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Glycoalkaloid and Calystegine Contents of Eight Potato Cultivars

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Diverse procedures have been reported for the separation and analysis by HPLC of the two major glycoalkaloids present in potatoes, α-chaconine and α-solanine. To further improve the usefulness of the HPLC method, studies were carried out on the influence of several salient parameters on the analysis of the two potato glycoalkaloids. Effects on retention (elution, separation) times of the (a) composition and pH of the mobile phase (acetonitrile and phosphate buffer), (b) concentration of the phosphate buffer, (c) capacity values of column packing of four commercial HPLC amino columns, (d) column temperature were studied. Except for pH, all of the variables significantly influenced the retention times. The results make it possible to select analysis conditions that produce well-separated as well as symmetrical peaks of the two glycoalkaloids. This improved HPLC method (limit of detection of ∼150 ng) was evaluated with extracts from the cortex of one whole potato variety (May Queen) grown in Japan and the freeze-dried peel and flesh from the following eight cultivars grown in the United States: Atlantic, Dark Red Norland, Ranger Russet, Red Lasoda, Russet Burbank, Russet Norkota, Shepody, and Snowden. In addition, the same samples were analyzed by GC-MS for the presence of two water-soluble nortropane alkaloids, calystegine A_3 and calystegine B_2, reported to be potent glycosidase inhibitors. The following ranges for the eight varieties of total glycoalkaloid and calystegine levels were observed: dry flesh, 5−592 and 6−316 mg/kg; dry peel, 84−2226 and 218−2581 mg/kg; dry whole potatoes, 40−883 and 34−326 mg/kg; wet flesh, 1−148 and 1−68 mg/kg; wet peel, 12−429 and 35−467 mg/kg; wet whole potatoes, 7−187 and 5−68 mg/kg. The possible significance of the results to plant and food sciences is discussed.

KEYWORDS: Potatoes; potato peel; potato flesh; *Solanum tuberosum*; glycoalkaloids; α-chaconine; α-solanine; HPLC; calystegine A_3; calystegine B_2; GC-MS

INTRODUCTION

Steroidal glycoalkaloids are naturally occurring, secondary plant metabolites that are found in a number of foods including potatoes, tomatoes, and eggplants, reviewed in refs *1*−*4*. Although they are reported to be potentially toxic, glycoalkaloids and hydrolysis products without the carbohydrate side chain (aglycons) also have beneficial effects. These include lowering of cholesterol (*5*, *6*), protection against infection by *Salmonella typhimurium* (*7*) as well as against cancer (*8*, *9*), and potentiation of general anesthetics that act by inhibiting cholinesterase (*10*) and of a malaria vaccine (*11*).

In commercial potatoes (*Solanum tuberosum*) there are two major glycoalkaloids, α-chaconine and α-solanine, both trisaccharides of the common aglycon solanidine. Solanidine, but not the parent glycolkaloids, exhibited estrogenic activity in an in vitro assay (*12*). Although glycoalkaloids appear to be largely unaffected by food-processing conditions such as baking, cooking, and frying, the content of the glycoalkaloids can vary greatly in different potato cultivars and is enhanced postharvest by environmental factors such as light, mechanical injury, and storage.

The toxicity of glycoalkaloids at appropriate high levels may be due to adverse effects such as anticholinesterase activity on the central nervous system and to disruption of cell membranes adversely affecting the digestive system and general body metabolism. Folic acid, glucose-6-phosphate, and nicotine adenine dinucleotide (NADP) are reported to protect frog embryos against α-chaconine-induced developmental toxicity (*13*, *14*). Other complicating factors regarding the glycoalkaloid content of the diet that must be taken into account are that (a) α-chaconine appears to be more biologically active by a factor of ∼3−10 than is α-solanine and (b) certain combinations of the two glycoalkaloids can act synergistically (*15*, *16*). These considerations have led to the establishment of informal guidelines limiting the total glycoalkaloid concentration of new potato cultivars to 200 mg/kg of fresh weight of potatoes.

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The complex nature of glycoalkaloid−dietary relationships suggests the need for accurate methods to measure the content of individual glycoalkaloids and their metabolites in fresh and processed potatoes as well as in body fluids such as plasma and tissues such as liver. HPLC methods are now widely used to determine the concentrations of individual glycoalkaloids of fresh and processed potatoes and different parts of the potato plant such as leaves and sprouts, as well as glycoalkaloid hydrolysis (glycolysis) products (*17*−*43*). However, the peaks on HPLC chromatograms are not always well separated and frequently also do not exhibit good symmetry or minima (tailing bands at the baseline). To further improve and optimize the HPLC method, we systematically evaluated several parameters expected to influence the chromatographic separation of α-chaconine and α-solanine in pure form and in extracts of potatoes. These parameters were the composition and pH of the mobile phase, the capacity of the column packing, and column temperature.

The water soluble nortropane alkaloids named calystegines were first discovered in 1988 from transformed root cultures of the nonfood plant *Calystegia sepium* (*44*), and their structures were elucidated in 1990 (*45*). Since then, they have been found in a number of other plant families including the Solanaceae, specifically in *Solanum melongena* (eggplant) and *Solanum tuberosum* (potato), reported to contain calystegine A_3 and calystegine B_2 (*46*−*50*). At least eight calystegines are currently known, and many exhibit potent specific inhibition of glycosidases that are universally required for normal cell function. Although no human toxicity data for calystegines have been reported, polyhydroxylated alkaloids are reported to have therapeutic effects in the treatment of cancer, diabetes, and bacterial and viral infections and to stimulate the immune system (*51*, *52*).

Calystegine structures and bioactivities resemble those of the polyhydroxylated indolizidine alkaloid swainsonine, a glycosidase inhibitor found in locoweeds and shown to be responsible for causing the irreversible brain lesions in cattle known as locoism (*53*, *54*). Biosynthetically, calystegines appear to be derived from the tropane alkaloids atropine and scopolamine also present in some Solanaceae plants such as *Datura stramonium* (jimsonweed) (*55*−*57*).

As mentioned above, one objective of this study was to devise an improved HPLC method to measure the two potato glycoalkaloids in freeze-dried dark potato peel and white potato flesh samples. A second objective was to assess variations in the levels of both glycoalkaloids and calystegines in eight cultivars in order to examine possible compositional relationships between the two classes of secondary metabolites present in potatoes. We therefore also determined the contents of calystegines A_3 and B_2 of the same potato flesh and peel samples by GC-MS.

MATERIALS AND METHODS

Materials. May Queen potatoes were obtained from a local market in Himji City, Japan. The other eight potato cultivars used to prepare freeze-dried flesh and peel were provided by Dr. J. J. Pavek (USDA, ARS, Potato Breeding Program, Aberdeen, ID). α-Chaconine and α-solanine were obtained from Sigma (St. Louis, MO). Authentic calystegine samples were kindly provided by Professor Naoki Asano (Hokuriku University, Kanazawa, Japan). HPLC grade acetonitrile, methanol, tetrahydrofuran, analytical grade potassium dihydrogen phosphate (KH_2PO_4), sodium dihydrogen phosphate (NaH_2PO_4), and ammonium hydroxide (NH_4OH) were obtained from commercial sources. The solvents were filtered through a 0.45 μM membrane filter (Millipore, Bedford, MA) and degassed with an ultrasonic bath before use.

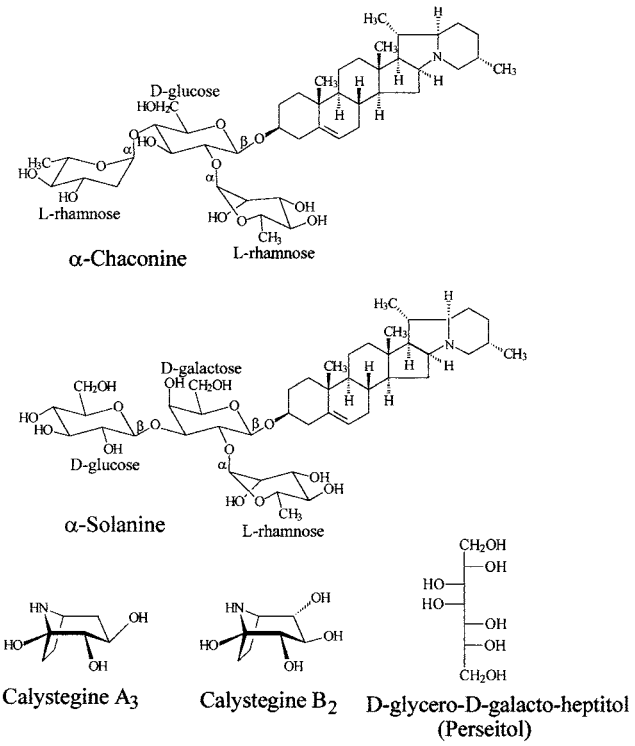

Figure 1. Structures of potato glycoalkaloids α-chaconine and α-solanine, of calystegine A_3 and calystegine B_2, and of the internal standard perseitol.

Preparation of Freeze-Dried Potatoes. Samples of eight potato varieties were received and stored at 4 °C in the dark. The potatoes were peeled to a depth of ∼1 mm with a vegetable peeler. Fresh peel weights amounted to 7−11% of the total weight of the fresh potatoes. The flesh was cut into slices 4 mm thick and immediately immersed in liquid nitrogen, as were the peels; then both were freeze-dried. Dry weights of the flesh and peels were 20−25 and 15−18% of the fresh weights, respectively. The dried samples were ground briefly in a blender and then in a Wiley mill to pass a 20-mesh screen.

Extraction of Glycoalkaloids from Fresh May Queen Potatoes for HPLC. The cortex layer (∼5 mm of peripheral tissue) from three uniform-size tubers was peeled and chopped with a knife. In the procedure for extraction of glycoalkaloids, adapted from a previously described method (*20*), the cortex preparation (20 g) was weighed and blended in a homogenizer with a mixture of chloroform/methanol (2:1, v/v) and then concentrated to 2−3 mL with the aid of a rotary evaporator. The concentrate was then dissolved in 40 mL of 0.2 N HCl, and the glycoalkaloids were precipitated with concentrated NH_4-OH. The ammonia was dissipated and, after centrifugation, the pellet was dissolved in 2 mL of a mixture of tetrahyhdrofuran/acetonitrile/20 mM KH_2PO_4 (50:30:20, v/v). The suspension was centrifuged, and the supernatant (20 μL) was used for HPLC analysis.

Extraction of Glycoalkaloids from Freeze-Dried Potatoes for HPLC. The freeze-dried potato powders (17 mg−1 g, depending on availability) were extracted with 40 mL of 5% acetic acid accompanied by ultrasonication for 10 min at room temperature. After filtration through a 3G3-glass filter, the residue was rinsed three times with 30 mL of 5% acetic acid each time. The washings were combined with the original filtrate. The filtrate was transferred to a 200-mL Erlenmeyer flask to which was added 10 mL of concentrated NH_4OH to precipitate the glycoalkaloids. The basic solution was placed in a 70 °C water bath for 50 min and then refrigerated overnight. The precipitate was collected by centrifugation at 18000*g* for 10 min at 1 °C and washed twice with a 2% solution of NH_4OH. The pellet was dried at 30 °C under reduced pressure, then dissolved in 1 mL of a mixture of tetrahydrofuran/acetonitrile/20 mM KH_2PO_4, and centrifuged at 18000*g* for 10 min at 1 °C. The supernatant (50 μL) was used for HPLC.

HPLC Analysis of Glycoalkaloids. *HPLC Columns.* The following four columns were used to evaluate the effectiveness of different column packing materials to separate the glycoalkaloids under the same

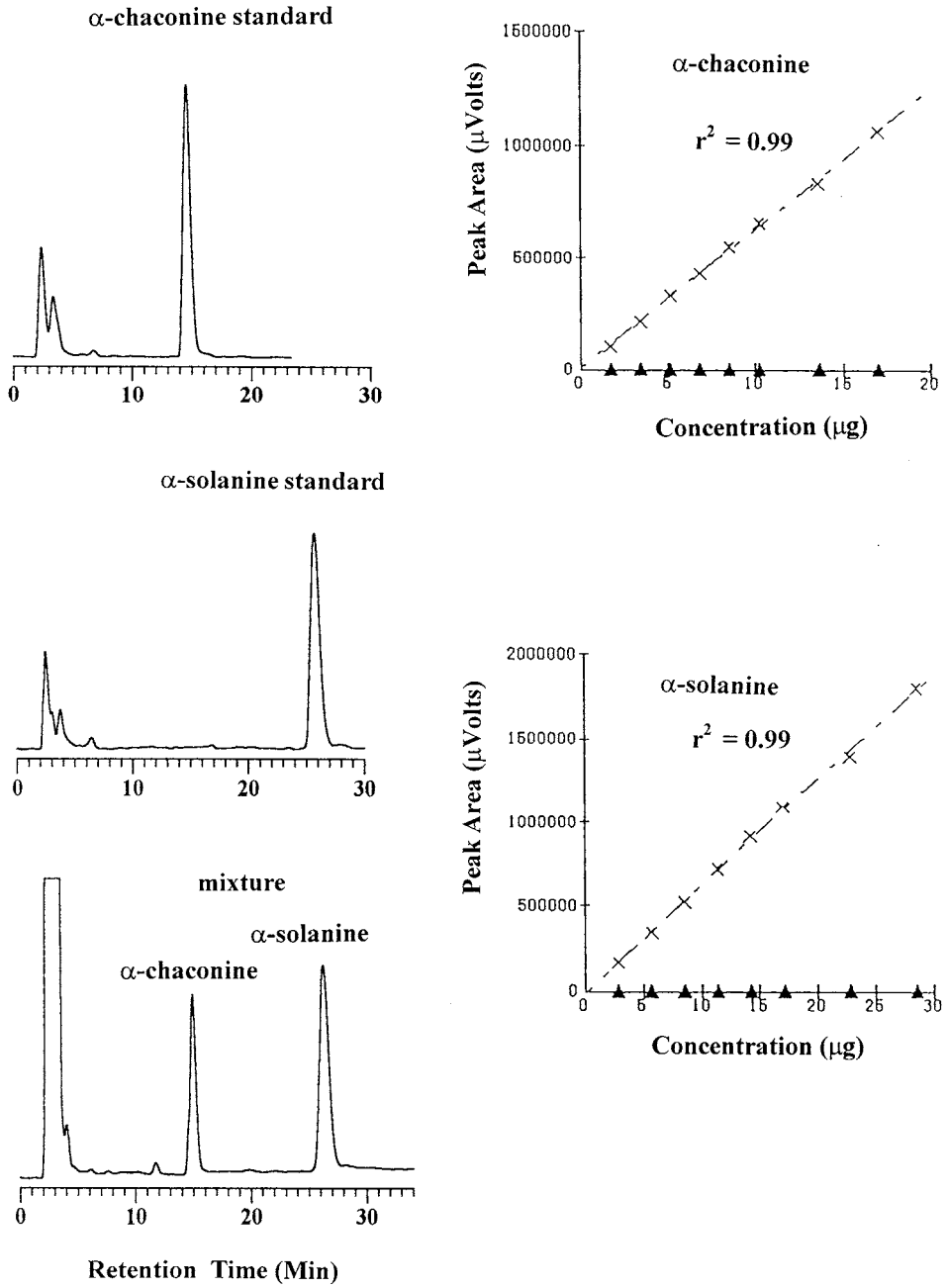

Figure 2. HPLC chromatogram of standard α-chaconine and α-solanine. Conditions: column, Nucleosil NH_2 (5 μM, 4.0 × 250 mm); mobile phase, acetonirile/20 mM KH_2PO_4 (80:20, v/v); flow rate, 1.0 mL/ min; column temperature, 20 °C; UV detector, 208 nm; sample size, 20 μL.

analytical conditions: (A) Spherisorb S NH_2 [5 μM, 4.0 × 250 mm (Waters, Bedford, MA)]; (B) Nucleosil NH_2 [5 μM, 4.0 × 250 mm (Nagel, Germany)]; (C) LiChrosorb NH_2 [5 μM, 4.0 × 250 mm (Merck, Darmstadt, Germany)]; and (D) Inertsil NH_2 [5 μM, 4.0 × 250 mm (GL Science, Japan)]. Each column was equilibrated at a flow rate of 1 mL/min with the same mobile system for 3 h before it was used for chromatography of the glycoalkaloids.

HPLC Analysis. The method used was adapted from the literature (*48*, *50*, *55*). HPLC chromatography was carried out with the aid of a Hitachi liquid chromatograph model 665A-11 equipped with a model 655-40 autosampler and a UV detector (Hitachi model 655A UV monitor) set at 208 nm. Column temperature was controlled with a Coolnics model CTR-120 device (Komatsu Electronics, Tokyo, Japan). Chromatogram peak areas were integrated with a Hitachi D-2500 chromato-integrator. Chromatography was performed with the four columns mentioned above. The mobile phase was acetonitrile/20 mM KH_2PO_4 (80:20, v/v). For the aglycon solanidine, the mixture consisted of acetonitrile/2.5 mM KH_2PO_4 (93:7, v/v). The flow rate was 1 mL/ min at a column temperature of 20 °C. The influence of column packing and several parameters on the separation of individual glycoalkaloids present in potatoes is expressed by the retention time or the amount of time a solute spends on the column. The concentrations of α-chaconine and α-solanine in the potato extracts were calculated by comparison with the integrated peak areas of known amounts of the standards by a Hitachi chromato-intergrator.

Identification of Glycoalkaloids. The two potato glycoalkaloids in the potato extract were identified as follows: (a) comparison of thin-layer chromatography of standards α-chaconine and α-solanine and of samples of each peak collected from the HPLC column containing the individual glycoalkaloids; and (b) HCl hydrolysis of the HPLC samples into sugars and the aglycon solanidine. These were then identified by GLC-MS, as described in detail elsewhere (*34a,b*).

Calystegine Analysis by GC-MS. *Extraction and Isolation of Hydrophilic Alkaloid Fractions.* The procedure was adapted from that of Nash et al. (*46*). Weighed samples (1.0 g) of powdered potato flesh and peel were stirred at room temperature for 24 h with methanol/ water (4:1, v/v, 25 mL). Each sample was vacuum filtered through a pad of Celite diatomaceous earth to remove solids. The filtrate was

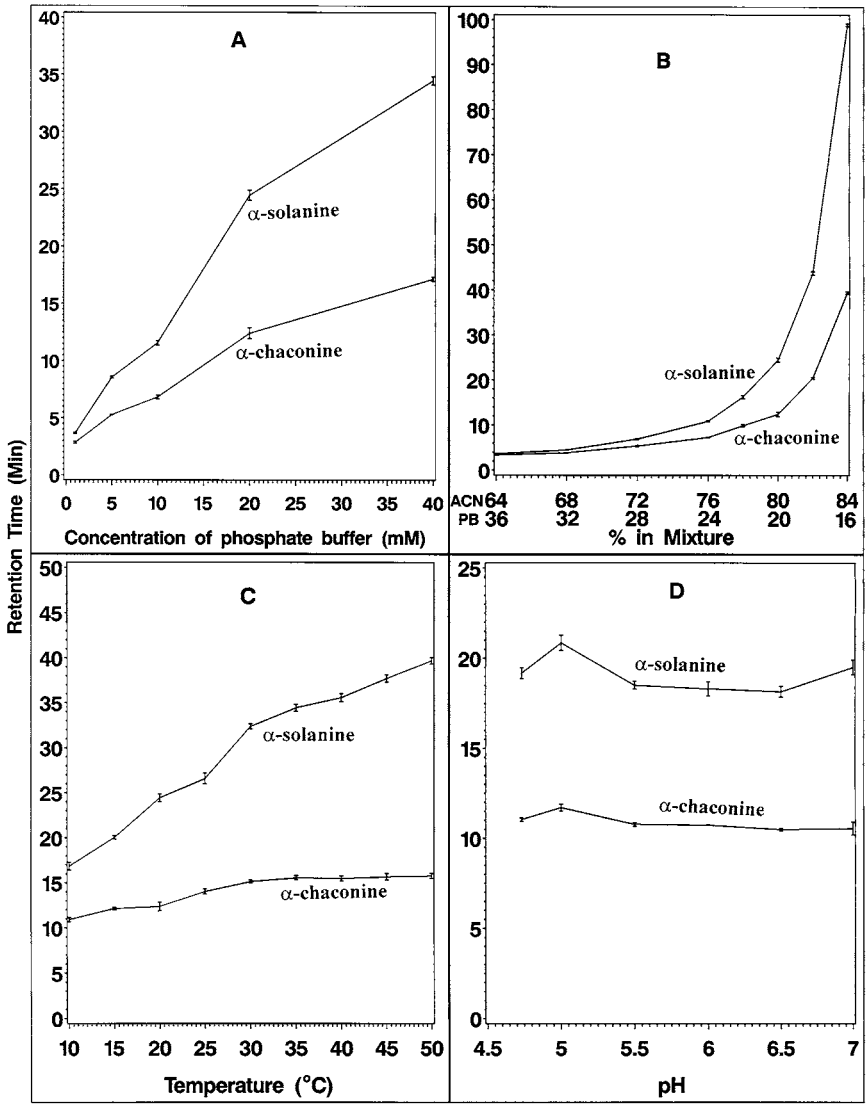

Figure 3. Effect on retention times of α-chaconine and α-solanine: (A) concentration of phosphate buffer (1–40 mM) [mobile phase, acetonitrile/phosphate buffer (80:20)]; (B) concentration of acetonitrile (ACN) and 20 mM KH_2PO_4 (phosphate buffer, PB); (C) column temperature; (D) pH of mobile phase [mobile phase, acetonitrile/20 mM KH_2PO_4 (pH 4.73–7.0) (80:20, v/v); other conditions as in **Figure 2**].

concentrated to ~3 mL by rotary evaporation at 45 °C. The residue was transferred quantitatively to a 10-mL beaker with deionized water and the pH adjusted to 4.0 with HCl. The more or less cloudy solution was introduced directly onto a 150 mm long × 12 mm diameter bed of cation-exchange resin (Dowex AG 50W X 8, Bio-Rad, Hercules, CA) at a flow rate of ~1 mL/min. After the column had been rinsed at the same flow rate with 55 mL of deionized water (~3 bed volumes), 0.5% NH_4OH (55 mL) was introduced and the alkaline eluent was collected and concentrated to 2−6 mL by rotary evaporation at 45 °C. The resulting solution was transferred quantitatively to a 10-mL volumetric flask and made up to 10.0 mL with deionized water from which 1.0-mL aliquots were transferred to 4-mL borosilicate glass screw cap vials, frozen in liquid nitrogen and freeze-dried.

Preparation of Trimethylsilyl (TMS) Ethers. To each vial was added dry pyridine (45 μL), *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) (Pierce, Rockford, IL) (45 μL), and as internal standard 10 μL of a solution of 250 μg of perseitol (Aldrich) in 50 μL each of pyridine and MSTFA that had been previously warmed for 1 h at 100 °C. The vials were then heated for 1 h in a Reacti-Therm block heater (Pierce).

GC-MS Analysis. The derivatized samples were analyzed on a Hewlett-Packard 5890 series II GC (helium carrier gas) coupled to a Hewlett-Packard 5971 mass-selective detector (MSD). A 60 m × 0.32 mm i.d., 0.25-μm film, SE-30 fused silica capillary column (J&W Scientific, Folsom, CA) was installed in the GC, and an on-column injector (SGE model OC1-3) held at ambient temperature was fitted to the column inlet. Samples (0.5 μL) were injected directly into the column held at 105 °C for 0.2 min. The column was ramped at 30 °C/min for 0.5 min, programmed from 120 to 300 °C at 10 °C/min, and held at the final temperature for 10 min. The MSD was operated at 70 eV in the EI mode with scans taken every 1.5 s from 75 to 600 amu. A postinjection delay of 7.0 min was set to allow solvent and derivatizing agent to elute before mass spectral data acquisition began.

Retention times and mass spectra of calystegine standards confirmed the presence of the trihydroxy nortropane alkaloid, calystegine A_3, and the tetrahydroxy nortropane, calystegine B_2, as TMS ethers in all eight of the potato cultivars examined. The amounts of the two alkaloids were calculated by comparison of the integrated total ion current peak areas with the peak area of the internal standard, perseitol-TMS.

RESULTS AND DISCUSSION

To facilitate discussion of the results, we will first briefly summarize the experimental data shown in the figures and tables. **Figure 1** illustrates the structures of the secondary metabolites evaluated in this study: the glycoalkaloids α-chaconine and α-solanine and the nortropane alkaloids calystegine A_3 and calystegine B_2. **Figure 2** shows the separation of standard α-chaconine and α-solanine on an HPLC chromatogram, and

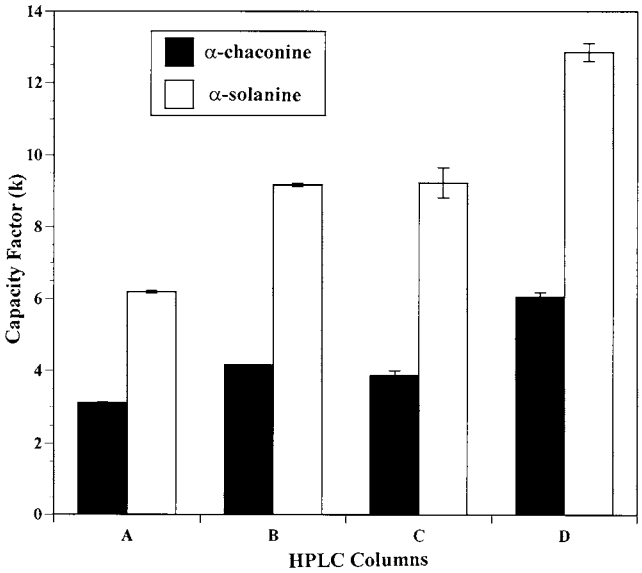

Figure 4. Capacity factors (*k*) of four different columns for the separation of α-chaconine and α-solanine: (A) Spherisorb S NH_2; (B) Nucleosil NH_2; (C) LiChrosorb NH_2; (D) Inertsil NH_2. Conditions were as in **Figure 2**.

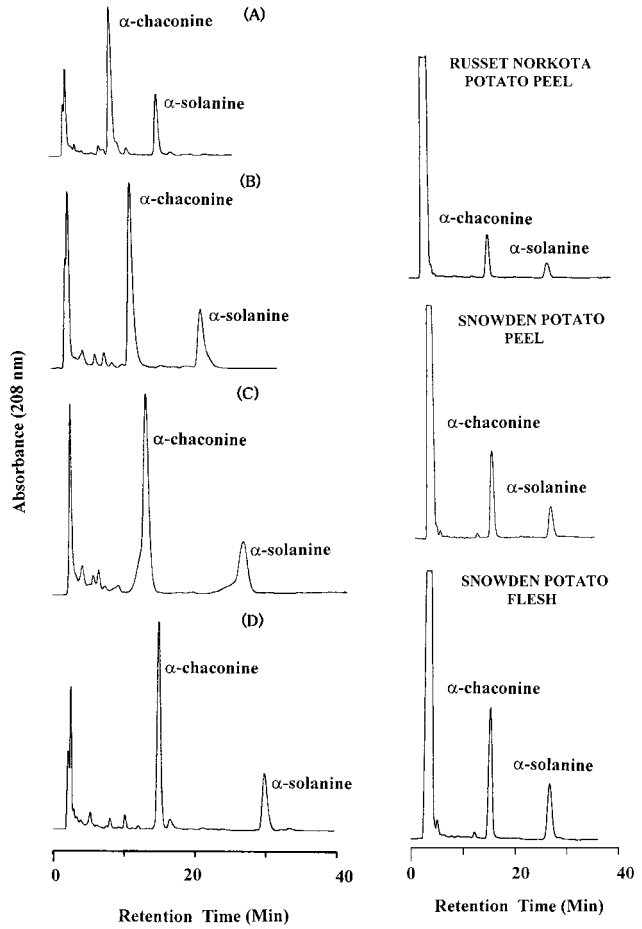

Figure 5. (Left) HPLC chromatograms of α-chaconine and α-solanine isolated from the tuber cortex of fresh May Queen potatoes on four different columns: (A) Spherisorb S NH_2; (B) Nucleosil NH_2; (C) LiChrosorb NH_2; (D) Inertsil NH_2. (Right) Conditions were as in **Figure 2** except for chromatography on the Inertstil NH_2 column.

Figures 3 and **4** depict effects of different parameters on the retention times. **Figure 5** shows chromatograms of α-chaconine and α-solanine extracted from fresh May Queen potatoes and from extracts of freeze-dried potatoes. **Figure 6** illustrates the

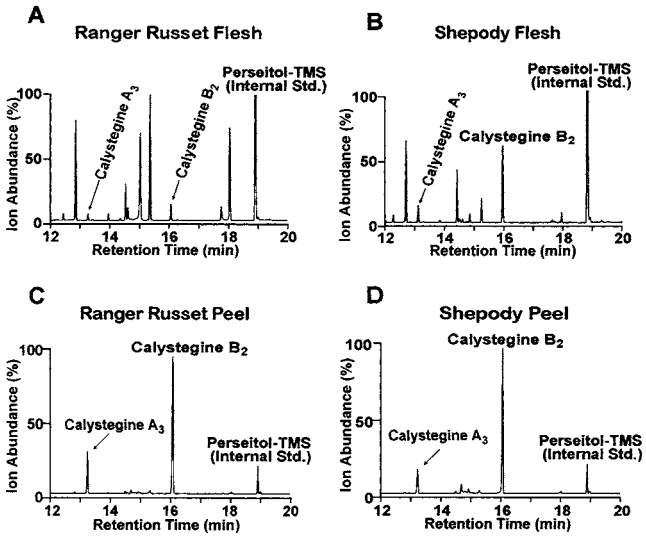

Figure 6. GC-MS total ion chromatograms of the hydrophilic alkaloid fraction extracted from freeze-dried potato flesh and peel.

Table 1. Glycoalkaloid Content of an Extract of the Cortex Tissues of Fresh May Queen Potatoes Chromatographed on Four Different HPLC Columns

	mg/kg of fresh cortex wt		
column	α-chaconine (A)	α-solanine (B)	A/B
Spherisorb S NH_2	211 ± 9[a]	88 ± 4[a]	2.4
Nucleosil NH_2	219 ± 9	102 ± 6	2.2
LiChrosorb NH_2	215 ± 6	103 ± 7	2.1
Inertsil NH_2	250 ± 10	111 ± 1	2.2

[a] Values are averages ± SD for triplicate analyses from separate extracts.

separation on GC-MS total ion chromatograms of calystegines A_3 and B_2 extracted from potato powders. **Table 1** lists the glycoalkaloid content of the same extract of May Queen potatoes chromatographed on four different columns. **Table 2** lists wet−dry relationships of flesh and peel of eight potato varieties. **Table 3** lists the α-chaconine and α-solanine contents of freeze-dried (dry) flesh and peel samples determined experimentally as well as the calculated values before freeze-drying (wet samples) using their water content shown in **Table 2**. **Table 4** lists calystegine levels of the same samples.

GLYCOALKALOIDS

Reproducibility of the Analyses with Standards. Figure 2 shows chromatograms on a Nucleosil NH_2 column of authentic standard α-chaconine and α-solanine in pure form and in a synthetic mixture. The figure also shows calibration plots for the two glycoalkaloids, both with correlation coefficients of 0.99. Results from seven analyses of the standards showed that the retention times of the analyses were highly reproducible, to within ~1% for both glycoalkaloids. The limit of detection for α-chaconine is estimated to be 150 ng and that for α-solanine, 160 ng. The average recovery of α-chaconine added to a freeze-dried sample was 91.9 ± 4.2% ($n = 5$). The corresponding value for α-solanine was 90.3 ± 1.1%. An evaluation of the different factors that were found to influence the HPLC analysis are outlined below.

Effect of Phosphate Buffer Concentration of Mobile Phase on Retention Times. Figure 3A shows the effect of varying the concentration of the phosphate buffer in the range of 1−40 mM on retention times of the two glycoalkaloids with a constant

Table 2. Summary of Total, Wet-Dry, and Flesh–Peel Weights of Eight Potato Cultivars

potato cv.	total wet[a] (g)	wet flesh[a] (g)	wet peel[a] (g)	wet peel and (flesh) total (%)	dry flesh[b] (g)	dry peel[b] (g)	dry/wet flesh (%)	dry/wet peel (%)
Atlantic	2214	2032	182	8.2 (91.8)	424	27	20.9	14.8
Dark Red Norland	2488	2220	268	10.8 (89.2)	469	40	21.1	14.9
Ranger Russet	2004	1806	198	9.9 (90.1)	383	36	21.2	18.1
Red Lasoda	2189	1992	197	9.0 (91.0)	404	32	20.3	16.2
Russet Burbank	2794	2608	186	6.7 (93.3)	558	30	21.4	16.1
Russet Norkota	2180	1994	186	8.5 (91.5)	399	31	20.0	16.7
Shepody	1800	1650	149	8.3 (91.7)	394	23	24.0	15.4
Snowden	2410	2171	239	9.9 (90.1)	535	36	25.0	15.4
av (%)				8.9 (91.1)			21.7	15.9

[a] Weights of whole fresh potatoes, potato flesh, and potato peel before freeze-drying. [b] Weights after freeze-drying.

mobile phase ratio of acetonitrile/phosphate buffer of 80:20 and under conditions shown in **Figure 2**. The separation of the two glycoalkaloids increased markedly with increasing phosphate buffer concentration. A compromise value of 20 mM concentration with an analysis time of ~25 min was selected as being optimal for this study.

Effect of Acetonitrile/Phosphate Ratio of Mobile Phase on Retention Times. To further optimize the separation of the two glycoalkaloids, we examined the trend in retention times as a function of the ratios of acetonitrile/KH_2PO_4 (mobile phase) under the elution conditions shown in **Figure 3B**. The results show that there is essentially no difference in retention times (no separation) for the two glycoalkaloids in the range of 64−76% acetonitrile/24−36% phosphate. The separation then increases with increasing acetonitrile concentration. We arbitrarily selected the 80:20 acetonitrile/phosphate ratio as offering good separation at a convenient 25 min analysis time.

Figure 3B also shows that further increases in this ratio can be used to achieve a wider separation at the expense of time (up to ~100 min) needed to complete the analyses. Such wider separation may be especially useful (a) for processed potato products such as coated, formed, or mashed potatoes with skin and/or condiments and (b) in situations when the analyses may contain contaminating compounds eluting between the two glycoalkaloids.

Effect of Column Temperature on Retention Times. Figure 3C illustrates the dependence of the retention times of the two glycoalkaloids on column temperature from 10 to 50° C under the conditions described in **Figure 2**. Surprisingly, the dependence is quite narrow for α-chaconine, ranging from about 11 to 16 min. By contrast the retention time for α-solanine increased significantly from ~17 to 40 min. At the lowest temperature (10 °C), the separation required ~6 min and at the highest (50 °C), ~24 min. Column temperature therefore appears to be another parameter that can significantly affect the separation of the two glycoalkaloids. To minimize column damage caused by higher temperatures, we recommend the use of a column temperature of 20 °C, which results in a separation time of ~12−25 min for the two glycoalkaloids on HPLC chromatograms.

The minor effect of column temperature on the retention (elution) time of α-chaconine, compared to a nearly linear increase in retention time for α-solanine, is probably due to the difference in the composition of the trisaccharide side chains attached to the common aglycon solanidine (**Figure 1**). Because α-chaconine elutes first, the side chain of this glycoalkaloid evidently has a lower affinity for the column packing material than does the corresponding side chain of α-solanine. We did not study temperature effects on retention times using the packing materials of the other three columns.

Effect of pH of Mobile Phase on Retention Times. Eight different phosphate buffers in the pH range of 4.73−8.0 were prepared by combining 20 mM KH_2PO_4 and 20 mM NaH_2PO_4. The α-chaconine and α-solanine standards were then chromatographed in mixtures of 80% acetonitrile and 20% phosphate buffers of different pH values. The effect of pH on the chromatographic separation of the glycoalkaloids had to be examined at pH values of 7.0 or lower because at pH >7.0 the pressure in the column increased markedly, to >400 atm.

Figure 3D illustrates the dependence of retention times of the two glycoalkaloids on the pH (in the range of 4.73−7.0) of the mobile phase (80:20 acetonitrile/20 mM phosphate buffer). The results show that the pH of the mobile phase in the cited range does not change the retention times of the two glycoalkaloids.

Differences among Columns. In addition to retention time, another measure of how much time a compound spends on the column is the capacity factor of a chromatographic column (k value), defined by the following equation: $k = (t_R - t_R')/t_R'$, where t_R = the retention time of the sample peak and t_R' = the retention time of the solvent peak. The capacity factor is a quantitative measure of an HPLC column's ability to resolve or separate the peaks associated with α-chaconine and α-solanine from the solvent front. To quantitatively define to what extent column packing influences the retention times, we determined the capacity factors (k) for each of the four columns for the α-chaconine and α-solanine in the extracts. **Figure 4** shows the following trend in k values for the four columns: (Spherisorb S NH_2, α-chaconine, 3.12 ± 0.01 (n = 3); α-solanine, 6.18 ± 0.03) < (Nucleosil NH_2, α-chaconine, 4.16 ± 0.01; α-solanine, 9.18 ± 0.05) = (Lichosorb NH_2, α-chaconine, 3.86 ± 0.14; α-solanine, 9.24 ± 0.42) < (Inertsil NH_2, α-chaconine, 6.06 ± 0.11; α-solanine, 12.86 ± 0.25). The cited values permit the selection of columns with different k values for various applications.

Application to Potatoes. Figure 5 depicts HPLC chromatograms of the two glycoalkaloids present in extracts of the tuber cortex of commercial May Queen potatoes obtained with four different HPLC columns. Although all four columns effectively separated the two glycoalkaloids, they differed in the time needed for a complete analysis in the following increasing order: Spherisorb S NH_2 column (~25 min) < Nucleosil NH_2 column (~30 min) < Lichosorb NH_2 column (~35 min) < Inertsil NH_2 column (~38 min). The columns therefore appear to vary in their ability to separate the two glycoalkaloids under otherwise the same experimental conditions. **Figure 5** also shows the excellent separation on the Inertsil NH_2 column of the two glycoalkaloids isolated from freeze-dried potato peel and flesh.

Table 3. Glycoalkaloid Content (Milligrams per Kilogram) of Freeze-Dried (Dry) and Fresh (Wet) Potato Flesh, Potato Peel, and Whole Potatoes of Eight Potato Cultivars[a]

potato cv.		α-chaconine (A)	α-solanine (B)	A + B	A/B
Atlantic	dry flesh	22.6 ± 2.8	13.9 ± 2.8	36.5	1.6
	dry peel	59.4[b]	24.4[b]	83.8	2.4
	dry whole	25.6	14.8	40.4	1.7
	wet flesh	4.7	2.9	7.6	1.6
	wet peel	8.8	3.6	12.4	2.4
	wet whole	5.0	8.0	8.0	1.7
Dark Red Norland	dry flesh	16.0 ± 0.6	6.1 ± 0.5	22.1	2.6
	dry peel	859 ± 35.4	405 ± 12.3	1264	2.1
	dry whole	107	49.2	156	2.2
	wet flesh	3.4	1.3	4.7	2.6
	wet peel	128	60.3	188	2.1
	wet whole	16.8	7.7	24.5	2.2
Ranger Russet	dry flesh	60.3 ± 1.6[c]	33.2 ± 0.3[c]	93.5	1.8
	dry peel	1273 ± 191	606 ± 105	1879	2.1
	dry whole	180	89.9	270	2.0
	wet flesh	12.8	7.0	19.8	1.8
	wet peel	230	110	340	2.1
	wet whole	34.3	17.2	51.5	2.0
Red Lasoda	dry flesh	21.6 ± 2.2[c]	13.7 ± 1.1[c]	35.3	1.6
	dry peel	827 ± 101	596 ± 51	1423	1.4
	dry whole	94.1	66.1	160	1.4
	wet flesh	4.4	2.8	7.2	1.6
	wet peel	134	96.6	231	1.4
	wet whole	16.0	11.2	27.2	1.4
Russet Burbank	dry flesh	121 ± 5.7[c]	100 ± 6.1[c]	221	1.2
	dry peel	1128 ± 107	638 ± 103	1766	1.8
	dry whole	188	136	325	1.4
	wet flesh	25.9	21.4	47.3	1.2
	wet peel	182	103	285	1.8
	wet whole	36.3	26.8	63.1	1.4
Russet Norkota	dry flesh	3.7 ± 0.4	2.7 ± 0.4	6.4	1.4
	dry peel	288 ± 17.9	138 ± 7.3	426	2.1
	dry whole	27.9	14.2	42.1	2.0
	wet flesh	0.74	0.54	1.3	1.4
	wet peel	48.1	23.0	71.1	2.1
	wet whole	4.8	2.5	7.3	1.9
Shepody	dry flesh	7.7 ± 0.6[c]	4.5 ± 1.3	12.2	1.7
	dry peel	1829 ± 126	957 ± 81	2786	1.9
	dry whole	159	83.6	243	1.9
	wet flesh	1.8	1.1	2.9	1.7
	wet peel	282	147	429	1.9
	wet whole	25.1	13.2	38.3	1.9
Snowden	dry flesh	366 ± 6.0	226 ± 2.5	592	1.6
	dry peel	2414[b]	1112[b]	3526	2.2
	dry whole	569	314	883	1.8
	wet flesh	91.5	56.5	148	1.7
	wet peel	372	171	543	2.2
	wet whole	119	67.9	187	1.8

[a] Unless otherwise indicated, $n = 3 \pm$ SD for analyses from separate extracts. The first two values for each sample are experimental data for freeze-dried flesh and peel. All other values are calculated from these values by taking into account the water content shown in **Table 2**. [b] $n = 1$. [c] $n = 2$.

Table 4. Calystegine Content (Milligrams per Kilogram) of Freeze-Dried (Dry) and Fresh (Wet) Potato Flesh, Potato Peel, and Whole Potatoes of Eight Potato Cultivars[a]

potato cv.		calystegine A_3	calystegine B_2	$A_3 + B_2$	B_2/A_3
Atlantic	dry flesh	5 ± 2[b]	7 ± 3	12	1.4
	dry peel	211 ± 37[b]	951 ± 220	1162	4.5
	dry whole	21.9	84.4	106	3.8
	wet flesh	1.1	1.5	2.6	1.4
	wet peel	31.2	141	172	4.5
	wet whole	3.5	12.9	16.4	3.7
Dark Red Norland	dry flesh	0 ± 1	6 ± 2	6	
	dry peel	43 ± 16	221 ± 83	266	5.1
	dry whole	4.6	29.4	34	6.3
	wet flesh	0	1.3	1.3	
	wet peel	6.4	33.3	39.7	5.2
	wet whole	0.7	4.7	5.4	6.7
Ranger Russet	dry flesh	5 ± 2	11 ± 4	16	2.2
	dry peel	481 ± 53	2100 ± 500	2581	4.4
	dry whole	52.1	218	270	4.2
	wet flesh	1.1	2.3	3.4	2.1
	wet peel	87.1	380	467	4.4
	wet whole	9.6	39.7	49.3	4.1
Red Lasoda	dry flesh	7 ± 3	21 ± 8	28	3.0
	dry peel	65 ± 19	153 ± 51	218	2.4
	dry whole	12.2	32.8	45.0	2.7
	wet flesh	1.4	4.3	5.7	3.1
	wet peel	10.5	24.8	35.3	2.4
	wet whole	2.2	6.1	8.3	2.8
Russet Burbank	dry flesh	52 ± 13	264 ± 77	316	5.1
	dry peel	41 ± 13	421 ± 130	462	10.3
	dry whole	51.3	275	326	5.4
	wet flesh	11.1	56.5	67.6	5.1
	wet peel	6.6	67.8	74.4	11.8
	wet whole	10.8	57.3	68.1	5.3
Russet Norkota	dry flesh	1 ± 1	4 ± 1	5.0	4.0
	dry peel	201 ± 63	775 ± 195	976	3.9
	dry whole	18.0	69.5	87.5	3.9
	wet flesh	0.2	0.8	1.0	4.0
	wet peel	33.6	129	163	3.9
	wet whole	3.0	11.9	14.9	4.0
Shepody	dry flesh	9 ± 3	38 ± 14	47.3	4.2
	dry peel	286 ± 85	1940 ± 475	2226	6.8
	dry whole	32.0	196	228	6.1
	wet flesh	2.2	9.1	11.3	4.1
	wet peel	44.0	299	343	6.8
	wet whole	5.6	33.1	38.7	5.9
Snowden	dry flesh	3 ± 2	3 ± 2	6.0	1.0
	dry peel	350 ± 100	625 ± 177	975	1.8
	dry whole	37.4	64.6	102	1.7
	wet flesh	0.8	0.8	1.7	1.0
	wet peel	54.2	96.3	150	1.8
	wet whole	5.8	10.2	16.0	1.8

[a] $n = 2 \pm$ SD for duplicate analyses from a single extract. [b] The first two values for each sample are experimental data for freeze-dried flesh and peel. All other values are calculated from these values by taking into account the water content shown in **Table 2**.

Table 1 summarizes the calculated individual α-chaconine and α-solanine contents of the same potato cortex extract chromatographed on the four different columns. The concentrations obtained on chromatography on the Spherisorb S NH_2, Nucleosil NH_2, and LiChrosorb NH_2 columns were identical, within experimental error. In contrast, the values obtained with the Inertsil NH_2 column were 12−14% higher than with the other three columns. We have no obvious explanation for this difference, except that because the peak shapes appear to be more symmetric with the Inertsil NH_2 column than with the other columns, the use of the Inertsil NH_2 column yields better quantitative results. The ratios of concentrations of α-chaconine to α-solanine for the four columns varied by ∼10%, ranging from 2.2 to 2.4.

Although detailed discussion of the dietary significance of the results listed in **Table 3** is beyond the scope of this paper, it should be noted that none of the wet whole potatoes exceeded the limit of 200 mg of total glycoalkaloids/kg of potatoes (see A + B column). However, this was not the case for potato peel. The values for three wet peel samples (Atlantic, Dark Red

Norland, and Russet Norkota) are <200 mg/kg and for the other five >200 mg/kg. High levels of glycoalkaloids in potato skins may be a concern for commercial products that have high skin/flesh ratios, for example, when flesh has been mostly removed and the skin is used to scoop up condiments such as salsa. Peel from potato-processing plant wastes may also be a concern if the peel is not thoroughly mixed with other waste streams.

The ratios of α-chaconine to α-solanine for the potato samples ranged from 1.2 to 2.6. The ratio for peel, generally in the range of ~2, was much higher than for flesh with values near ~1.5. Because, as mentioned earlier, α-chaconine is more toxic than α-solanine, it is desirable to have this ratio as low as possible. We can only speculate about possible reasons for the wide variations in these ratios. Because the two glycoalkaloids, which share the common aglycon solanidine but not the same trisaccharide side chain (**Figure 1**), appear to be synthesized via distinctly different (discrete) biosynthetic channels (*58*), it is possible that the rates of biosynthesis of the two glycoalkaloids in the different channels are cultivar-dependent. Another possible rationalization for the varying ratios is that the rate of metabolism of the two glycoalkaloids is also cultivar-dependent. These considerations imply that alteration of the genes encoding enzymes involved in the biosynthesis of α-chaconine and/or α-solanine may be mutually dependent.

CALYSTEGINES

Results of the GC-MS analyses for calystegines A_3 and B_2 are shown in **Table 4**. In terms of milligrams per kilogram, the ranges are as follows: dry flesh, A_3 from 0 (Dark Red Norland) to 52 (Russet Burbank) and B_2 from 3 (Snowden) to 264 (Russet Burbank); dry peel, A_3 from 41 (Russet Burbank) to 481 (Ranger Russet) and B_2 from 153 (Red Lasoda) to 2100 (Ranger Russet); dry whole potatoes, A_3 from 4.6 (Dark Red Norland) to 52.2 (Ranger Russet) and B_2 from 29.4 (Dark Red Norland) to 275 (Russet Burbank); wet flesh, A_3 from 0 to 11.1 (Russet Burbank) and B_2 from 1.3 (Dark Red Norland) to 56.5 (Russet Burbank); wet peel, A_3 from 6.4 (Dark Red Norland) to 87.1 (Ranger Russet) and B_2 from 33.3 (Dark Red Norland) to 380 (Ranger Russet); wet whole potatoes, A_3 from 0.7 (Dark Red Norland) to 10.8 (Russet Burbank); and B_2, from 4.7 (Dark Red Norland) to 57.3 (Russet Burbank). For wet whole potatoes, there is a 12-fold variation from lowest to highest values for A_3 and a 15-fold variation for B_2.

For the sum of the two calystegines (**Table 4**, $A_3 + B_2$ column), the ranges are as follows: dry flesh, from 5 (Russet Norkota) to 316 (Russet Burbank); dry peel, from 218 (Red Lasoda) to 2581 (Ranger Russet); dry whole potatoes, from 34 (Dark Red Norland) to 326 (Russet Burbank); wet flesh, from 1 (Russet Norkota) to 67.6 (Russet Burbank); wet peel, from 35.3 (Red Lasoda) to 467 (Ranger Russet); wet whole potatoes, from 5.4 (Dark Red Norland) to 68.1 (Russet Burbank), a 13-fold variation from lowest to highest values.

For calystegine B_2/A_3 ratios, the ranges are as follows: dry flesh, from 1.0 (Snowden) to 5.1 (Russet Burbank); dry peel, from 1.8 (Snowden) to 10.3 (Russet Burbank); dry whole potatoes, from 1.7 (Snowden) to 6.3 (Dark Red Norland); wet flesh, from 1.0 (Snowden) to 5.1 (Russet Burbank); wet peel, from 1.8 (Snowden) to 11.8 (Russet Burbank); wet whole potatoes, from 1.8 (Snowden) to 5.9 (Shepody), a 3-fold variation from lowest to highest values.

It is also instructive to calculate the following ratios of total glycoalkaloid to total calystegine content for wet whole potatoes: Russet Norkota, 0.19; Atlantic, 0.49; Russet Burbank, 0.93; Shepody, 0.99; Ranger Russet, 1.0; Red Lasoda, 3.3; Dark Red Norland, 4.5; Snowden, 11.7. The data show that the biosynthesis of glycoalkaloids seems to parallel that of calystegines in some varieties but not in others.

As is the case with the glycoalkaloids, the results show that the concentrations of both the calystegines are greatest in the peel, and their levels in the flesh and peel as well as their sum vary widely among the cultivars evaluated. The fact that the glycoalkaloid/calystegine ratio also varies implies that the synthesis of the two classes of secondary metabolites may be under separate genetic control. Moreover, because the individual calystegine isomers differ in their biological activities (*47*, *52*), both their ratios as well as total amounts present in different potato cultivars may be important in the assessment of the role of calystegines in the diet.

CONCLUSIONS

Experiments were carried out to devise an improved HPLC method for the separation and analysis of potato glycoalkaloids extracted from fresh and dried potatoes. Factors that can be effectively used to manipulate the separation of glycoalkaloids include column packing, column temperature, and composition of the mobile phase, whereas changing the pH of the mobile phase has no effect on the separation. In parallel studies, we measured the calystegine content of the same potato samples by GC-MS. The described analyses should be useful to measure the glycoalkaloid and calystegine contents in fresh processed potatoes and potato products as well as in biological samples such as plasma and liver. Other possible applications include plant breeding and plant molecular biology studies designed to follow the inheritance of glycoalkaloids and calystegines and/or to alter genes that encode enzymes that govern their biosynthesis. Finally, because the biological activities as well as their roles in host-plant resistance of glycoalkaloids and calystegines could be interrelated, there is a need to define the levels of both glycoalkaloids and calystegines in different potato cultivars and to study individual and combined effects in animals and humans. Possible therapeutic applications of high-calystegine potato diets also merit study.

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