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Rapid High-Performance Liquid Chromatographic Determination of the Potato Glycoalkaloids α-Solanine and α-Chaconine

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A method for the determination of the glycoalkaloids α-solanine and α-chaconine in fresh potato tubers and processed potato products is described. The glycoalkaloids are extracted with dilute aqueous acetic acid in the presence of an ion-pairing reagent. The crude extract is prepared for analysis by ion-pair column chromatography on a commercially available disposable C-18 cartridge column. Recoveries of added α-solanine and α-chaconine averaged 90% and 96%, respectively, with a coefficient of variation of approximately 4.5%. An assay time of 20 min and the possibility of preparing up to eight samples concurrently for analysis facilitate the assay of large numbers of samples in a short time. Survey data are presented for fresh potato samples collected from the three major potato growing areas in the U.S. and for commercially prepared frozen potato skins collected from one of these areas.

Glycoalkaloids are toxic, naturally occurring compounds commonly found in plants that are members of the Solanaceae or "Nightshade" family. Well-known members of this family are the potato, eggplant, tomato, capiscum, nightshade, and thorn apple. Of the more than 10 different glycoalkaloids found in these plants α-solanine and α-chaconine account for more than 95% of the glycoalkaloids found in the tuber (edible portion) of the potato plant (Guseva and Paseshnichenko, 1957). The generally accepted safe upper limit for glycoalkaloids in potato tubers in the U.S. is 20 mg of total glycoalkaloids/100 g of tuber. However, some researchers feel that the safe upper limit is much lower, 6 mg/100 g (Morris and Lee, 1984).

A number of analytical methods have been reported for the determination of individual and total glycoalkaloids in potato products. These methods commonly employ extraction by aqueous/organic or bisolvent systems followed by precipitation of the glycoalkaloids with aqueous base. The determinative steps involve thin-layer chromatography (Cadle et al., 1978), gas–liquid chromatography (Herb et al., 1975), high-performance liquid chromatography (HPLC) (Bushway et al., 1979; Morris and Lee, 1981), colorimetry (Wang et al., 1972), or titrimetry (Fitzpatrick and Osman, 1974). These methods can suffer from one or more of the following problems: large volumes of expensive or hazardous solvents, nonspecificity, and long analysis times. In contrast, the method described here is rapid (20-min assay time), simple, and economical. In addition, with minor changes, the proposed method accommodates samples of widely varying size and has a range of detection for each glycoalkaloid (α-solanine and α-chaconine) from 0.2 to 20 mg/100 g. As many as eight samples can be prepared for determination by HPLC at one time.

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EXPERIMENTAL SECTION

Materials. α-Solanine, α-chaconine, and 1-heptanesulfonic acid were purchased from Sigma Chemical Co. (St. Louis, MO). The standards were assumed to be 98% pure (Bushway, 1983) and were not further purified or tested. All other solvents and reagents were ACS reagent grade except for acetonitrile, which was HPLC (UV) grade purchased from Mallinckrodt Chemical Co. (Paris, KY). Distilled water was used for the preparation of the extracting solution, and HPLC-grade water was used for the preparation of the HPLC mobile phase.

Fresh potatoes for method development and recovery studies were purchased from local supermarkets. Fresh potatoes for the survey were collected by Food and Drug Administration (FDA) investigators from packers, growers, or shippers located in the potato-growing areas of Texas, Idaho, and Maine. Commercially prepared frozen potato skins were collected from shippers in Maine. Fresh potatoes and frozen potato skins were shipped within several days after collection. Fresh potato samples were shipped at ambient temperature, and frozen potato skin samples were packed in dry ice for shipment. Upon receipt, the fresh samples were stored at 4 °C, and the frozen samples were stored at −8 °C until analyzed. All samples were analyzed within 30 days of receipt.

Apparatus. The C-18 cartridge columns and Sep-Pak column manifold system were obtained from Waters Associates, Inc, (Milford, MA).

An Altex/Beckman, Inc. (Berkeley, CA), Model 322 MP system equipped with an Altex/Beckman, Inc., Model 210 injection valve fitted with a 20-μL injection loop was used for HPLC separations. An Altex/Beckman 4.6 mm × 25 cm HPLC column packed with 5-μm octyl (C-8) spherical silica was used for all separations, which were performed at a flow rate of 1.0 mL/min and ambient temperature.

A Hitachi, Ltd. (Tokyo, Japan), Model 100-40 UV/visible variable-wavelength detector was operated at 202 nm and fitted with an Altex/Beckman flow cell. Detector

sensitivity was adjusted to either 0.5 or 1.0 AUFS. Detector response was monitored on a Kipp and Zonen, Inc. (Delft, Holland), Model BD-41 strip chart recorder operated at a sensitivity of 10-mV full scale and a speed of 0.5 cm/min. Peak areas were measured using a Hewlett-Packard, Inc. (Avondale, PA), Model 3390A integrator.

Solutions. *Extracting Solution.* The extracting solution was prepared by dissolving 4.0 g of 1-heptanesulfonic acid, sodium salt, in 1 L of distilled water containing 10 mL of acetic acid.

HPLC Mobile Phase. The mobile phase was prepared by dissolving 0.29 g of ammonium phosphate in 250 mL of HPLC-grade water and then adding 250 mL of acetonitrile. The mobile phase was filtered through a 0.7-μm glass fiber filter and degassed before use.

Glycoalkaloid Standard Solutions. Stock solutions were prepared by dissolving α-solanine (1.0 mg/mL) and α-chaconine (1.5 mg/mL) in the extracting solution. The stock solutions were stable for 60 days when stored at 4 °C. HPLC working standards were prepared by diluting the stock solutions in the HPLC mobile phase.

Procedures. *Sample Preparation.* Fresh potato samples consisted of approximately 2 kg of fresh, whole potatoes (six to nine potatoes) of a single variety collected from a grower, packer, or shipper. Any potatoes with obvious physical damage were discarded. From this sample, four to six potatoes (800–1000 g) were selected for analysis. The potatoes were washed, wiped dry, cut into ca. 1–2 cm pieces, and ground to a smooth consistency in a Waring commerical food processor. One gram of sodium bisulfite/100 g of sample was added to retard oxidation during sample preparation and analysis. Frozen potato samples consisted of ca. 1 kg collected from a shipper. From each sample, ca. 500 g was selected at random and prepared as described above. The variety of the frozen skin samples is unknown.

Extraction. A 100-g portion of the prepared sample was removed for analysis and placed in a Waring blender with 120 mL of the extracting solution. The sample was blended at high speed for 3 min and filtered through coarse paper to obtain a crude filtrate. For weight/volume calculations, all samples were assumed to have a water content of 80% (Watt and Merrill, 1963). This resulted in a sample/solvent ratio of 100 g/200 mL.

Ion-Pair Chromatography. A C-18 cartridge column was conditioned by elution with 5 mL of methanol followed by 5 mL of the extracting solution. To the conditioned column was added 10 mL of the sample extract; it was allowed to pass through the column, and then was followed by 5 mL of 20% acetonitrile in water. All of the previous eluates were discarded. The glycoalkaloids were eluted from the column with two 1.0-mL volumes of the HPLC mobile phase. The vacuum inside the Sep-Pak rack was controlled so that the flow rate for all of the eluants was 1–2 drops/s. The sample solution was then mixed by shaking, and 20 μL was injected onto the liquid chromatograph. The final sample volume was determined to be 2.0 mL (±2.5%) by measuring a large number of samples during initial method development. Once these data were obtained, final sample volumes were no longer measured.

Spike Sample Recoveries. Spiked sample recoveries were performed by adding the appropriate amounts of the stock standard solutions to 10-mL aliquots of extracted samples prior to ion-pair chromatography. Recoveries were performed in duplicate for each spike level. Results were corrected for glycoalkaloids found in blank samples.

Confirmation of Identity. The identity of α-solanine and α-chaconine was confirmed by thin-layer chromatography. A 10-μL portion of the sample solution used for the HPLC determination was spotted on a silica gel G plate along with the appropriate standard. The spots were dried for 2 min with the aid of a warm-air blower. The spotted plate was developed in an equilibrated tank, with the lower layer prepared by mixing chloroform, methanol, and 1% ammonium hydroxide (2:2:1). The developed plate was sprayed with a solution of 0.1 g of paraformaldehyde in 1 L of 85% phosphoric acid and then heated in an oven for 5 min at 105 °C. As little as 1 μg of each glycoalkaloid could be visualized in this manner under room light (pink to purple spot), and less than half this amount could be detected under long-wave UV light (blue spot).

Table I. Recovery of Glycoalkaloids from Fresh, Raw Potatoes

spike level, mg/100 g of total glycoalkaloid	recovery,[a] %	
	α-solanine	α-chaconine
2.5	87	101
5	87	96
10	96	98
20	91	90
	$\bar{x}$ = 90	$\bar{x}$ = 96
	CV = 4.3%	CV = 4.6%

[a] Average of two determinations. CV between duplicate determinations at each level <2%.

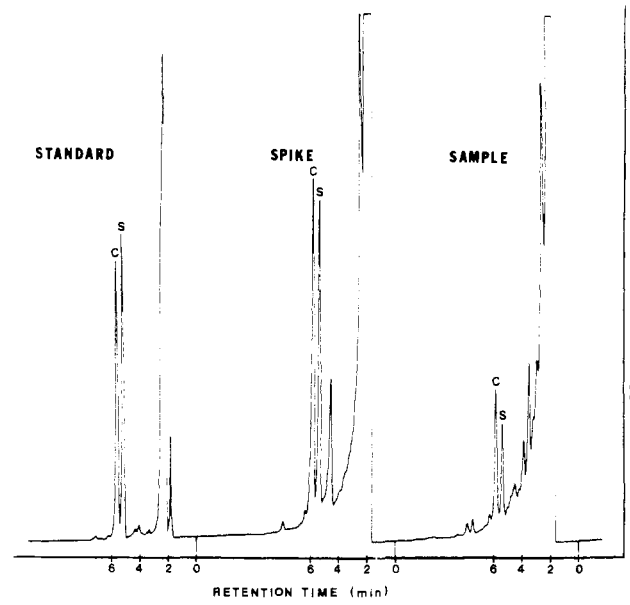

Figure 1. Chromatograms of α-solanine (S) and α-chaconine (C) standards, spiked sample, and unspiked sample. The standard injection represents the response obtained from 2 μg of α-solanine and 2 μg of α-chaconine. The spike injection represents the response from a sample spiked with α-solanine and α-chaconine at the 4 mg/100 g level. The sample injection represents the response obtained for the unspiked sample in which 0.6 mg of α-solanine/100 g and 0.6 mg of α-chaconine/100 g were found. HPLC conditions are as described in the text.

RESULTS AND DISCUSSION

Before any survey work was attempted, the performance of the method was evaluated by determining the recovery of glycoalkaloids added to potato samples. The results are given in Table I. Figure 1 illustrates the results obtained for standard, spiked sample, and unspiked sample injections. The precision of the method was evaluated by performing replicate assays on spiked (duplicate assays) and unspiked samples (triplicate assays). For both types

of samples, the coefficient of variation (CV) calculated for replicate assays of the same sample was less than 2%.

The method was compared against two accepted procedures for the assay of glycoalkaloids in potatoes: Fitzpatrick and Osman (1974) and Bushway et al. (1980). Three samples of locally purchased potatoes were assayed by each procedure. The method of Fitzpatrick and Osman gave results for total glycoalkaloids of 70% (CV = 22%) of that found by the proposed method. The method of Bushway et al. yielded results of 80% (CV = 9%) of that found by the proposed method. The most likely explanation for the higher recoveries by the proposed method is that there is much less extensive sample handling and fewer steps in the assay than in either of the two other methods.

All data reported for the recoveries and the survey were obtained by performing a linear least-squares fit for at least three standard data points. The concentration of glycoalkaloids in the sample was calculated from the computed equation for the line obtained by the least-squares fit. The correlation coefficient for the standard data fit to the line was generally greater than 0.999.

Survey data for 29 fresh potato samples (major growing areas represented as follows: (Texas, 9; Idaho, 10; Maine, 10) analyzed by this method are given in Table II.

It is apparent from the data that the potatoes collected from the southwest are significantly lower in glycoalkaloids than those from the other two growing areas. Because of the relatively small size of the survey, no conclusions as to variety vs. total glycoalkaloids were made.

Nine frozen potato skin samples collected from Maine had α-solanine levels of 0.5, 0.7, 1.1, 1.2, 1.8, 2.3, 2.4, 3.0, and 3.1 mg/100 g. In these samples, the α-chaconine levels were 0.7, 1.3, 1.5, 1.9, 2.5, 3.0, 3.3, 3.8, and 4.8 mg/100 g, respectively ($\bar{x}$, total glycoalkaloids, 4.3 mg/100 g; range, total glycoalkaloids, 1.2–7.9 mg/100 g). Although the levels of glycoalkaloids in a product such as potato skins would be expected to be much higher than in fresh potatoes (Bushway et al., 1983), the survey data do not support this hypothesis. The most tenable explanation for these results is that commercial producers of potato skin products select potato varieties that are naturally low in glycoalkaloids from which to produce their products. Research elsewhere (Bushway et al., 1983) indicates that levels of total glycoalkaloids in baked russet potato skins fall within this range.

Although recovery data are not presented for products other than fresh protatoes and fresh–frozen potato skins, frozen french fried, hash browned, canned, and dry (instant mashed) potatoes were analyzed by using this method. Spiked sample recoveries for these products yielded recoveries within the same range as that for fresh potatoes.

One problem arose during the analysis of processed samples. The samples were difficult to filter after extraction due to a gellike consistency that bound the aqueous extractant tightly, probably caused by a partial hydrolysis of the potato starches during processing. It was solved by adding diatomaceous earth filter aid during extraction and vacuum filtration. Dried (instant mashed) potato products gave the best recoveries if rehydrated with warm water and allowed to stand at least 10 min before extraction.

The addition of sodium bisulfite as an antioxidant during sample preparation was necessary to prevent browning of the ground sample and darkening of the filtrate. The latter was accompanied by an increased number of peaks in the final chromatogram (including those from the partially hydrolyzed glycoalkaloids, e.g., β-chaconine).

Table II. Results of Survey of Fresh Potatoes from Three U.S. Growing Areas

growing area	variety	α-solanine, mg/100 g	α-chaconine, mg/100 g
	Southwest		
Frio, TX	Viking Red	0.4	0.8
Hereford, TX	unknown	1.0	1.5
Muleshoe, TX	Norgold Russet	1.2	1.8
Muleshoe, TX	Norgold Russet	2.6	3.0
Hereford, TX	Nortgold Russet	1.3	2.2
Cotton Ctr, TX	Nortgold Russet	0.7	1.2
Littlefield, TX	Viking Red	0.8	1.6
Amherst, TX	Viking Red	0.4	0.8
Portales, NM	unknown	0.9	1.3
$\bar{x}$, total glycoalkaloids = 2.5 mg/100 g range total glycoalkaloids = 1.2–5.6 mg/100 g			
	Midwest		
Oakley, ID	Russet Burbank	2.5	3.1
Oakley, ID	Russet Burbank	5.2	5.6
Oakley, ID	Russet Burbank	8.3	7.5
Burley, ID	Russet Burbank	5.7	5.7
Burley, ID	Russet Burbank	6.9	6.6
Burley, ID	Russet Burbank	4.6	5.0
Murtaugh, ID	Russet Burbank	1.7	2.1
Murtaugh, ID	Russet Burbank	8.0	6.8
Declo, ID	Russet Burbank	2.6	3.2
Paul, ID	Russet Burbank	3.8	4.2
$\bar{x}$, total glycoalkaloids = 9.9 mg/100 g range total glycoalkaloids = 3.8–15.8 mg/100 g			
	Northeast		
Dresden, ME	Superior	2.0	4.2
Dresden, ME	Katahdin	1.5	2.9
Aroostook County, ME	Ontario	4.1	5.1
Aroostook County, ME	Kennebec	3.6	6.6
Aroostook County, ME	Russet Burbank	10.3	8.3
Aroostook County, ME	Russet Burbank	6.7	7.8
Aroostook County, ME	Russet Burbank	2.8	3.9
Aroostook County, ME	Russet Burbank	5.6	7.4
Aroostook County, ME	Shepody	1.4	2.7
Belfast, ME	Kennebec	3.4	6.6
$\bar{x}$, total glycoalkaloids = 9.7 mg/100 g range total glycoalkaloids = 4.1–18.6 mg/100 g			

Several HPLC solvent systems were evaluated before choosing one for this method. Tetrahydrofuran–water–acetonitrile on an NH_2/CN column was tried but did not give sufficient resolution of the glycoalkaloids from the coextractives. The same solvent system was tried on a C-18 column and a carbohydrate analysis column (Bushway et al., 1979). The C-18 column gave neither sufficient separation of α-solanine from α-chaconine nor sufficient separation of the glycoalkaloids from the coextractives. The carbohydrate analysis column gave good separation of the glycoalkaloids from one another but marginal separation of the glycoalkaloids from the sample coextractives. Several other problems were experienced with this solvent system. A significant loss in sensitivity was experienced due to the necessity of operating at a minimum wavelength of 212 nm because of the high background absorbance caused by tetrahydrofuran (UV cutoff 210 nm). Because of the lack of inhibitors in UV- (HPLC-) grade tetrahydrofuran, it was necessary to use freshly opened bottles. The formation of decomposition products caused the background absorbance to become so high that the solvent mixture was not useable. The level of peroxides in the tetrahydrofuran is also sufficiently high to cause a potential safety problem. Two different lots each from three different manufacturers were tested, and peroxide levels ranged from 20 to 80 parts per million (ppm) in freshly opened bottles. Levels in bottles opened for longer than

3 days were noticeably higher, >200 ppm. Acetonitrile–water containing ethanolamine on C-8 and C-18 columns (Morris and Lee, 1981) gave adequate separation but was inconvenient to use because of the necessity of carefully adjusting the pH of the mobile phase. The system described in this paper gives good separation with the advantages of not having to adjust the pH of the mobile phase and a slightly lower background absorbance compared to ethanolamine. The selectivity of the system can be adjusted by changing the concentration of ammonium phosphate in the mobile phase.

Some work has been performed in this laboratory to evaluate the utility of the method for determining the glycoalkaloids that are present in other members of the Solanaceae. Slight changes in the strength of the wash and final elution solvents permitted the determination of the glycoalkaloids tomatine (present in green tomatoes) and soalsonine (present in eggplant). However, the minimum detectable quantity of tomatine is approximately one-tenth that of solanine and chaconine (detection range 2–200 $\mu g/g$) due to the lack of the unsaturated double bond at the Δ^5 position, which results in a lower molar absorptivity. Adjustments in the mobile phase composition would also be necessary for optimum separation if all four of the above glycoalkaloids were to be determined simultaneously. This would not likely be necessary as all four glycoalkaloids do not occur in any sizable amounts in any one of the aforementioned commodities. The method may also be applicable to the determination of other glycoalkaloids, including those that are not readily precipitated by base (Zitnak, 1968).

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Possible Misidentification of Pipecolic Acid in HPLC Analysis of Legume *Phaseoleae* Seed Oligosaccharides

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This paper describes the purification, identification, and quantitation by high-pressure liquid chromatography (HPLC) of L-pipecolic acid, an unusual amino acid that was mainly found in common bean (*Phaseolus vulgaris*). Seeds from various species from *Phaseolus* and *Vigna* genera were examined for both their L-pipecolic acid and sugar contents by HPLC.

INTRODUCTION

Dry beans constitute a preponderant portion of traditional diet in many areas including India, Africa, and Central and North America. Apart major constituents (protein and starch) legume seeds contain free amino acids among which uncommon amino acids such as α- and γ-diaminobutyric acid may exhibit strong metabolic interactions in animals fed with legume-containing diets (Ressler et al., 1961). They contain also other minor components such as oligosaccharides, mainly α-D-galactosides, which have been also extensively investigated (Cerning-Beroard and Filiatre, 1976; Naivikul and d'Appolonia, 1978; Schweizer et al., 1978; Aman, 1979; Quemener and Mercier, 1980; Fleming, 1981; Sosulski et al., 1982; Sathe et al., 1983). High-performance liquid chromatography (HPLC) is the choicest method for analysis of oligosaccharides, detection being performed by differential refractometry. However, some noncarbohydrate compounds may interfere by yielding a refractive index response. Quemener and Brillouet (1983) have reported an unidentified compound in common bean (*P. vulgaris*) eluted before the succrose peak in HPLC analysis. We now report the purification, identification, and quantitation of

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