein content measured in tubers derived from these NewLeaf Y varieties do not impact the nutritional value of the tubers derived from NewLeaf Plus and Newleaf Y potato varieties.

Proximates. The levels of macronutrients in the three NewLeaf Plus and conventional Russet Burbank varieties are presented in Table 4. There were no statistically significant differences between macronutrients in the genetically modified tubers versus tubers derived from the conventional Russet Burbank control variety except for fat in NewLeaf Plus lines RBMT21-129 and RBMT22-082. The levels of macronutrients in NewLeaf Y Shepody, conventional Shepody, NewLeaf Y Russet Burbank, and conventional Russet Burbank are presented in Tables 5 and 6. The level of fat in NewLeaf Y Shepody line SEMT15-15 was statistically significantly higher than the level of fat in the conventional Shepody tuber; no other values were statistically significantly different from the control. According to Scherz (1989), the levels of fat in potato tubers vary from 0.1 to 0.8 g/100 g of tuber dry weight. The difference in fat levels in the NewLeaf Plus and NewLeaf Y varieties was as great as 35% (Table 5; SEMT15-15 versus conventional Shepody). However, these difference are seen as minor considering that fat can vary by up to 8-fold in conventional varieties. Therefore, the minor differences observed are not considered to be important to the nutritional quality of the tubers produced by the NewLeaf Plus and NewLeaf Y clones.

Table 6. Comparison of Proximate Values for NewLeaf Y Russet Burbank and Conventional Russet Burbank Potato Tubers[a,b]

parameter	NewLeaf Y clone RBMT15-101 [mean (SE)[c]]	RB control	literature range[d]
total protein	11.75 (0.183)	12.3 (0.183)	7.1−14.6
fat	0.19 (0.082)	0.21 (0.032)	0.1−0.8
ash	5.81 (0.250)	6.04 (0.250)	2.2−9.5
crude fiber	1.69 (0.082)	1.66 (0.082)	0.2−3.5
total carbohydrates	82.25 (0.303)	81.44 (0.303)	84.5 (av)
calories	377.7 (1.06)	376.9 (1.06)	350 (av)

[a] Samples were collected from tubers harvested in 1996 from a field site located near Lethbridge, AB, Canada. Statistical analyses were conducted as described in the text. [b] Except for calories, reported values are in g/100 g of dry weight (corrected for moisture content in the tuber powder). Calories is reported in calories per 100 g of dry weight. [c] Literature ranges for total protein, fat, ash, total carbohydrates and calories are from Sherz et al. (1989). Values for crude fiber are from Talburt and Smith (1987). [e] Numbers in parentheses are standard error of the mean.

Minor Nutrients and Amino Acids. The results from the analysis of the levels of vitamins B_6 and niacin, the minerals copper, magnesium, and potassium, and amino acids in the NewLeaf Plus and NewLeaf Y Russet Burbank Lines are shown in Tables 7 and 8. The mean value for each mineral, vitamin, or amino acid was compared to the control (conventional Russet Burbank or Shepody planted at the same site and grown under identical conditions) and to the previously reported range for each value. In all cases, the levels of minerals and vitamins in the conventional and transgenic varieties were comparable to the range previously reported

Table 7. Vitamin, Mineral, and Amino Acid Composition of NewLeaf Y Shepody and Shepody Potato Tubers[a]

component (mg/200 g of FW)	NewLeaf Y SE clone SEMT15-02 mean	SEMT15-02 range max	SEMT15-02 range min	SEMT15-15 mean	SEMT15-15 range max	SEMT15-15 range min	Shepody control mean	Shepody control range max	Shepody control range min	literature range[b]
vitamin B_6	0.56	0.62	0.49	0.50	0.72	0.32	0.52	0.62	0.40	0.26−0.82
niacin	4.55	5.05	4.14	4.78	5.86	3.98	4.43	5.15	3.73	0.18−6.2
copper	0.41	0.61	0.20	0.48	1.10	0.23	0.39	0.53	0.20	0.03−1.4
magnesium	53.13	67.16	48.17	56.95	90.00	47.85	54.22	65.54	48.95	22.5−110
potassium	1097.24	1326.78	996.82	1135.14	1634.40	971.46	1162.01	1259.30	1105.92	700−1250
aspartic acid	919.24	1151.50	614.88	994.27	1404.00	702.24	1001.97	1324.80	670.72	677−1476
threonine	185.54	220.50	142.13	202.22	278.64	157.47	183.00	225.77	138.75	102−214
serine	191.19	231.77	147.67	201.53	286.56	149.18	187.80	230.18	141.06	125−255
glutamic acid	865.48	1073.10	665.28	976.81	1173.60	856.52	966.06	1181.28	773.12	583−1207
proline	171.46	232.06	127.01	181.36	272.16	130.34	164.45	201.48	118.78	89−366
glycine	165.64	196.97	133.06	179.23	249.84	147.90	161.84	184.92	133.12	92−195
alanine	149.36	171.99	117.94	163.20	219.60	133.53	146.35	172.22	119.30	87−238
cystine	78.86	89.99	71.57	83.82	108.72	75.54	76.36	86.55	66.56	96−185
valine	219.00	271.68	186.48	249.09	346.32	223.44	225.81	247.84	200.70	196−363
methionine	75.21	85.28	63.00	83.49	105.84	72.35	72.13	83.90	55.30	57−100
isoleucine	159.52	207.72	129.53	183.88	259.20	159.60	164.08	187.15	137.22	119−238
leucine	303.91	363.37	227.30	331.62	460.80	252.17	291.80	359.35	213.50	171−346
tyrosine	147.49	170.93	128.02	170.91	228.24	151.62	150.98	160.63	137.22	114−236
phenylalanine	200.41	239.98	161.28	226.08	315.36	193.12	201.51	227.98	165.38	138−272
histidine	84.53	93.96	72.58	94.17	128.16	81.93	87.05	96.60	76.29	33−117
lysine	276.80	318.09	231.34	304.06	410.40	265.47	274.90	314.64	225.79	154−342
arginine	220.30	259.21	173.88	250.47	339.84	200.56	241.84	314.09	171.52	175−362
tryptophan	43.23	49.30	38.56	46.09	67.18	36.27	42.75	48.96	34.51	29−70

[a] Samples were collected from Island Falls, ME, and two sites in Canada (Hartland, NB; Summerside, PE). Plots were replicated four times at Hartland, NB. Plots were not replicated at the other two locations. Values presented represent the mean calculated from all six values. [b] For vitamins and minerals, reported by Storey and Davis (1978) and Lisinska and Leszczynski (1989); for amino acids, reported by Talley et al. (1984). Fresh weight concentration for literature range was determined by assuming that potatoes are composed of ~75% water. All values are reported as mg/200 g of FW.

for potato. For tubers derived from both NewLeaf Y Shepody clones, all of the amino acids were within the range reported in the literature and were comparable to those observed for the nonmodified Shepody control variety. With the exception of valine, methionine, and isoleucine, the levels of amino acids in NewLeaf Plus and NewLeaf Y Russet Burbank were within the literature range reported for the respective amino acid. In cases where levels were outside the published range, levels of the amino acid were comparable to levels observed for the conventional Russet Burbank variety. Environmental effects are known to influence amino acid concentrations in potato tubers (Lisinska and Leszczynski, 1989; Storey and Davis, 1978; Talley et al., 1984). Therefore, the concentration differences observed between the published values for valine, methionine, and isoleucine and the concentration of these amino acids generated in this study are likely attributable to interassay variation and environmental factors that contribute to the concentration of these amino acids in tubers.

DISCUSSION

Some rationale as to the reason for why these nutrients and antinutrients were selected is warranted. Components were selected after careful consideration of the role potato plays as a source of these nutrients in the human diet and on the basis of what potato breeders consider to be important for release of a new potato variety. Potato breeders traditionally place very little importance on nutritional constituents as a selection criteria or as a basis for release decisions. However, several important components are considered prior to release of a new variety. For instance, according to Love et al. (2000), a review of 257 release documents for North American potato varieties revealed that there were two important components that were consistently measured. One is the glycoalkaloids, natural toxicants with known mammalian toxicity. The second constituent is total tuber solids, which is composed of 80% carbohydrates. Total solids are measured as tuber dry matter or estimated as a measure of tuber specific gravity. Total solids is an important quality factor and the single most important determinant of culinary appeal in potatoes (Murphy et al., 1967). Dry matter content is documented in virtually all release documents published since 1958 and in many as early as 1935.

Only four other nutritional constituents have been published in the release documents of traditionally bred varieties, although a much longer list is required on consumer packaging. These are reducing sugars (glucose and fructose), sucrose, protein, and vitamin C. The sugar content of tubers contributes to the quality of chips and French fries produced from the tubers (Talburt and Smith, 1987). The accumulation of high concentrations of excessive reducing sugars produced from enzymatic hydrolysis of starch and sucrose during cold storage of tubers produces a nonenzymatic browning reaction upon frying, which detracts from the finished quality of chips or fries (Sowokinos, 1989). Potatoes are an excellent source of vitamin C, and fresh potatoes may contain as much as 30 mg/100 g when harvested fresh (Kadam et al., 1991). Therefore, measurement of vitamin C is important. Potatoes are also considered to be a good source of protein (Kadam et al., 1991). In addition to gross protein measurements, detailed analysis of the amino acid composition provided further insight into the nutritive value of the protein component of the tubers.

Table 8. Vitamin, Mineral, and Amino Acid Composition of NewLeaf Plus and NewLeaf Y Russet Burbank Potato Lines and Russet Burbank Potato Tubers[a]

component (mg/200 g of FW)	NewLeaf Plus RB clone												RB control			literature range[b]
	RBMT15-101			RBMT21-129			RBMT21-350			RBMT22-082						
		range			range			range			range			range		
	mean	max	min	mean	max	min	mean	max	min	mean	max	min	mean	max	min	
vitamin B_6	0.52	0.54	0.46	0.51	0.58	0.32	0.53	0.57	0.31	0.52	0.78	0.30	0.52	0.56	0.45	0.26−0.82
niacin	4.11	4.46	3.34	4.03	5.10	2.81	3.99	4.44	3.20	4.28	5.11	2.67	4.06	4.60	3.49	0.18−6.2
copper	0.30	0.42	0.11	0.39	0.61	0.14	0.30	0.50	0.14	0.33	0.64	0.11	0.32	0.50	0.14	0.03−1.4
magnesium	49.77	52.32	48.04	52.18	56.60	48.54	45.73	50.80	42.99	46.98	67.81	38.47	51.54	66.12	47.12	22.5−110
potassium	996.59	1151.94	826.50	1072.68	1185.60	955.86	1026.63	1120.24	966.72	1038.22	1512.64	931.20	1080.74	1202.70	979.20	700−1250
aspartic acid	1193.86	1346.40	1020.44	1279.99	1822.08	811.68	1095.46	1419.00	680.96	1296.17	1982.08	814.80	1250.12	1630.20	728.16	677−1476
threonine	138.04	148.41	125.19	146.72	192.82	115.88	134.10	161.70	106.40	152.41	211.25	114.65	147.19	172.71	118.99	102−214
serine	144.63	157.08	135.66	151.29	196.56	108.65	146.53	174.90	120.99	158.68	214.51	121.06	154.59	185.25	123.73	125−255
glutamic acid	750.61	826.20	644.10	770.79	1017.12	550.02	700.62	858.00	491.26	778.67	1114.92	587.82	792.52	1054.50	515.63	583−1207
proline	116.82	134.13	96.90	123.61	159.74	91.85	111.19	140.80	77.82	128.61	185.82	83.81	119.25	160.17	88.21	89−366
glycine	114.23	116.84	105.73	122.12	147.89	100.39	111.76	126.50	96.06	125.45	178.00	102.43	121.27	142.50	106.56	92−195
alanine	107.29	113.73	101.52	117.62	146.64	95.59	111.88	132.55	91.81	123.86	170.82	101.27	116.46	135.09	98.86	87−238
cystine	59.26	64.98	54.18	61.49	66.77	55.54	59.01	62.73	55.21	63.96	78.24	56.22	62.09	69.54	57.12	96−185
valine	208.37	224.77	192.78	216.62	248.20	170.88	198.91	245.02	162.34	237.42	374.25	191.48	217.70	284.43	175.23	196−363
methionine	54.30	57.99	49.93	58.53	66.14	47.79	52.40	62.24	42.44	62.43	99.76	48.25	56.36	83.79	41.03	57−100
isoleucine	130.99	141.36	118.83	139.86	162.24	108.94	123.39	150.67	100.32	147.69	229.50	116.98	139.43	177.84	116.62	119−238
leucine	206.96	219.81	183.05	224.38	286.42	173.02	200.40	240.35	155.65	229.31	323.39	168.78	220.30	262.77	176.42	171−346
tyrosine	121.01	133.87	99.96	127.50	161.62	96.65	128.54	150.18	110.66	149.34	208.64	121.64	143.54	177.84	116.62	114−236
phenylalanine	157.52	166.97	144.12	166.86	202.80	133.50	156.05	184.26	130.11	179.85	267.97	140.84	167.96	208.05	132.61	138−272
histidine	78.45	88.92	66.80	78.14	94.85	64.08	71.66	82.50	57.27	83.86	122.58	66.93	82.23	100.32	65.71	33−117
lysine	226.92	243.39	203.04	234.76	283.92	194.38	211.39	240.90	175.10	244.62	359.90	197.88	233.34	291.27	192.99	154−342
arginine	186.81	194.14	169.90	195.57	253.34	147.38	179.61	218.90	131.33	214.91	319.48	169.94	199.55	253.65	145.04	175−362
tryptophan	42.53	51.64	35.65	42.75	46.11	37.52	39.17	42.30	35.87	45.14	64.35	36.67	42.38	54.38	33.51	29−70

[a] Samples were collected from Island Falls, ME, and two sites in Canada (Hartland, NB; Summerside, PE). Plots were replicated four times at Hartland, NB. Plots were not replicated at the other two locations. Values presented represent the mean calculated from all six values. [b] For vitamins and minerals, reported by Storey and Davis (1978) and Lisinska and Leszczynski (1989); for amino acids, reported by Talley et al. (1984). Fresh weight concentration for literature range was determined by assuming that potatoes are composed of ~75% water. All values are reported as mg/200 g of fresh weight.

Although potatoes are rich in carbohydrates, vitamin C, sugars, and protein, potatoes also contribute a considerable amount of vitamin B_6, niacin, copper, magnesium, and potassium to the total recommended daily intake (RDI) for these compounds (National Academy Sciences, 1989). For instance, using the mean value of these nutrients found in the conventional Russet Burbank potato tuber (Table 8), a 200 g serving of potatoes would contribute approximately 26% of the vitamin B_6, 21% of the niacin, 15% of the copper, 15% of the magnesium, and 68% of the potassium needed for the total RDI for a healthy adult. Given the role potato plays as a source for these minor nutrients, it was felt to be prudent to include them in this assessment. In addition, proximates (macronutrients) are measured for nearly every modified crop and are considered to be important compositional indicators.

Hundreds of potato varieties are currently in commerce in the United States, Canada, and Europe. Current varieties are the products of traditional breeding, come from diverse backgrounds, and contain considerable heterogeneity. These varieties vary widely for any given trait, including concentration of nutritional constituents and natural toxicants. Validation of the safety of the traditional breeding process is manifested historically based on years of consumption and variety development.

The process of agronomic selection, coupled with a detailed knowledge of the biochemistry associated with each trait and measurement of key nutritional, quality, and antinutritional components, is critical in determining the overall risk for food derived from plants produced from the techniques of modern biotechnology. This process provides assurance to regulatory agencies and consumers of the relative safety of the products produced from this new technology.

The compositional assessment of tubers produced by NewLeaf Plus and NewLeaf Y potato plants took into consideration and addressed the potential for changes in the levels of nutrients and antinutrients that could occur as a result of (1) changes due to direct interaction of these components with the products of the inserted genes, (2) changes due to interaction by the products of the inserted genes with metabolic processes associated with uptake/transport of nutritional or antinutritional components, (3) chance insertion of genetic material into a gene responsible for production and/or uptake/transport of nutritional or antinutritional components, and (4) selection of clones due to the tissue culture process.

On the basis of the detailed knowledge of the proteins produced (Cry3A, PVY coat protein, PLRV ORF1/ORF2, NPTII, and CP4 EPSPS), it was concluded that there should be no direct interaction between these proteins and the key nutrients or antinutrients produced in tubers. The gene products produced by the *cry3A*, *nptII*, and *CP4-EPSPS* genes are very well characterized and, when produced in genetically modified plants, are not expected to interfere or participate in any way with the metabolic pathways responsible for production and/or uptake/biosynthesis/transport of nutritional or antinutritional components. Thorough safety assessments for the CP4-EPSPS, NPTII, and Cry3A proteins have been previously performed (Fuchs et al., 1993; Harrison et al., 1996; Lavrik et al., 1995). In cases where these gene products have been produced in plants, no unexpected effects have been noted (Berberich et al., 1996; Nida et al., 1996; Padgette et al., 1996a). Both PLRV and PVY are commonly a part of the human diet via consumption of virus-infected potatoes. The proteins produced by PVY and PLRV are not known to cause health effects in persons consuming virus-infected potatoes. The PVY and the ORF1/ORF2 proteins are expected to be produced at extremely low concentrations in leaf and tubers from these transgenic lines. Even though mRNA of the correct size is made from the inserted viral transgenes (data not shown), neither of these proteins has been detected in leaf tissues derived from NewLeaf Y or NewLeaf Plus plants using highly sensitive immunological techniques, thus decreasing even further any remote chance for interaction with nutritional/antinutritional components or disruption of metabolic pathways in cells that contain the gene.

The potential for disruption of an endogenous gene responsible for a pathway for a key nutrient or antinutrient due to the insertion of T-DNA is possible. However, disruptions would be expected to lead to measurable changes that would be detected by nutritional analyses or through phenotypic and agronomic changes observed during clone selection. This possibility is greatly reduced given that potato is a tetraploid with multiple copies of each gene (Hawkes, 1990; OECD, 1997). To disrupt a pathway, a transgene would have to insert precisely in all four copies of the same gene on different chromosomes, which is extremely improbable.

Prior to commercialization of any potato clone, intensive agronomic evaluations are conducted by expert potato agronomists. Agronomic trials are conducted over several years in different environments and under various agronomic conditions. Clones that are not agronomically comparable (comparable yield, tuber type, phenotype, growth characteristics, and disease susceptibility) to the conventional variety are not advanced for commercialization. It is this selection process that removes off-type clones produced as a result of T-DNA insertion or through the selection of clones during tissue culture.

In conclusion, on the basis of the guidance of internationally accepted scientific recommendations for the establishment of substantial equivalence, detailed compositional analyses of tubers derived from NewLeaf Plus and NewLeaf Y were performed. Key nutrients, antinutrients, macronutrients, and minor nutrients produced by tubers harvested from NewLeaf Y and NewLeaf Plus clones were consistent with those found in conventional potatoes. Use of NewLeaf Y and NewLeaf Plus potato clones by potato producers has the potential to markedly decrease reliance on chemical pesticides, thereby diminishing the environmental impact of agriculture while ensuring high-quality tuber production. These traits are and will continue to be an important factor in maintaining and increasing the efficiency of tuber production and the quality of potato tubers produced throughout the world.

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