Analysis of Potato Glycoalkaloids by a New ELISA Kit

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Steroidal glycoalkaloids are naturally occurring compounds present in solanaceous plants including potatoes. They are reported to be toxic to animals and humans. The recognition of their potential toxicity has led to implementation of guidelines limiting glycoalkaloid content. The effectiveness of these guidelines is dependent upon reliable analytical methods for their analysis. The objective of this study was to develop a simple, rapid, and inexpensive immunoassay for potato glycoalkaloids that correlates with HPLC. This was successfully demonstrated with various potato samples, including eight fresh potato varieties; potato flesh, peel, sprouts, and leaves; and processed products such as French fries, chips, and skins. Storage of the ELISA kit in a refrigerator for >3 months did not affect its effectiveness. The use of a stable, accurate, and highly sensitive ELISA kit should facilitate (a) development of standard protocols for handling and sampling of potatoes to minimize pre- and postharvest glycoalkaloid formation; (b) analysis of foliar glycoalkaloids, thus saving plant breeders considerable time, effort, and cost; (c) marketing potatoes at lower cost; (d) measurement of the metabolism and distribution of glycoalkaloids in animals and humans; and (e) assurance to the consumer of eating a good quality potato.

Keywords: *α-Chaconine; ELISA kit; HPLC; potatoes; potato chips; potato fries; potato leaves; potato peel; potato sprouts; α-solanine*

INTRODUCTION

Steroid glycoalkaloids occur naturally in potatoes and other plants and are reported to be toxic to bacteria, fungi, insects, animals, and humans (Friedman and McDonald, 1999). They therefore must have important functions both in the plant and in the diet. The recognition of their potential human toxicity has led to the development of guidelines limiting glycoalkaloid content. New, improved potato cultivars such as Lenape, with desirable compositional properties, cannot be released for commercial use because of possible high levels of glycoalkaloids. The biosynthesis and accumulation of glycoalkaloids continues long after harvest. Following harvest, the glycoalkaloid content can increase during the marketing cycle under the influence of light, cutting, slicing, sprouting, mechanical injury, and exposure to phytopathogens.

The effectiveness of the guidelines is dependent upon reliable analytical methods for glycoalkaloids. Numerous analytical methods used to measure the glycoalkaloid content of fresh and processed potatoes and parts of the potato plant have not always been reliable or reproducible, as discussed in detail elsewhere (Friedman and McDonald, 1997). The objectives of the current research were (a) to evaluate the usefulness of a new enzyme-linked immunosorbent assay (ELISA) kit based on a monoclonal antibody described by Stanker et al. (1994, 1996) to measure potato glycoalkaloids extracted from different matrices including potatoes, potato leaves, sprouts, and processed potato products; and (b) to compare the results from the ELISA for total glycoalkaloids to those obtained by HPLC analysis of the same extracts for the sum of α-chaconine and α-solanine. Our findings demonstrate the validity and usefulness of the ELISA.

EXPERIMENTAL PROCEDURES

Materials. The Potato Glycoalkaloid plate kit (catalog no. AP 002) was provided by EnviroLogix Inc., Portland, ME. The kit contains the following items: 8 strips of 12 antibody-coated wells each, in a plate frame; 1 vial of negative control; 1 vial of 0.15 ppm α-solanine standard (calibrator); 1 vial of 0.75 ppm α-solanine standard (calibrator); 1 vial of 2.5 ppm α-solanine standard (calibrator); 1 bottle of solanidine−enzyme (HRP) conjugate; 1 bottle of 10× concentrated assay diluent; 1 bottle of substrate; 1 bottle of stop solution. The negative control and the calibrators were prepared in aqueous 2% acetic acid. α-Chaconine and α-solanine were isolated from potato sprouts, and β_2- and γ-chaconines and solanidine were prepared by partial acid hydrolysis of α-chaconine and purified as described previously (Friedman et al., 1993); solamargine was a gift of Prof. Adelia Emilia de Almeida, and solasonine was obtained from Biosynth, A.G., Switzerland. All other compounds were purchased from Sigma, Inc. (St. Louis, MO).

HPLC Analysis of Glycoalkaloids. The concentrations of the two main potato glycoalkaloids, α-chaconine and α-solanine, were determined by HPLC as described previously (Friedman and Dao, 1992; Friedman and Levin, 1992; Friedman and McDonald, 1995). Briefly, a Beckman (Fullerton, CA) model 334 liquid chromatograph with a Dionex (Sunnyvale, CA) 4400 integrator and a Beckman 165 UV−vis variable wavelength detector was used. The column was 3.9 × 300 mm with Resolve C_{18} packing (Waters, Milford, MA). Eluent was 100 mM monobasic ammonium phosphate in 35% acetonitrile, adjusted to pH 3.5 with phosphoric acid. Flow rate was 1 mL/min, and detection was at 200 nm.

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10.1021/jf980646z CCC: $15.00
Published on Web 11/13/1998

Glycoalkaloid Analysis by ELISA Kit. The following procedures illustrate the approach used in the development of the kit with the aid of a 96-well microplate ELISA reader (Molecular Devices Corp., Sunnyvale, CA).

Sample Extraction. (a) Fresh Whole Potato. Each fresh potato sample (10 g) in 60 mL of 2% acetic acid was stirred in a Cuisinart food processor for 1 h. The resulting homogenized sample was centrifuged, filtered, made basic with NH_4OH, and partitioned twice with 20 mL of water-saturated butanol. The combined butanol layers were then evaporated to dryness, and the residue was redissolved in 5 mL of the solvent (consisting of 2 mL of methanol/2 mL of acetonitrile/1 mL of H_2O) that was used for analysis in both methods. Final solution was equivalent to 2 g of potatoes/mL. The values represent averages of two separate extractions for ELISA; for HPLC, each sample was injected three times, and all values were averaged.

(b) Freeze-Dried Potato Sprouts, Leaves, Peel, and Flesh. Freeze-dried *sprouts* (20 mg) were stirred in 40 mL of 2% acetic acid for 2 h. The resulting suspension was then filtered, made basic with NH_4OH, and partitioned twice with 20 mL of water-saturated butanol. The combined butanol layers were evaporated to dryness, and the residue was redissolved in a 5 mL solution consisting of 2 mL of methanol/2 mL of acetonitrile/1 mL of H_2O. An aliquot of this solution was used for analysis in both methods. The procedure for *leaves* was identical to that used for sprouts except that a 50 mg sample was used. The freeze-dried *peel* (100 mg) was stirred in 30 mL of 2% acetic acid for 1 h. The resulting homogenate was centrifuged, and the supernatant was passed through a Sep-Pak column before analysis, as described by Friedman and Levin (1992). The procedure for *potato flesh* was identical to that of peel except that 1 g of sample was used.

(c) Potato Fries, Chips, and Skins. The sample (10 g) was first defatted by stirring in 50 mL of hexane for 1 h. The fries and skins were suspended in 60 mL and chips in 200 mL of 2% acetic acid, respectively, then stirred for 1 h in the food processor, centrifuged, filtered, made basic with NH_4OH, and partitioned twice with 20 mL of water-saturated butanol. The combined butanol layers were evaporated to dryness; the residues from potato skin samples were redissolved in 1 mL of methanol, and those from the fries and chips were redissolved in 2 mL of methanol, respectively. Aliquots of the methanol solutions were used for analyses.

Performing the ELISA Assay. The contents of the 10× concentrated assay diluent bottle (30 mL) was poured into a flask and diluted with 270 mL of H_2O. The suspension was stirred at room temperature to mix thoroughly, and the resulting assay diluent was stored in a refrigerator.

The glass test tubes or vials were labeled for negative control (NC), 0.15 ppm calibrator (C1), 0.75 ppm calibrator (C2), 2.5 ppm calibrator (C3), and sample extract to be assayed. A 50 mL Combitip was attached to a Repeater pipet, and the dispense dial was set at 5. Assay diluent was then added to each of the labeled tubes. Next, 10 μL of negative control and 10 μL of each calibrator and sample extract were dispensed into the assay diluent in the appropriate tube and mixed thoroughly. These dilutions were assayed within 1 h of preparation.

Diluted negative control (NC, 100 μL), each diluted calibrator (C1−C3, 100 μL), and each diluted sample extract (S1−S8, 100 μL) were added to their respective wells, followed immediately by addition of the solanidine−enzyme conjugate (100 μL) to each well. The contents of the wells were thoroughly mixed by moving the strip holder in a rapid circular motion on the benchtop for 20−30 s. The wells were then covered with tape or Parafilm to prevent evaporation and incubated at ambient temperature for 1 h (if a plate shaker is available, it should be set at 200 rpm). The wells were drained and then flooded completely with cool tap water, shaken, and emptied. This wash step was repeated four times. The plate was then slapped on a paper towel to remove as much water as possible (an automated microtiter plate washer can be used for the wash steps). Substrate (100 μL) was added to each well, and contents were thoroughly mixed; the wells were covered with new tape and incubated for 30 min at room temperature. An orbital shaker can be used, if available. Stop solution (100 μL, 1.0 N HCl) was then added, and the contents were mixed thoroughly (the well contents turn yellow). The plate was read within 30 min of addition of stop solution.

Calculation of Results. The wavelength of the ELISA plate reader was set at 450 nm. Absorbances at 600, 630, or 650 nm can be used as the "reference" wavelength. The instrument was zeroed against water (200 μL) in a blank well. The optical density (OD) of the contents in each well was then recorded. Using a plate reader, a semilog curve fitted for the standard curve can be used. The results were also calculated manually, as outlined below.

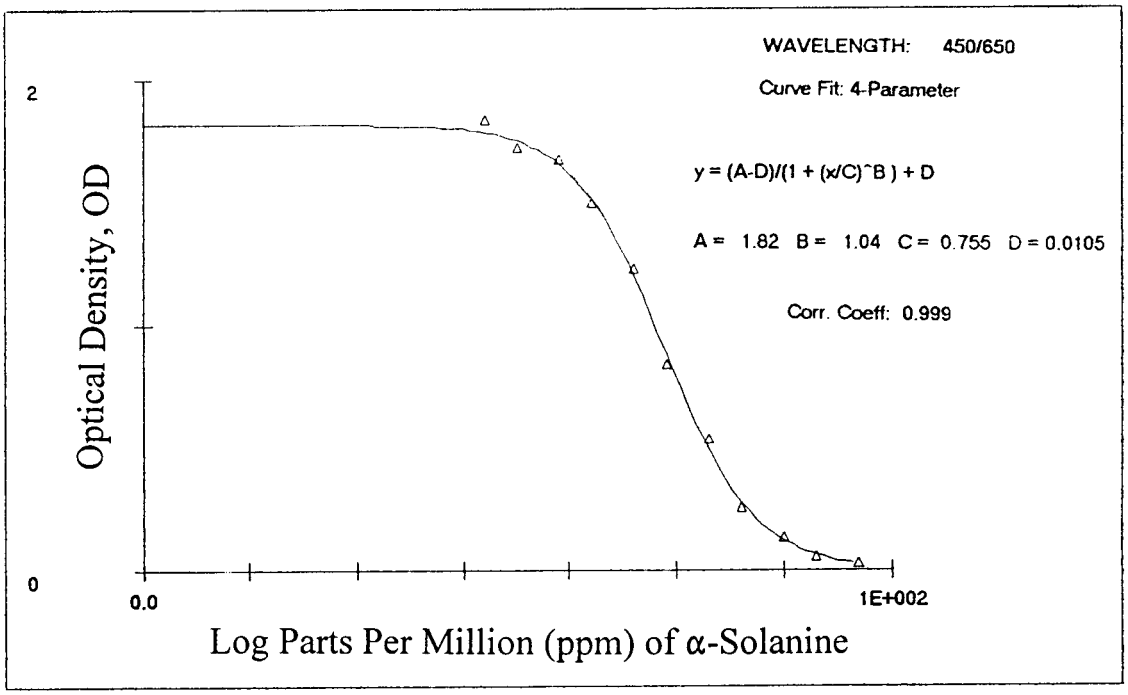
The OD values of each set of calibrators and samples were averaged and the percent bound (%Bo) values were calculated as follows: %Bo = average OD of calibrator or sample × 100/average OD of negative control; 100% Bo = negative control, defined as the maximum amount of glycoalkaloid−enzyme conjugate that is bound by the antibody in the absence of any glycoalkaloid.

The %Bo relationship of calibrators or samples to the negative control remains fairly constant in the following ranges: calibrator (ppm), %B (a) 0.15, 75−88; (b) 0.75, 40−53; (c) 2.5, 19−27. The coefficient of variation (CV) for each pair of calibrator and sample OD values did not exceed 15%. Note that interpolation of sample concentration is only possible if the %Bo of the sample falls within the range of %Bo of the calibrators. If the %Bo of the sample is *higher* than that of the *lowest* calibrator, the sample is reported as less than the lowest calibrator. If the %Bo of a sample is *lower* than that of the *highest* calibrator, the sample is reported as greater than the highest calibrator. In these cases, the assay is repeated with appropriate dilutions. The %Bo of each calibrator against its glycoalkaloid concentration was then automatically or manually plotted on a semilog scale and the glycoalkaloid concentration of each diluted sample extract was determined by finding its %Bo value and its corresponding concentration level on the graph. The result was multiplied by the dilution factor used during sample extraction.

RESULTS AND DISCUSSION

Immunochemical Assays. Analysis of food or other materials by immunoassays is based on the interaction between an antibody and a corresponding analyte followed by detection of that interaction using enzymes (Brandon et al., 1987; Henle, 1995). Generally, immunochemical assays are rapid and simple in design and do not require expensive instrumentation and the use of organic solvents. Previously, Stanker et al. (1994) developed a panel of monoclonal antibodies following murine immunization with a solanidine−bovine serum albumin (BSA) conjugate that binds the potato glycoalkaloids α-chaconine and α-solanine and their common aglycon, solanidine. Fifty percent inhibition of control reactions in a competition ELISA ranged as low as 30 ppb. Additional studies during the development of the ELISA kit based on one of these antibodies (Sol-129) resulted in further improvement of the assay in terms of long-term stability of the monoclonal antibodies and enhanced sensitivity of the assay, as described below. In related studies, other investigators (Ward et al., 1988; Sporns, 1996) raised antibodies using immunogens produced by conjugation of cleaved sugars associated with the glycoalkaloids. These conjugate strategies are more complex than that used in the present effort. Our findings showed that an immunogen prepared with a simple solanidine derivative produced antibodies capable of binding to certain potato glycoalkaloids.

Sensitivity and Reproducibility of the Assay by ELISA Kit. In this immunoassay, glycoalkaloids in the sample extract compete with horseradish peroxidase-



Std. Value	Well	OD	Mean	Std Dev	CV	Calc. Value
0 ppm	A1	1.845	1.791	0.076	4.264	<<<<<
	B1	1.737				0.041
0.016 ppm	A12	1.877	1.840	0.053	2.883	<<<<<
	B12	1.802				0.010
0.032 ppm	A6	1.766	1.731	0.049	2.859	0.027
	B6	1.696				0.062
0.08 ppm	A11	1.672	1.678	0.008	0.464	0.074
	B11	1.683				0.068
0.16 ppm	A5	1.515	1.500	0.022	1.462	0.163
	B5	1.484				0.182
0.4 ppm	A10	1.253	1.232	0.030	2.469	0.355
	B10	1.210				0.394
0.8 ppm	A4	0.869	0.846	0.033	3.845	0.835
	B4	0.823				0.921
2 ppm	A9	0.537	0.537	7.07e-4	0.132	1.785
	B9	0.536				1.790
4 ppm	A3	0.268	0.261	0.011	4.072	4.278
	B3	0.253				4.575
10 ppm	A8	0.145	0.140	0.008	5.576	8.617
	B8	0.134				9.416
20 ppm	A2	0.064	0.061	0.005	8.181	21.95
	B2	0.057				25.23
50 ppm	A7	0.035	0.034	0.002	6.332	47.37
	B7	0.032				53.82

Figure 1. Semilogarithmic plot of concentration of α-solanine versus OD (absorbance) in the ELISA (standard curve).

labeled solanidine for a limited number of antibody binding sites on the inside surface of the test wells. The outcome of the competition is visualized by color development, where sample concentration is inversely proportional to color; that is, darker color equals lower concentration and lighter color equals higher concentration. The assay is calibrated with α-solanine, as illustrated in Figure 1. Because the results correlated with the sum of α-chaconine and α-solanine (determined independently by HPLC, see below), which makes up >95% of glycoalkaloids in commercial potatoes, the results are interpreted as "total potato glycoalkaloids".

The kit contains α-solanine calibrators at 0.15, 0.75, and 2.5 ppm (or μg/mL). This is the range of detection of sample extracts. The limit of detection (LOD) of the kit is 0.07 ppm total glycoalkaloids after appropriate dilution. The LOD is determined by interpolation of 93.9% Bo from an α-solanine standard curve. This

Table 1. Precision of Analysis of α-Solanine by the ELISA Kit, Expressed as a Percent of CV of Recorded Dose (Dose) and OD for Repetitive Analyses of α-Solanine in a Single Assay (Intra-assays) and for Different Assays at Different Times (Inter-assays)

α-solanine (ppm)	dose CV (%)	OD CV
	Intra-assays ($n = 7$)	
0.25	6.8	1.5
1.25	3.3	1.9
	Inter-assays ($n = 7$)	
0.25	10.1	
1.25	6.3	

Table 2. LOD, Defined by the Amount of Test Compound−Enzyme Conjugate Bound by the Antibody (%Bo) to Potato Glycoalkaloids α-Solanine and α-Chaconine and Possible Cross-Reactants

	ppm in sample extract			cross-reactivity[b] (%)
test comp	LOD[a]	85% Bo	50% Bo	
α-solanine	0.07	0.15	0.77	100
α-chaconine	0.03	0.10	0.75	103
β_2-chaconine	0.015	0.05	0.36	214
γ-chaconine	0.03	0.07	0.37	208
solanidine	0.07	0.19	1.12	69
demissidine	0.08	0.27	1.9	41
α-tomatine	0.43	1.5	8.8	8.7
tomatidine	4.0	10	55	1.4
solasonine	10	38	220	0.35
solamargine	50	120	620	0.12
solasodine	100	470	≥2500	0.03

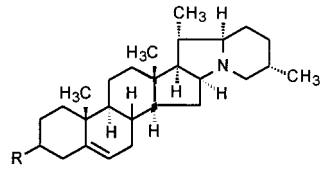
[a] 93.9% Bo (see text). [b] At 50% Bo concentration.

value was determined to be 3 standard deviations (SD) from the mean of $n = 11$ negative control samples. The 100% Bo equals the maximum amount of glycoalkaloid−enzyme conjugate that is bound by the antibody in the absence of any glycoalkaloid in the sample, that is, the negative control; %Bo = (OD of sample or calibrator/OD negative control) × 100. Because the extracts are extensively diluted before analysis, we estimate that the kit can detect 0.3 parts per billion (ppb) of α-chaconine and α-solanine, several orders of magnitude lower than the level detected by HPLC (Friedman and Levin, 1992).

To establish the precision of the assay, α-solanine-spiked control solutions were repetitively analyzed both within a single assay and in different assays on different days. Table 1 demonstrates excellent reproducibility.

Cross-Reactivity. Cross-reactivity occurs when antibodies bind to molecules having structures similar to those used as the immunogen. Generally, cross-reactivity should be determined with any compound with structural features similar to those of the analyte of interest. Initially, potential cross-reactants are assayed at a concentration 3 orders of magnitude higher than the highest calibrator. For those compounds showing inhibition greater than that of the negative control, serial dilutions are run and LOD and 50% Bo are determined.

Table 2 shows the 50 and 85% Bo (the level equivalent to the lowest kit calibrator) of α-solanine and α-chaconine; two hydrolysis products of α-chaconine (β_2- and γ-chaconines); the glycoalkaloids α-tomatine (from tomatoes); α-solasonine and solamargine (from many plants including eggplant); and the aglycons demissidine, solanidine, and tomatidine (Figure 2). The results indicate that the reactivity of the chaconine hydrolysis products with the antibody is more than twice that of the parent compound α-chaconine, and the cross-reactivity of the other compounds is low with the exceptions of demissidine and solanidine. Table 3 lists a typical data set used to calculate the glycoalkaloid concentration of a potato sample.



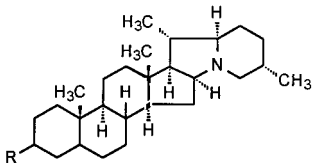
solanidine R = HO

α-chaconine R = rhamnose–rhamnose–glucose–

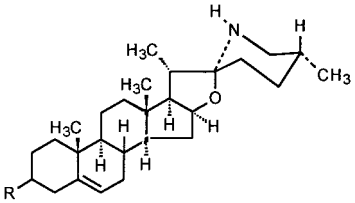
β_2-chaconine R = rhamnose–glucose–

γ-chaconine R = glucose–

α-solanine R = rhamnose–glucose–galactose–



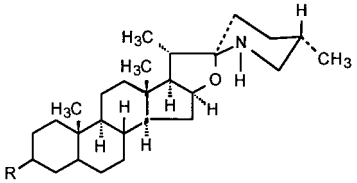
demissidine R = HO



solasodine R = HO

α-solamargine R = rhamnose–rhamnose–glucose–

α-solasonine R = rhamnose–glucose–galactose–



tomatidine R = HO

α-tomatine R = xylose–glucose–glucose–galactose–

Figure 2. Structures of compounds evaluated for cross-reactivity (see Table 2).

Applications. Table 4 compares the glycoalkaloid concentrations of eight commercially available potato varieties (obtained from a grocery store) determined with the same extract by both HPLC and the ELISA kit. The results show that (a) within experimental error, the glycoalkaloid values determined by both methods are consistent; (b) HPLC measures the two major glycoalkaloids α-chaconine and α-solanine, whereas the ELISA measures the total glycoalkaloid content, which may include small amounts of hydrolysis products

Table 3. Typical Data Used To Calculate Glycoalkaloid Content Determined with the ELISA Kit

well contents	OD (av ± SD)	CV %	% Bo	glycoalkaloid concn (ppm)
negative control	2.042 ± 0.017	0.8	100	
0.15 ppm α-solanine[a]	1.741 ± 0.034	1.9	85	
0.75 ppm α-solanine	1.024 ± 0.029	2.8	50	
2.5 ppm α-solanine	0.488 ± 0.017	3.5	24	
potato sample[b]	1.188 ± 0.023	2.0	58	0.52

[a] Used as calibrator (see text). [b] Potato peel sample with dilution of 1:300 during extraction followed by an additional 1:10 dilution in extraction solvent. The observed 0.52 ppm value is therefore multiplied by 3000 to obtain an actual value of 1560 ppm in freeze-dried peel.

Table 4. Glycoalkaloid Content of Potato Tubers (in Milligrams per Kilogram of Fresh Weight) Analyzed by HPLC (Sum of α-Chaconine and α-Solanine) and the ELISA Kit

	assay method	
potato variety	HPLC	ELISA kit
Russet, organic	5.8 ± 1.4[a]	5.1 ± 0.5
Russet	22 ± 3	24 ± 3
Yukon Gold	40 ± 3	38 ± 4
Small Purple	45 ± 1	37 ± 6
Small Red	101 ± 6	128 ± 7
Small Gold	105 ± 5	113 ± 19
Large White	125 ± 3	132 ± 11
Small White	203 ±16	209 ± 12

[a] The values represent averages from two extractions ± SD.

Table 5. Glycoalkaloid Content (in Milligrams per Kilogram of Freeze-Dried Sample) of Potato Peel, Leaves, and Sprouts Determined by HPLC and the ELISA Kit

	assay method	
potato plant part	HPLC	ELISA kit
flesh, peeled Red Lasoda	45.6 ± 1.7[a]	51.6 ± 4.4
peel, Shepody	1432 ± 118	1251 ± 100
sprouts, Shepody	7641 ± 56	6218 ± 542
leaves, NDA-1725[b]	9082 ± 105	8851 ± 470

[a] The values represent averages from two extractions ± SD. [b] From a high-glycoalkaloid variety (Friedman and Dao, 1992).

resulting from the cleavage of the glycosidic bonds of the carbohydrate side chains of the two glycoalkaloids (these may appear on HPLC chromatograms); and (c) the glycoalkaloid content of the eight varieties varied from ~5 mg/kg for the organic Russet potatoes to ~200 mg/kg of fresh weight for the Small White potatoes, a 40-fold variation. It is not apparent why the glycoalkaloid content of organically grown Russet potatoes is ~4 times lower than that of the same variety not grown organically.

Table 5 shows that, within experimental error, HPLC and ELISA also gave similar results for the glycoalkaloid concentrations in potato flesh, peel, sprouts, and leaves. This finding implies that the extracts of these tissues (used for analysis) do not contain any cross-reacting compounds which could adversely affect the ELISA.

The analysis of glycoalkaloids in potato leaves deserves special comment. Our observation that the ELISA kit can be used to measure the glycoalkaloid content of potato leaves after a simple extraction with dilute acetic acid should find immediate application in plant breeding studies in which an inexpensive, simple, field-portable kit could be used to analyze thousands of samples in multigeneration breeding programs (Dao and Friedman, 1996). The ELISA kit appears to overcome the interference of pigments with the analysis, a common occurrence with other methods.

Table 6. Comparison of Glycoalkaloid Content (in Milligrams per Kilogram of Original Material) for Processed Potato Products Determined by HPLC and the ELISA Kit

	assay method	
potato product	HPLC	ELISA kit
French fries, A	0	1.22 ± 0.14
French fries, B	24.1 ± 2.5[a]	22.7 ± 6.3
potato chips, low-fat	15.2 ± 0.1	14.5 ± 0
potato skins, A	43.3 ± 0.5	35.0 ± 6.1
potato skins, B	37.2 ± 1.9	41.0 ± 1.6

[a] The values represent averages from two extractions ± SD.

Table 6 lists the glycoalkaloid levels of French fries, potato chips, and potato skins. The results show good correlation between HPLC and ELISA. These obsevations demonstrate that the kit can be used to measure processed potato products after extraction of fats and oils with hexane.

It is worth noting that for French fry A, HPLC was not sufficiently sensitive to detect any glycoalkaloids, whereas the more sensitive ELISA was capable of measuring the low level (1.22 mg/kg) present. It is also striking that the glycoalkaloid content of French fry B, obtained from a different restaurant, was ~20 times greater than that of French fry A as determined by both methods. Although cooking and frying of potatoes leave the glycoalkaloid content largely unaffected (Bushway et al., 1983; Friedman and McDonald, 1997), the high value of French fry B can perhaps be explained by the observation of Chungcharoen (1988) that, during frying, the glycoalkaloids first leach out from the potato into the oil. If the oil is reused many times, it can become saturated with glycoalkaloids. Further addition of fresh batches of fries, sliced potatoes, or peel to the oil can then result in diffusion of the glycoalkaloids from the glycoalkaloid-saturated oil into the potato matrix. Thus, too frequent reuse of frying oils may lead to an increase in glycoalkaloid content of potato products.

The glycoalkaloid content of french fries is also influenced by the variety of the potatoes used in their preparation and by the presence of high-glycoalkaloid potato peel pieces as a result of imperfect peeling of the tubers (Sizer et al., 1980).

Benefits to Agriculture and Food Safety. Fast and low-cost methods for glycoalkaloid analysis will facilitate monitoring of glycoalkaloids in raw and processed potatoes and potato products. Using these data to minimize pre- and postharvest glycoalkaloid formation in potatoes will provide several benefits, including the following:

(1) With worldwide breeding programs and molecular biology techniques being used to constantly improve disease resistance, growing characteristics, and composition, methods are needed to ensure that new cultivars do not have higher than allowable levels of known glycoalkaloids or have not produced new significantly toxic ones. The described immunochemical method can provide quick screening of immature plants and cell cultures, thereby saving time and effort on the part of breeders and geneticists and thus providing information to ensure safety. Breeders will be able to rapidly test germplasm lines at early stages and discard any that contain high levels of glycoalkaloids.

(2) Adverse postharvest increases in glycoalkaloid content of potatoes can be detected with the aid of the kit. These increases are reported to occur during storage and shipping, under the influence of light and radiation, and as a result of mechanical injury and other stress conditions. Using the kit can thus ensure that the product that reaches the marketplace remains completely safe.

(3) The distribution and fate of glycoalkaloids and metabolites can be followed at nanomolar concentrations in body tissues and fluids of animals and humans after consumption, thus facilitating studies on the pharmacology and toxicology of glycoalkaloids.

ACKNOWLEDGMENT

We thank Prof. Adelia Emilia de Almeida of the Universidade Estadual Paulista, Brazil, for the solamargine and Gary M. McDonald for the potato glycoalkaloids used in the development of the kit. We also thank Carol E. Levin for advice with the HPLC analyses. Presented at the National Meeting of The Potato Association of America, Fargo, ND, July 28, 1998.

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Received for review June 15, 1998. Revised manuscript received October 2, 1998. Accepted October 6, 1998.

JF980646Z