Distribution of Glycoalkaloids in Potato Plants and Commercial Potato Products

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As part of a program to control the biosynthesis of *Solanum* glycoalkaloids in potatoes, we used a modified extraction–HPLC assay to measure the α-chaconine and α-solanine content of commercial and new potato varieties, different parts of the potato plant, and commercial potato products. The improved assay was accomplished by extracting, precipitating, and filtering the hot methanol extract through a 0.45-μm membrane before HPLC analysis. Recoveries of spiked samples ranged from 89 to 95%. The combined α-chaconine and α-solanine contents of different parts of the new NDA 1725 potato cultivar (in milligrams per 100 g of fresh weight) were as follows: tubers, 14.7; main stems, 32.0; small stems, 45.6; roots, 86; leaves, 145; and sprouts, 997. The α-chaconine content of several other potato cultivars ranged from 1.17 to 13.5 mg/100 g of fresh weight and the corresponding α-solanine content from 0.58 to 5.9 mg/100 g of fresh weight. The corresponding values for potato berries were 22.1 and 15.9 mg/100 g of fresh weight, respectively. The total glycoalkaloid content determined by titration with bromophenol blue was 12–30% greater than the sum of α-chaconine and α-solanine determined by HPLC. The extraction–HPLC method was adapted to measure the glycoalkaloids in freeze-dried french fries (0.08–0.84 mg/100 g of product), skins (3.1–20.3 mg/100 g of product), potato chips (2.4–10.9 mg/100 g of product), and potato pancake powders (4.5–6.5 mg/100 g of product). The presence of the two glycoalkaloids in commercial foods was also confirmed by thin-layer chromatography. The possible significance of these findings to food safety and plant physiology is discussed.

INTRODUCTION

Alkaloids are nitrogen-containing secondary plant metabolites found in numerous plant species. At least 20 structurally different alkaloids have been recognized in potatoes and tomatoes and about 300 in other Solanaceae species (Schreiber, 1979).

Glycoalkaloids are usually present at low levels in commercial potatoes. However, they can accumulate to high levels in greened, stored, damaged, and irradiated potatoes (Maga, 1980; Jadhav et al., 1981; Mondy and Seetharaman, 1990). Unresolved is the question of whether increases in glycoalkaloid biosynthesis always parallel light- and storage-induced greening (Kozukue and Mizuno, 1989, 1990; Maine et al., 1988; Swallow, 1991). The literature reports that 14–27% of the U.S. potato crop has been rejected due to greening (Morris and Lee, 1984). Identification and elimination of the toxic glycoalkaloids while retaining natural defenses against pests would allow modification of existing new cultivars with superior processing characteristics, currently commercially unavailable due to unacceptable alkaloid content (Norris, 1989; Stapleton et al., 1991).

The two major glycoalkaloids in commercial potatoes, α-chaconine and α-solanine, are both glycosylated derivatives of the aglycon solanidine. Methodologies for analysis of glycoalkaloids and hydrolysis products have been extensively studied (Bushway, 1982a–c; Bushway and Ponnampalam, 1981; Bushway et al., 1980a,b, 1981, 1983, 1985, 1986; Cadle et al., 1978; Carman et al., 1986; Coxon, 1984; Filadelfi and Zitnak, 1982, 1983; Fitzpatrick and Osman, 1974; Hellenas, 1986; Jones and Fenwick, 1981; Kobayashi et al., 1989; Morgan et al., 1985; Morris and Lee, 1981; Saito et al., 1990; Van Gelder et al., 1989; Zitnak and Johnson, 1970). No single method has gained widespread acceptance, but high-performance liquid chromatography (HPLC) is increasingly used to analyze both individual glycoalkaloids and hydrolysis products.

A useful analysis method consists of three major steps: extraction of the alkaloids with aqueous or nonaqueous solvents, removal of interfering substances (impurities), and analysis by HPLC or gas chromatography. The literature suggests that glycoalkaloids can be extracted by methanol (Kobayashi et al., 1989; Saito et al., 1990), methanol–chloroform (2:1 v/v) (Bushway and Ponnampalam, 1981), water containing 1-heptanesulfonic and acetic acids (Carman et al., 1986), 1% acetic acid (Filadelfi and Zitnak, 1982), and tetrahydrofuran (THF)–water–acetonitrile with 1% acetic acid (Bushway et al., 1980, 1981, 1983, 1986). The alkaloid extract is then precipitated under basic conditions or purified by using a Sep-Pak cartridge prior to HPLC analysis.

A disadvantage of using C_{18} Sep-Pak cartridges is that the cleanup of the extract is not always effective because of the extract's high lipid content. Instead, Sep-Pak NH_2 cartridges have been suggested as an alternative (Saito et al., 1990). We find that performance of the Sep-Pak cartridges can also vary from batch to batch (Crabbe and Fryer, 1980; unpublished results). Although previous workers report good separations of potato glycoalkaloids, we and Saito et al. (1990) encountered some problems when using THF in HPLC. For example, in our hands the presence of tetrahydrofuran in the mobile-phase mixture results in high baselines and in poor separation on HPLC chromatograms unless oxygen is rigorously excluded from the system. Bonded-phase columns (amino or carbohydrate) are easily contaminated and have a much shorter life span than the C_8 and C_{18} reversed-phase columns (Morris and Lee, 1981).

We adapted and modified reported procedures to determine the two major potato glycoalkaloids. In this paper, we evaluate the effectiveness of the modified

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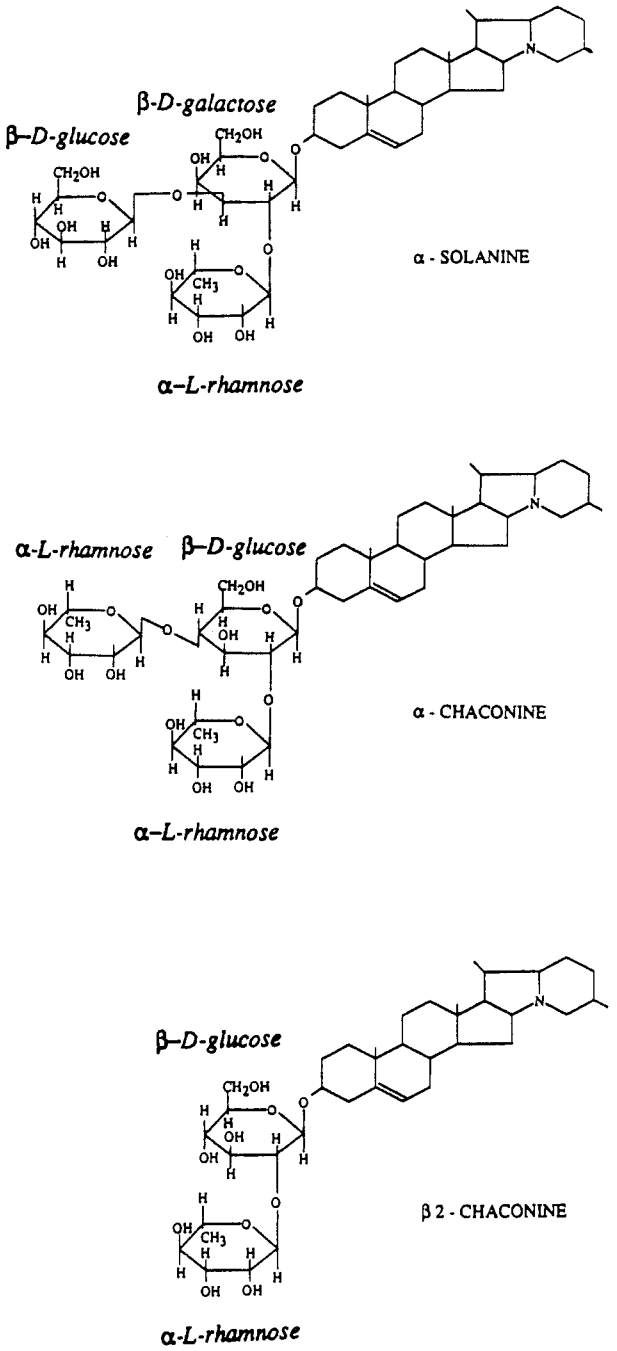

Figure 1. Structures of glycoalkaloids measured by HPLC and TLC.

method for analyzing the distribution of potato glycoalkaloids in various parts of high-glycoalkaloid-containing potatoes such as Lenape and one of its progenies, NDA 1725. For comparison, we also measured the glycoalkaloid content of commercial potatoes and processed potato products.

MATERIALS AND METHODS

Materials. Potato berries were obtained from the Potato Breeding Program, University of Idaho, Aberdeen, ID (D. Corsini). Red, white, and Russet potatoes were obtained from a local store and new varieties from Aberdeen, ID, and the Simplot Co., Caldwell, ID (R. Henderson). Potato sprouts were harvested after long-term (3–5 months) storage of potatoes.

The NDA 1725 potato plants were harvested in a greenhouse 2.5 months after planting. The plants were then cut up into tubers, roots, main stems, small stems, and leaves. The roots and tubers were cleaned with water and dried with a paper towel. Potato chips (skinless), french-fried potatoes (skinless), potato

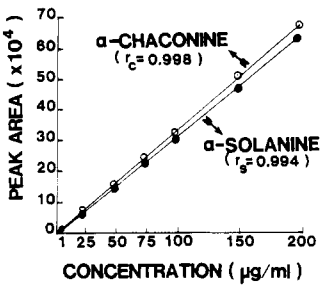

Figure 2. α-Chaconine and α-solanine peak area response by HPLC.

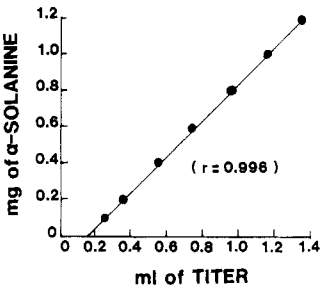

Figure 3. Titration of α-solanine by bromophenol blue.

pancake powders, frozen twice-cooked potatoes, and frozen potato wedges were obtained from local stores. Baked potato skins were purchased in local restaurants.

The flesh and toppings of potato skins were removed to leave skins with a final thickness of about 2 mm. Double-baked potatoes were treated in a microwave oven according to the instructions of the manufacturer prior to removal of the topping. All potato samples except chips were cut up into small cubes, placed in a freeze-drying jar, and quickly frozen with liquid nitrogen. The frozen samples were then lyophilized. The dried samples were then ground in a Wiley mill, passed through a 40-mesh screen, and stored in a refrigerator at 4 °C.

A glycoalkaloid stock solution (1 mg/mL) was prepared by dissolving α-solanine and α-chaconine (Sigma Chemical Co., St. Louis, MO) in methanol. Working standard solutions were prepared by dilution with methanol. All solvents were of HPLC grade.

Extraction Method. The extraction technique was adapted from Bushway et al. (1980) with some modifications. Briefly, 0.5–10 g of freeze-dried sample powder was stirred with 40–50 mL of tetrahydrofuran–water–acetonitrile (5:3:2 v/v) in a Sorvall Omni mixer for 15 min. The extracts were centrifuged, and the supernatant was collected and concentrated to approximately 10–15 mL by evaporation on a air vacuum rotary evaporator. To the concentrate was then added 10–15 mL of 0.2 N HCl with stirring. Next, the suspension was centrifuged and the pH was adjusted to 10–11 with concentrated ammonium hydroxide before the sample was placed into a water bath at 70 °C for 30 min. The solution was then refrigerated overnight or cooled in an ice–water bath for 1 h prior to centrifugation. The precipitate was washed with 5 mL of 1% ammonium hydroxide and centrifuged, and the pellet was collected. The air-dried pellet was then dissolved in 20 mL of methanol and boiled for 10 min. The hot suspension was filtered through a 0.45-μm filter membrane. The filtrate was evaporated to a final volume of 1 mL. To this solution was then added 5.5 mL of acetonitrile and 3.5 mL of H_2O. This mixture was used for HPLC.

For potato chips and french-fried potatoes, 25-g samples were stirred with 70 mL of tetrahydrofuran–water–acetonitrile. The extracts were filtered through a No. 4 filter paper, and the filtrate was then transferred to a separatory funnel. The lower layer was collected after 20 min and the extraction continued as described.

Spiking Experiments. A series of spiking experiments were carried out to establish the extent of recovery of added glycoalkaloids from potatoes. Specifically, to 10 g of freeze-dried Russet potato powder were added in separate experiments 2, 5, 10, and 15 mg each of α-solanine or α-chaconine. The samples were thoroughly mixed, extracted, and analyzed by HPLC for recovery of added glycoalkaloids.

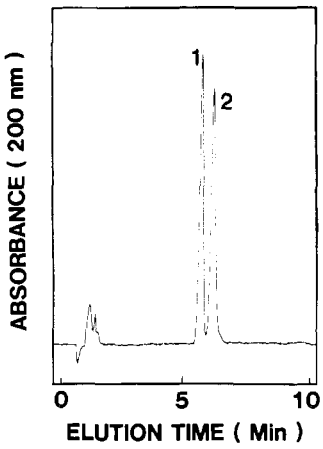

Figure 4. HPLC chromatogram of a mixture of α-solanine and α-chaconine standard. The mixture contained 100 μg/mL of each glycoalkaloid. Peaks: 1, α-solanine; 2, α-chaconine.

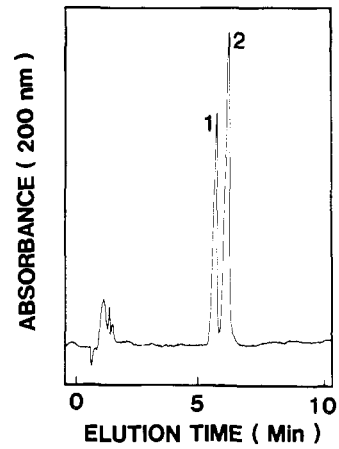

Figure 5. Typical HPLC chromatogram of α-solanine and α-chaconine in a potato extract. Peaks: 1, α-solanine; 2, α-chaconine.

HPLC Procedure. A Beckman 334 HPLC system with a 427 integrator and a 165 UV–vis variable-wavelength detector was used (Friedman and Levin, 1989). The column was C_{18} Perkin-Elmer coupled cartridges. The mobile phase consisted of 50% acetonitrile containing 5 mM sodium lauryl sulfate and 5 mM sodium sulfate decahydrate. The pH was adjusted to 4.5 with 1% sulfuric acid. The solvent flow rate was 1 mL/min, and the UV absorbance was monitored at 200 nm.

Total Glycoalkaloid Content. The total glycoalkaloid content (TGA) of potato berries and three varieties of potatoes was also determined according to the bromophenol blue colorimetric dye binding procedure (Fitzpatrick and Osman, 1974). Briefly, the above-described methanol extract was evaporated to dryness with a stream of nitrogen and the residue redissolved in 5 mL of absolute methanol and titrated with 0.067% bromophenol blue and 10% phenol in absolute methanol. The TGA content was determined as solanine equivalent (milligrams per 100 g of fresh weight) by reference to a standard curve for α-solanine.

TLC Procedure. Preparative thin-layer chromatography (TLC) was performed on silica gel precoated plates, 0.25 mm × 20 cm × 20 cm (Merck, Darmstadt, Germany). A 25-μL methanol extract was spotted on the plate along with α-chaconine and α-solanine standards. The plate was then developed with a bottom layer of methanol–chloroform–1% ammonium hydroxide (2:2:1 v/v). Next, the dried plate was sprayed with 1% 8-anilinonaphthalene-1-sulfate (ANS) and visualized in a UV chamber.

RESULTS AND DISCUSSION

Figure 1 depicts the structures of the glycoalkaloids measured by HPLC. Figure 2 shows linear relationships in the range 1–200 μg/mL between concentrations of α-chaconine (r = 0.998) and α-solanine (r = 0.994) and the respective peaks areas on HPLC chromatograms. Figure 3 is a linear plot of the titration of α-solanine in the range 0.1–1.2 mg by bromophenol blue. The correlation coefficient was r = 0.996. Figures 4 and 5 illustrate the separation of these two glycoalkaloids on the HPLC columns both in pure form and in a potato extract. These data suggest that minimal error is associated with the HPLC part of the analytical procedure.

Twenty-five microliters of the methanol extracts was also spotted on a TLC plate along with α-chaconine and α-solanine standards. A total of 11 different potato extracts were analyzed this way. After development, all plates, except the one with potato root extract, showed only two spots corresponding to α-solanine (R_f = 0.22) and α-chaconine (R_f = 0.28). The potato root sample had a third spot on the TLC plate (R_f = 0.45) and a peak with retention time of 8.03 min on the HPLC column. An authentic sample prepared according to the method of Filadelfi and Zitnak (1983) was used to confirm that this compound is β_2-chaconine.

Table I. Recoveries of α-Chaconine and α-Solanine Added to Freeze-Dried Red Potato Powder[a]

	recovery,[b] %	
glycoalkaloid added, mg	α-chaconine	α-solanine
2	89.4 ± 1.0	90.1 ± 3.9
5	88.8 ± 2.0	91.7 ± 5.0
10	89.1 ± 0.4	92.1 ± 0.3
15	91.6 ± 0.6	95.4 ± 1.2
av ± SD	89.7 ± 1.3	92.3 ± 2.2

[a] To 10 g of powder was added the indicated amount of glycoalkaloid. The samples were mixed, extracted, and analyzed by HPLC. [b] Average from two separate determinations ± standard deviation.

Table II. α-Chaconine and α-Solanine of NDA 1725 Cultivar (n = 3)

	α-chaconine, mg/100 g of fresh wt	α-solanine, mg/100 g of fresh wt	ratio of α-chaconine to α-solanine
tubers	9.27 ± 0.08	5.35 ± 0.09	1.64
main stems	19.61 ± 0.17	12.40 ± 0.16	1.58
small stems	26.71 ± 1.65	18.91 ± 0.84	1.41
roots	44.86 ± 2.60	41.19 ± 2.75	1.09
leaves	84.57 ± 4.65	60.51 ± 2.75	1.40
sprouts	520.18 ± 12.85	476.88 ± 12.69	1.09

Spiking experiments (Table I) revealed that the modified method affords an 89–95% recovery of added glycoalkaloids. In the range 2–15 mg of glycoalkaloid/10 g of freeze-dried potato powder, the extent of recovery increased with the amount added before extraction.

Recovery of added and native glycoalkaloids was further increased by 3–6% by not washing the precipitate with 1% ammonium hydroxide. Washing is used to remove mostly brown pigments in the final extraction product when potato peels or sprouts are used as starting materials. The brown pigment does not seem to interfere in the analysis except for producing a dark product.

To minimize possible interference of chlorophyll present in leaves and stems, the green precipitate formed during evaporation of the extracts was first removed by centrifugation and filtration. The partially evaporated clear filtrate was then analyzed for glycoalkaloid content.

To overcome losses during precipitation, the hot methanol solution was filtered through a 0.45-μm membrane after refluxing. This filtrate was injected directly into the HPLC system.

The modified method was then applied to a series of freeze-dried potato products including established commercial and potential new varieties, potato skins and flesh, potato berries, and potato sprouts. Tables II and III show

Table III. α-Chaconine, α-Solanine, and Total Glycoalkaloid (TGA) Content of Potato Products

material	α-chaconine,[c] mg/100 g of fresh wt	α-solanine,[c] mg/100 g of fresh wt	ratio of α-chaconine to α-solanine	TGA,[d] mg/100 g of fresh wt
potato (no. 3194) sprouts	150.4 ± 6.0	123.4 ± 5.6	1.22	
potato berries	22.1 ± 1.43	15.9 ± 0.80	1.39	44.6 ± 2.71
potato (Lenape)	13.5 ± 0.38	5.91 ± 0.13	2.28	22.6 ± 2.65
potato peel (Lenape)	62.3 ± 3.61	23.0 ± 1.81	2.70	
potato flesh (Lenape)	8.02 ± 0.20	3.95 ± 0.03	2.03	
potato (no. 3194)[a]	3.68 ± 0.43	1.95 ± 0.50	2.45	6.38 ± 0.71
potatoes (Simplot I)[a]	3.85 ± 0.43	1.72 ± 0.18	2.24	
potatoes (Simplot II)[a]	2.75 ± 0.16	1.07 ± 0.02	2.57	
red potatoes[b]	2.52 ± 0.21	1.09 ± 0.07	2.31	
white potatoes[b]	1.17 ± 0.12	0.58 ± 0.03	2.02	
Idaho Russet potatoes[b]	1.34 ± 0.08	0.65 ± 0.02	2.06	2.86 ± 0.54
Washington Russet potatoes[b]	1.30 ± 0.01	0.58 ± 0.01	2.24	

[a] New genetic varieties. [b] Commercial varieties. [c] Determined by HPLC. Average ± standard deviation. Triplicate determinations for all samples except red, white, and Washington Russet potatoes ($n = 2$). [d] Determined by a bromophenol blue colorimetric procedure.

the distribution of the glycoalkaloids in potato plants, the commercial potatoes, and new potato varieties. The total glycoalkaloid content (in milligrams per 100 g of fresh weight) ranged from 2 for tubers to 997 for sprouts. In addition to sprouts, roots, stems, leaves, and potato berries have high levels of glycoalkaloids.

Table III also shows that the total glycoalkaloid content of potato berries measured according to the bromophenol blue method was about 15% greater than the sum of α-chaconine and α-solanine measured by HPLC. For potato varieties, the difference was 12% for Lenape, 15% for No. 3194, and 30% for Idaho Russet potatoes. These differences may result from (a) the presence of interfering compounds that may react with dye and (b) the large standard deviations in the values obtained with the colorimetric procedure.

Historically, the potato cultivar Lenape and its progeny are instructive examples of some of the problems glycoalkaloid biosynthesis can introduce into potato breeding programs. This cultivar was shown to have a high solid content and a low level of reducing sugars, which resulted in excellent chipping and storage properties (Akeley et al., 1968). However, because of its high glycoalkaloid content (Table III), Lenape had to be withdrawn from the market (Zitnak and Johnston, 1970). This cultivar remains such a superior chipper that it is apparently still widely used in potato breeding programs. In fact, Lenape is a progenitor of the NDA 1725 cultivar.

Since french fries and potato chips contain high concentrations of lipids, we evaluated different ways to separate the fats from the extracts before analysis. The oils can be readily removed by placing the tetrahydrofuran–water–acetonitrile extract into a separatory funnel and, after about 20 min, separating the top oily layer formed. Additional studies showed that the oily layer contained only trace amounts of glycoalkaloids.

Table IV lists the alkaloid content of several commercial potato products in milligrams per 100 g. Although the french-fried potatoes contained low levels of glycoalkaloids, this was not the case for potato chips and skins. For chips (not freeze-dried), the total glycoalkaloid content measured as the sum of α-chaconine and α-solanine ranged from 2.5 to 11 mg/100 g for the three samples tested. The values for potato skins ranged from 3.1 to 20.3 mg/100 g for the five products tested and for commercial potato pancake mixes from 4.5 to 6.5 mg/100 g for the three products tested. Freeze-dried potato wedges contained 4.4 mg/100 g. Whether the difference in glycoalkaloid content among the various products is due to the use of different potato varieties in the preparation or to changes in alkaloid content during processing is not known.

Table IV. α-Chaconine and α-Solanine of Processed Potato Products ($n = 3$)

	α-chaconine, mg/100 g of product	α-solanine, mg/100 g of product	ratio of α-chaconine to α-solanine
commercial french fries, I[a]	0.04	0.04	1.00
commercial french fries, II[a]	0.42 ± 0.02	0.42 ± 0.02	1.00
commercial wedges[a]	2.39 ± 0.43	2.01 ± 0.29	1.18
skins from twice-baked potatoes[b]	2.04 ± 0.12	1.08 ± 0.04	1.89
skins, I[b]	3.89 ± 0.13	1.74 ± 0.09	2.23
skins, II[b]	4.40 ± 0.47	2.36 ± 0.23	1.86
skins, III[b]	11.61 ± 0.56	7.23 ± 0.18	1.60
skins, IV[b]	11.95 ± 0.57	8.35 ± 0.38	1.43
pancake powder, I[b]	2.05 ± 0.37	2.41 ± 0.32	0.82
pancake powder, II[b]	2.48 ± 0.18	1.94 ± 0.11	1.27
mushed flakes, III[b]	3.17 ± 0.20	3.29 ± 0.17	0.96
commercial chips, I[b]	1.30 ± 0.27	1.05 ± 0.15	1.23
commercial chips, II[b]	3.16 ± 0.30	1.76 ± 0.04	1.79
commercial chips, III[b]	5.88 ± 0.37	5.02 ± 0.44	1.17

[a] Values are for freeze-dried powders. [b] Values are for original products.

CONCLUSIONS

The modified extraction–HPLC procedure described can accurately measure α-chaconine and α-solanine in different parts of the potato plant, potato berries, and potato sprouts. The concentration of these glycoalkaloids varied by a factor of about 500. The ratio of α-chaconine to α-solanine varied widely, ranging from 1.2 to 2.7. In contrast, previous studies (Maga, 1980) report this ratio to be near 1.5. These findings may have implications for food safety and plant breeding since α-chaconine is more embryotoxic and has a greater ability to induce liver enzymes than α-solanine (Caldwell et al., 1991; Friedman, 1991; Friedman et al., 1991, 1992). It may therefore be preferable to use potato varieties with a low α-chaconine to α-solanine ratio to enhance food safety.

The ratios of the two major glycoalkaloids in commercial potato products, especially potato chips and skins, also varied widely, ranging from 1.0 to 2.2. Possible causes for this variation need to be ascertained.

The total glycoalkaloid content of potatoes and potato berries determined by colorimetric procedure is greater than the sum of α-chaconine and α-solanine determined by HPLC. Possible causes for this difference are not known. The more information we have on the specific and total alkaloid content, the more effective we will be in devising new ways to prevent the accumulation of the more toxic ones in the potato. The described distribution of α-chaconine and α-solanine in commercial and potential new potato varieties and in commercial potato products currently being consumed should facilitate development of new varieties and products with both low total glycoalkaloid content and low α-chaconine to α-solanine ratios.

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