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Review

Analysis of biologically active compounds in potatoes (*Solanum tuberosum*), tomatoes (*Lycopersicon esculentum*), and jimson weed (*Datura stramonium*) seeds

Mendel Friedman*

Western Regional Research Center, Agricultural Research Service, US Department of Agriculture, 800 Buchanan Street, Albany, CA 94710, USA

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Abstract

Potatoes and tomatoes, members of the *Solanaceae* plant family, serve as major, inexpensive low-fat food sources providing for energy, high-quality protein, fiber, vitamins, pigments, as well as other nutrients. These crops also produce biologically active secondary metabolites, which may have both adverse and beneficial effects in the diet. This limited overview, based largely on our studies with the aid of HPLC, TLC, ELISA, GC–MS, and UV spectroscopy, covers analytical aspects of two major potato trisaccharide glycoalkaloids, α-chaconine and α-solanine, and their hydrolysis products (metabolites) with two, one, and zero carbohydrate groups; the potato water-soluble nortropane alkaloids calystegine A_3 and B_2; the principal potato polyphenolic compound chlorogenic acid; potato inhibitors of digestive enzymes; the tomato tetrasaccharide glycoalkaloids dehydrotomatine and α-tomatine and hydrolysis products; the tomato pigments β-carotene, lycopene, and chlorophyll; and the anticholinergic alkaloids atropine and scopolamine present in *Datura stramonium* (jimson weed) seeds that contaminate grain and animal feed. Related studies by other investigators are also mentioned. Accurate analytical methods for these food ingredients help assure the consumer of eating a good-quality and safe diet.
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Keywords: Reviews; Potatoes; Tomatoes; Jimson weed; Food analysis; Alkaloids; α-Chaconine; α-Solanine

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* Tel.: +1-510-559-5615; fax: +1-510-559-5777.
E-mail address: mfried.@pw.usda.gov (M. Friedman).

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1. Introduction

All plants including *Solanaceous* plants such as potato, tomato, jimson weeds, eggplants, and peppers synthesize a variety of compounds which serve as natural defenses against plant phytopathogens including fungi, viruses, bacteria, insects, and worms; reviewed in [1–10]. This review summarizes and integrates several analytical techniques we developed to analyze biologically active compounds of *Solanum* plants consumed by animals and humans. Our cited studies and those by other investigators offer an entry into the extensive analytical literature on these subjects.

The described results should facilitate measuring changes in levels of these biologically active plant compounds (a) as influenced by different plant growing conditions and locations; (b) during large-scale plant breeding and molecular biology studies designed to develop improved plant varieties; (c) during harvesting and post-harvest handling, storing, and sampling of the crops; and (d) in nutritional and toxicological assessment of dietary roles following consumption by animals and humans.

For the purpose of this study, we define the following terms: *glycoalkaloids*—naturally occurring, nitrogen-containing plant steroids each with a carbohydrate side chain attached to the 3-OH position, e.g. α-chaconine and α-solanine from potatoes, dehydrotomatine and α-tomatine from tomatoes; *aglycones*—the steroidal part of the glycoalkaloid, lacking the carbohydrate side chain, e.g., solanidine from α-chaconine and α-solanine, tomatidenol from dehydrotomatine, and tomatidine from α-tomatine; *tomatine*—mixture of dehydrotomatine and α-tomatine; *alkaloids*—aglycones as above, calystegines from potatoes, and atropine and scopolamine from jimson weed seeds. The following abbreviations are also used: ELISA, enzyme-linked immunosorbent assay; GC–MS, gas chromatography–mass spectrometry; HPLC-PAD, high-performance liquid chromatography with pulsed-amperometric detection; HPLC-UV, HPLC with UV detection; TLC, thin layer chromatography; and UV, ultraviolet spectroscopy.

2. Results and discussion

Figs. 1–3 depict the structures of the compounds evaluated in this study and Figs. 4 and 5 shows HPLC chromatograms and UV spectra of biologically active potato, tomato, and jimson-weed-seed-ingredients. Concentrations of compounds in various matrices based on the analyses are shown in Tables 1–5 for potatoes and in Tables 6–12 for tomatoes.

2.1. Biological effects of potato glycoalkaloids

The potato glycoalkaloids α-chaconine and α-solanine serve as natural defenses against insects and other pests. In some varieties and under certain storage conditions, the concentration of these steroidal glycosides can be toxic to humans as well as to insects. To reduce the content of the most toxic alkaloids in potatoes, methods are needed to analyze and eventually decrease levels of these compounds.

To meet this need, studies were carried out that included defining the relative safety of glycoalkaloids using the Frog Embryo Teratogenesis (FETAX) and membrane potential assays as well as feeding studies in mice designed to elucidate molecular mechanisms of glycoalkaloid toxicity and its prevention [11–29]. The data show that: (a) mixtures of two glycoalkaloids act synergistically; (b) for glycoalkaloids, the nature and number of carbohydrate groups in the side chain influence potency; removal of one or two carbohydrates from the trisaccharide side chain lowers toxic potency; (c) the change in membrane potential correlated with the teratogenicity and survival of the embryos; (d) folic acid, glucose-6-phosphate, and nicotine adenine dinucleotide (NAD) protected frog embryos against the most potent potato glycoalkaloid α-chaconine; (e) the glycoalkaloids do not interact with DNA or chromosomes (Ames and micronucleus tests); (f) glycoalkaloids inhibit the growth of *Phytophthora infestans* potato fungi [30]. Other studies have shown that potato glycoalkaloids adversely affect inflammatory bowel disease in mice [31].

Beneficial effects of potato and tomato glycoalkaloids include inactivation of the herpes simplex virus [32], protection of mice against infection by *Salmonella typhimurium* [33], enhancement of general anesthetics that inhibit cholinesterase [34], potentiation of a malaria vaccine [35], lowering of plasma cholesterol in hamsters [27], and inhibition of growth of human colon and liver cancer cells [36].

The discovery that alkaloid-induced liver enlargement in mice is reversed after the alkaloid is removed from the diet suggests that the change in liver size is a benign event, i.e. an adaptive response [22,29]. Our studies also imply that dietary folic acid, glucose 6-phosphate, and NAD may protect humans against adverse effects of glycoalkaloids. These protectors as food supplements are expected to have a beneficial impact on food safety and nutrition. The observed

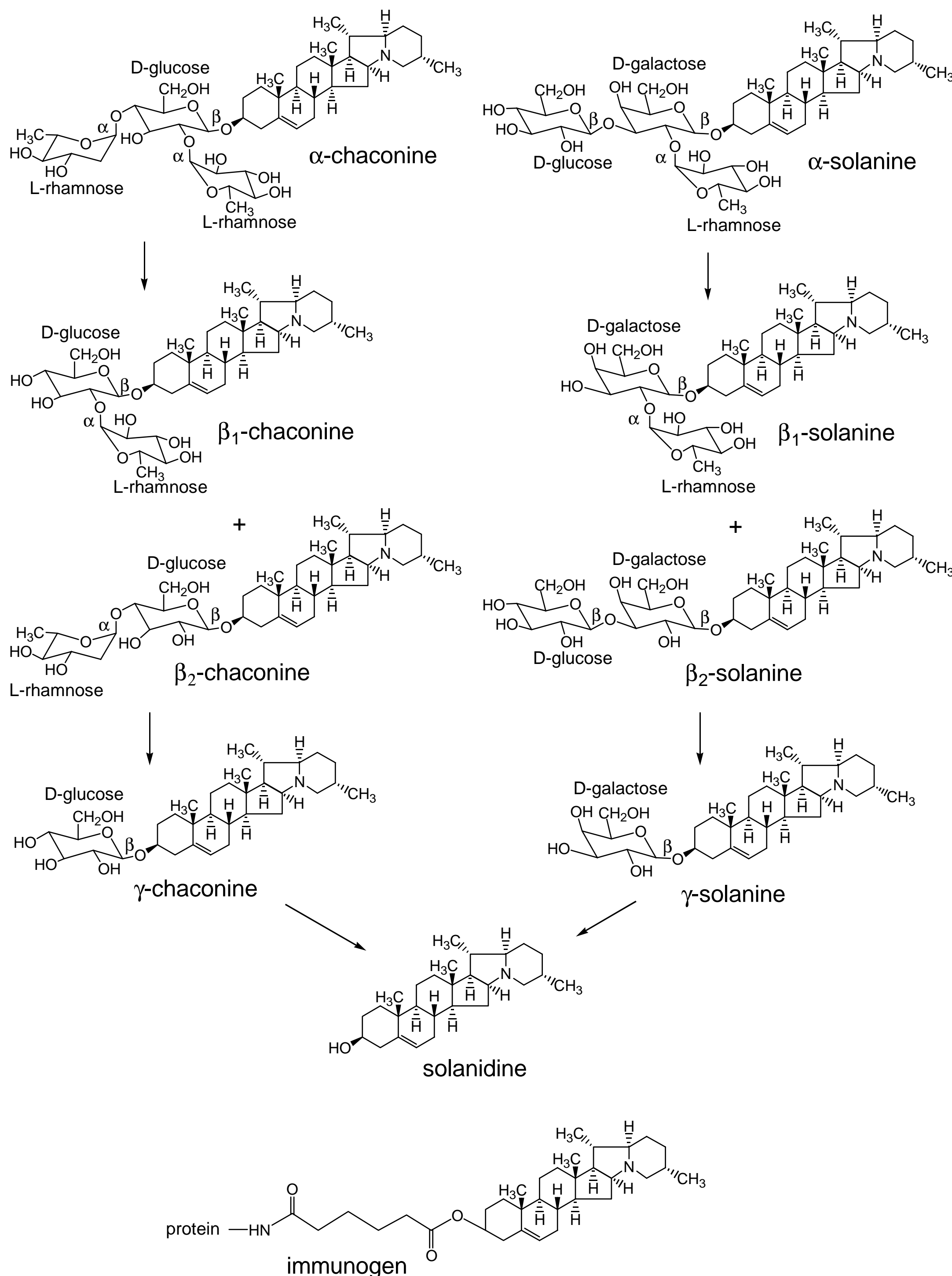

Fig. 1. Structures of potato glycoalkaloids α-chaconine and α-solanine, glycoalkaloid hydrolysis products, and of the solanidine immunogen used to generate monoclonal antibodies in mice for immunoassays of potato and tomato glycoalkaloids.

structure-activity relationships make it easier to (a) predict developmental toxicities of compounds of dietary interest using in vitro FETAX, thus minimizing the use of live animals and (b) suppress the formation of the most toxic compounds in potatoes through plant molecular biology programs.

2.2. Glycoalkaloid content of potatoes and potato products

Although glycoalkaloids appear to be largely unaffected by food processing conditions such as baking, cooking, and frying, the glycoalkaloid content can vary

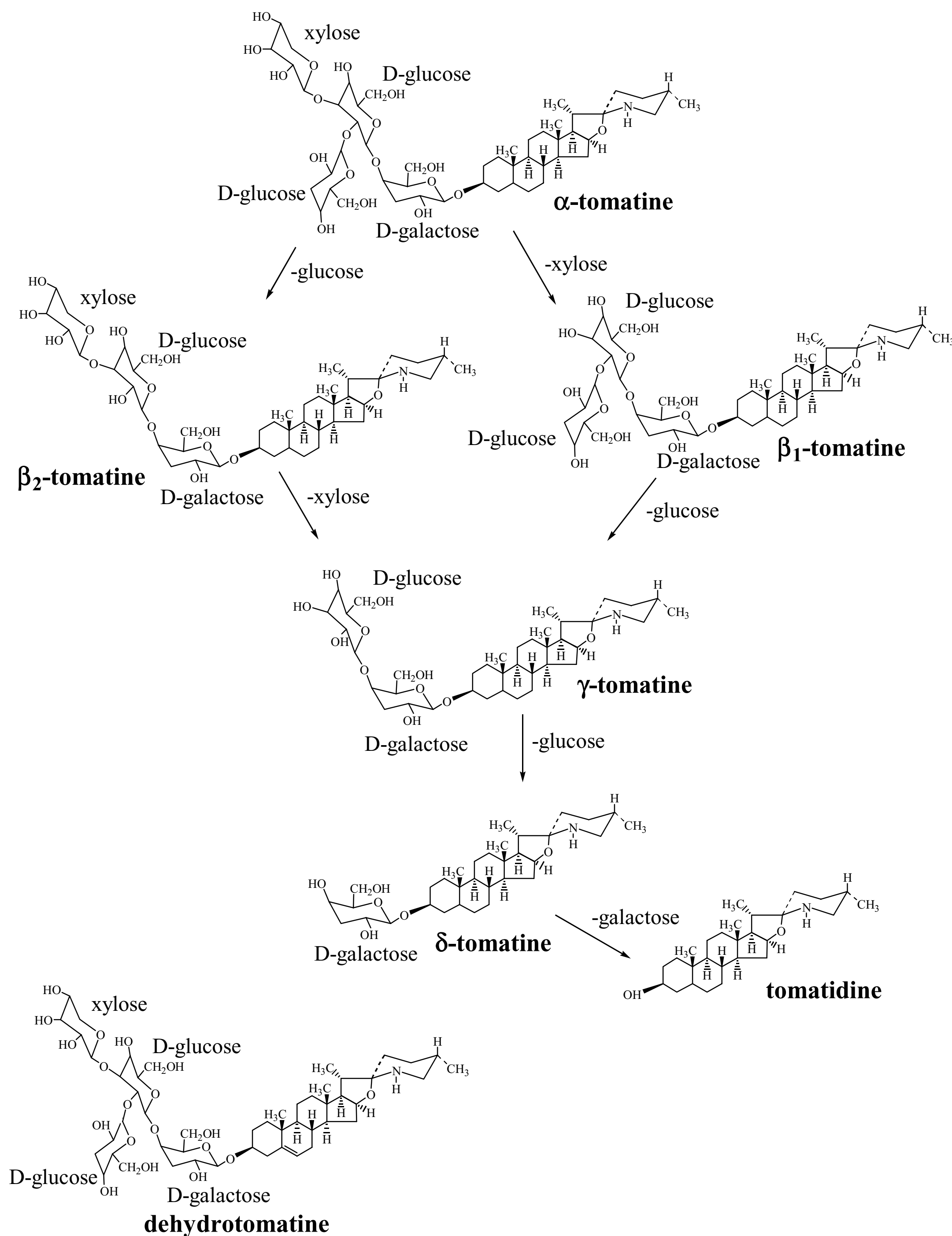

Fig. 2. Structures of tomato glycoalkaloids α-tomatine, α-tomatine hydrolysis products, and of dehydrotomatine.

greatly in different potato cultivars and may be influenced post-harvest by environmental factors such as light, irradiation, mechanical injury, and storage. 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.

Methods used for the analysis of potato extracts containing glycoalkaloids include spectrophotometry [37], TLC [38–41], isotachophoresis [42], GC [43,44], MS [45,46], GC–MS [47], electrophoresis–MS [48], immunoassays [49–55], biosensors [56,57], and HPLC [58–73]. Although these methods usually generate similar results, advantages of the widely used HPLC procedures include analysis at room temperature and simultaneous analysis of individual

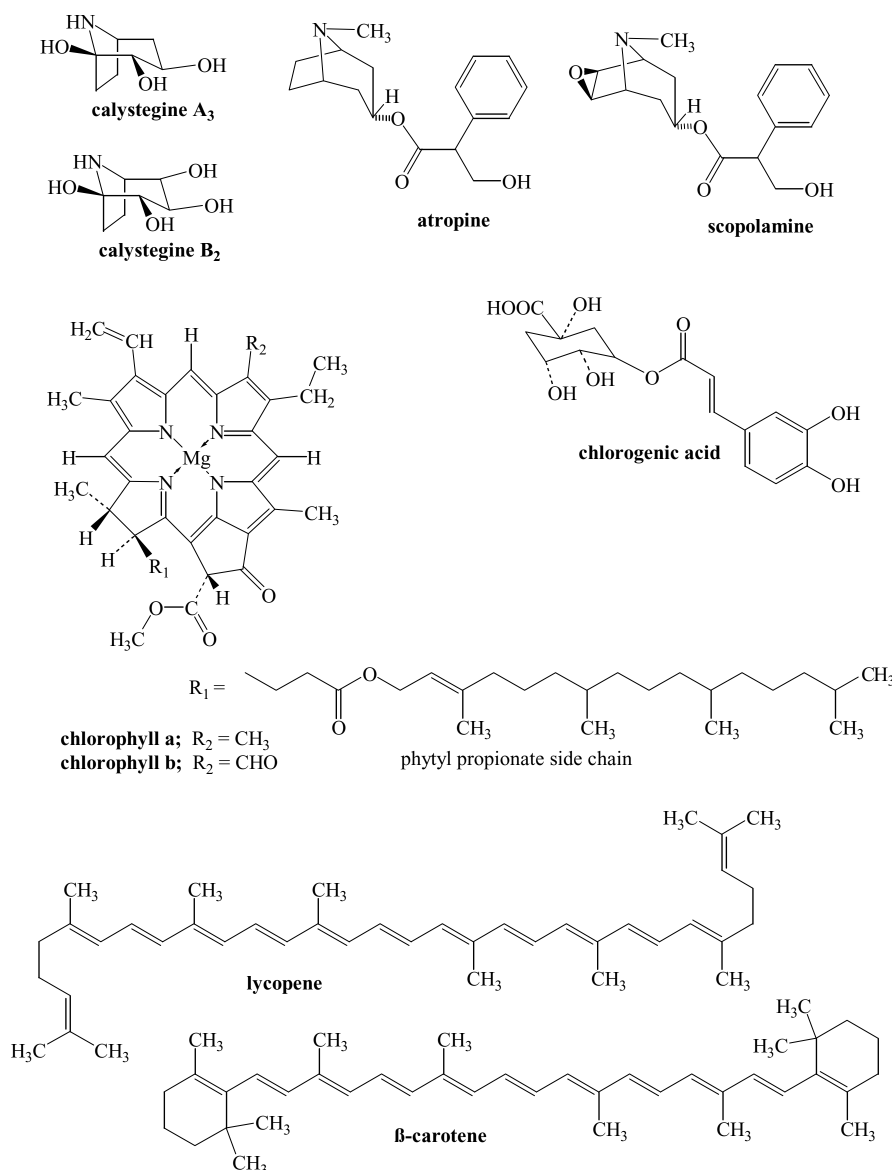

Fig. 3. Structures of nonsteroidal, biologically active plant compounds discussed in the text.

structurally related glycoalkaloids and hydrolysis products without derivatization.

The extraction-clean-up steps are of paramount importance in all analyses [5,74–76]. An assessment of the literature indicates that optimizing one part of the extraction-clean-up procedure may cause problems in another part. Thus, the most efficient extraction solvent may co-extract compounds that will interfere in the analysis or require extensive clean-up. Extracts of freeze-dried potatoes are also much easier to clean up since they are not subject to browning [77].

Advantages of using freeze-dried (dehydrated) compared to fresh potato samples include the cessation of enzyme-catalyzed, wound-induced, and moisture-dependent compositional changes that may affect glycoalkaloid content; the ability to store samples for analysis at different time periods and by different investigators; and the availability of portions of the same sample to measure other potato ingredients (protein, carbohydrate, vitamins) for nutritional and other studies.

Extensive efforts have been made to improve HPLC analyses with respect to sample preparation and clean-up, column selection, and peak detection. For example, based on an evaluation of ten commercially available HPLC columns with reversed-phase packing, Friedman and Levin [71] found that column acidity caused by active silanol sites on the surface of the packing strongly influenced the chromatographic separation of *Solanum* glycoalkaloids. Because this brief review is limited to describing our own efforts to improve the analyses, discussion of incremental improvements in the cited HPLC methods is beyond the scope of this paper.

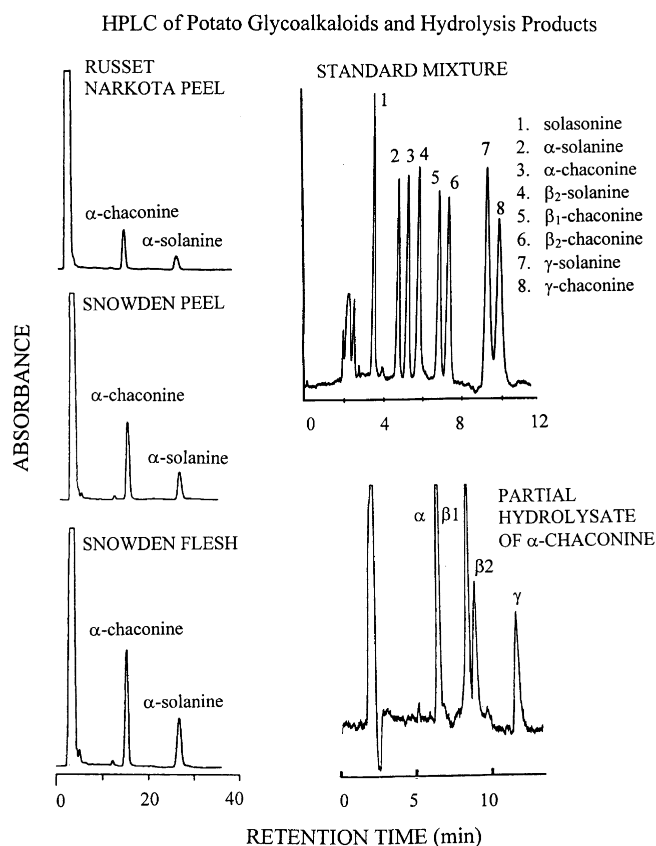

Fig. 4. HPLC chromatograms of potato glycoalkaloids and their hydrolysis products. Adapted from [73] (glycoalkaloids); [71,82] (glycoalkaloid hydrolysis products).

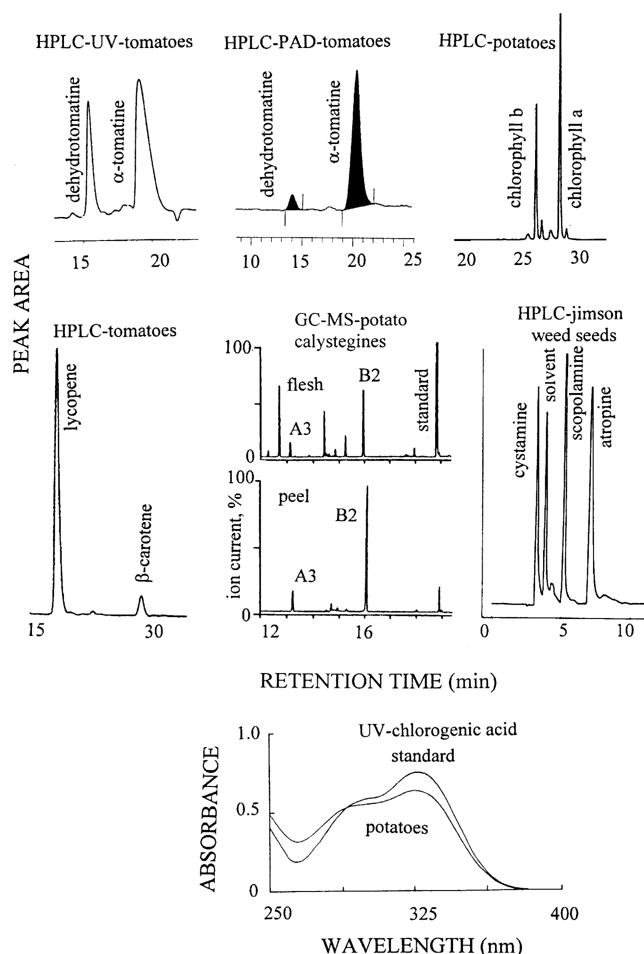

Fig. 5. HPLC chromatograms and UV spectra of biologically active compounds in potatoes, tomatoes, and jimson weed seeds. Adapted from [99] (tomato glycoalkaloids); [101] (chlorophyll, lycopene, and β-carotene); [73] (calystegines); [109] (atropine and scopolamine with cystamine as internal standard); [78,83,116] (chlorogenic acid).

ELISA may have advantages over HPLC with respect to sensitivity, rapidity, and cost [53]. However, a major disadvantage is that ELISA measures total, not individual, glycoalkaloid content of potatoes. Below are described some of our efforts to improve analysis by HPLC.

HPLC methods are now widely used to determine the concentrations of individual glycoalkaloids of fresh and processed potatoes, different parts of the potato plant such as leaves and sprouts, and glycoalkaloid hydrolysis (glycolysis) products. However, the peaks on HPLC chromatograms are not always well separated and frequently do not exhibit good symmetry or minima (tailing bands at the baseline). To improve the HPLC method, we systematically evaluated several parameters expected to influence the chromatographic separation of α-chaconine and α-solanine mixtures in pure form and in extracts of potatoes, as described below.

Our initial studies developed HPLC methods for the analysis of the glycoalkaloid content in fresh and dehydrated potatoes, different parts of the potato plant, and processed potato products [70,71,78,79]. To further improve the separation and analysis by HPLC of the two major glycoalkaloids present in potatoes, additional studies were carried out on separation efficacy of the following parameters: elution times; composition and pH of the mobile phase (acetonitrile and phosphate buffer); concentration of buffer; capacity values of column packing of HPLC amino columns; and column temperature. All of the variables except pH significantly influenced the separation values [73]. The following conditions gave optimum separation of α-chaconine and α-solanine: column, Nucleosil NH_2 (5 μM, 4.0 × 250 mm); mobile phase, acetonirile/20 mM KH_2PO_4 80:20; flow rate, 1 mL/min; column temperature, 20 °C; UV detector, 208 nm; sample size, 20 μL. Calibration showed high linearity (r = 0.99) and spiking showed recovery rates between 90 and 92% with a lower limit of detection of ∼150 ng.

The improved method, based on the optimum efficiency of each parameter, was then evaluated with extracts of dehydrated whole potatoes, potato peel, and potato flesh. Fig. 4 depicts the excellent separation of mixtures of the two glycoalkaloids on the chromatographic the column. Tables 1–3 show the wide-ranging glycoalkaloid content of different potato cultivars.

Table 1
Glycoalkaloid content of extracts potato flesh, peel, and whole potatoes (in μg/g)

Sample (dehydrated powders)	α-Chaconine (A)	α-Solanine (B)	Total (A + B)	Ratio (A/B)
Atlantic potato peel	59.4	24.4	83.8	2.43
Atlantic potato flesh	22.6	13.9	36.5	1.63
Russet Narkota potato peel	288	138	425	2.09
Russet Norkota potato flesh	3.7	2.7	6.4	1.37
Dark Red Norland potato peel	859	405	1,264	2.12
Dark Red Norland potato flesh	16.0	6.1	22.1	2.62
Snowden potato peel	2,414	1,112	3,526	2.17
Snowden potato flesh	366	226	591	1.62
Russet whole potatoes	65.1	35.0	100	1.86
White whole potatoes	28.2	15.3	43.5	1.84
Benji whole potatoes	70.7	27.6	98.3	2.56
Lenape whole potatoes	413	216	629	1.91

Adapted from [73].

2.3. Glycoalkaloids in potato leaves

Attempts to measure potato leaf glycoalkaloids indicated variability among similar plants, suggesting that a single small sampling of a young plant might not be a reliable measure of composition [80,81]. A greenhouse study on the glycoalkaloid content of leaves of growing potato plants revealed that analyzing single leaves from the same stem position of each plant can minimize variability. By contrast, a comparison involving leaves obtained from different positions on the potato plant indicated that the glycoalkaloid content varied with time and position on the stem. Therefore, in large-scale breeding programs involving repeated planting and analyses, plants of one or more control and wild varieties should always be grown to enable comparison of levels present in the controls to those found in the experimental varieties. These studies also demonstrate the usefulness of extraction, purification, and analysis by HPLC of both fresh and dehydrated potato leaves.

2.4. Comparison of HPLC and ELISA for potato glycoalkaloids

The usefulness of ELISA, based on monoclonal antibodies, and using the immunogen shown in Fig. 1, was evaluated to measure potato glycoalkaloids extracted from potatoes, potato leaves, sprouts, and processed potato products (French fries, potato chips, potato skins) [50,51,53]. The results from the ELISA for total glycoalkaloids were then compared with those obtained by HPLC analysis of the same extracts for the sum of α-chaconine and α-solanine. The agreement of the two methods shown in Table 3 reinforces the validity and utility of both HPLC and ELISA.

2.5. Potato glycoalkaloid hydrolysis products

Since both the nature and number of sugar groups associated with glycoalkaloids influence biological activities, procedures were developed for preparation, isolation, and analysis by TLC, HPLC, and GC–MS of acid hydrolysis products derived from α-chaconine and α-solanine [72,82]. Figs. 1 and 2 show the compounds that theoretically could be derived from hydrolysis of the parent potato and tomato glycoalkaloids. Table 4 lists the TLC Rf values for the hydrolysis products; these Rfs ensured adequate separation with very little spreading of spots. The HPLC chromatograms in Fig. 4 show good separation of mixtures of hydrolysis products. The data also indicate that hydrolytic stabilities of the carbohydrate groups attached to

Table 2
Glycoalkaloid content of processed commercial potato products (in mg/kg)

Processed potato product	α-Chaconine (A)	α-Solanine (B)	Total (A + B)	Ratio (A/B)
French fries, A[a]	0.4	0.4	0.8	1.00
French fries, B[a]	4.2	4.2	8.4	1.00
Wedges[a]	23.9	20.1	44.0	1.18
Chips, A	13.0	10.5	23.8	1.23
Chips, B	31.6	17.6	49.2	1.79
Chips, C	58.8	50.2	109.0	1.17
Skins, A	38.9	17.4	56.3	2.23
Skins, B	44.0	23.6	67.6	1.86
Skins, C	116.1	72.3	188.4	1.60
Skins, D	119.5	83.5	203.0	1.43
Pancake powder, A	20.5	24.1	44.6	0.82
Pancake powder, B	24.8	19.4	44.2	1.27

Adapted from [6,70,79].
[a] Values are for dehydrated powders. All other values are for original products.

Table 3
Comparison of glycoalkaloid content of the same potatoes and potato products analyzed by HPLC (sum of α-chaconine and α-solanine) and ELISA

Sample	Assay method	
	HPLC Fresh (mg/kg)	ELISA Fresh (mg/kg)
Whole potatoes		
Russet, organic	5.8	5.1
Russet	22	24
Yukon Gold	40	38
Purple, small	45	37
Red, small	101	128
Gold, small	105	113
White, large	125	132
White small	203	209
	Dehydrated (mg/kg)	Dehydrated (mg/kg)
Potato plant parts		
Flesh, Red Lasoda	45.6	51.6
Peel, Shepody	1432	1251
Sprouts, Shepody	7641	6218
Leaves	9082	8851
	Original (mg/kg)	Original (mg/kg)
Processed potatoes		
French fries, A	0	1.2
French fries, B	24.1	22.7
Chips, low-fat	15.2	22.7
Skins, A	43.3	35.0
Skins, B	37.2	41.0

Adapted from [53].

α-chaconine and α-solanine in a potato matrix appear to be similar to those of the pure compounds.

It was possible to optimize the formation of specific compounds by varying the hydrolysis conditions in acidified aqueous-alcoholic solvents. These results should make it easier to characterize biosynthetic intermediates in plants and metabolites in animal tissues, and to assess relative safety. Attempts to isolate β_1-solanine from a partial hydrolysate of α-solanine were unsuccessful.

Table 4
Rf values determined by TLC for potato glycoalkaloids and hydrolysis products[a]

Compound	Rf	Rf/Rf of α-solanine
α-Solanine	0.11	1.00
β_2-Solanine	0.23	2.09
γ-Solanine	0.46	4.18
α-Chaconine	0.20	1.82
β_1-Chaconine	0.27	2.45
β_2-Chaconine	0.37	3.36
γ-Chaconine	0.50	4.55
Solanidine	0.84	7.64

Adaped from [71,72,82].
[a] Solvent: chloroform–methanol–2% NH_4OH, 70:30:5.

Table 5
Calystegine content of fresh potato flesh, potato peel, and whole potatoes (in mg/kg)

Potato cultivar	Potato part	Calystegine A_3	Calystegine B_2	$A_3 + B_2$	B_2/A_3
Atlantic	Flesh	1.1	1.5	2.6	1.4
	Peel	31.2	141	172	4.5
	Whole	3.5	12.9	16.4	3.7
Dark Red Norland	Flesh	0	1.3	1.3	-
	Peel	6.4	33.3	39.7	5.2
	Whole	0.7	4.7	5.4	6.7
Ranger Russet	Flesh	1.1	2.3	3.4	2.1
	Peel	87.1	380	467	4.4
	Whole	9.6	39.7	49.3	4.1
Red Lasoda	Flesh	1.4	4.3	5.7	3.1
	Peel	10.5	24.8	35.3	2.4
	Whole	2.2	6.1	8.3	2.8
Russet Burbank	Flesh	11.1	56.5	67.6	5.1
	Peel	6.6	67.8	74.4	11.8
	Whole	10.8	57.3	68.1	5.3
Russet Norkota	Flesh	0.2	0.8	1.0	4.0
	Peel	33.6	129	163	3.9
	Whole	3.0	11.9	14.9	4.0
Shepody	Flesh	2.2	9.1	11.3	4.1
	Peel	44.0	299	343	6.8
	Whole	5.6	33.1	38.7	5.9
Snowden	Flesh	0.8	0.8	1.7	1.0
	Peel	54.2	96.3	150	1.8
	Whole	5.8	10.2	16.0	1.8

Adapted from [73].

Table 6
Comparison of chlorogenic and glycoalkaloid content of parts of the NDA1725 potato plant (in mg/kg of fresh weight)

Sample	Chlorogenic acid	Glycoalkaloids
Potato tuber	174	150
Potato roots	263	860
Potato leaves	2235	1450
Potato sprouts	7540	9970

Adapted from [78,83].

Table 7
Dehydrotomatine and α-tomatine content of parts of the tomato plant (in mg/kg of fresh weight)

Tomato plant part	Dehydrotomatine	α-Tomatine	Dehydrotomatine (%)
Large immature green fruit	14	144	8.8
Roots	33	118	23
Small immature green fruit	54	465	10
Calyxes	62	795	7.3
Leaves	71	975	6.9
Small stems	138	896	13
Large stems	142	465	25
Flowers	190	1100	15
Senescent leaves	330	4900	6.1

Adapted from [99,102].

Table 8
Tomatine content of store-bought tomatoes

Tomato	mg/kg	
	Dry weight	Fresh weight
Beefsteak	15	0.9
Roma	7	0.4
Standard tomato	4	0.3
Cherry	42	2.8

Adapted from [96,97].

Table 9
Tomatine content per fruit

Variety	Fruit weight (g)	mg/kg	mg/fruit
Ripe			
Sungold cherry	3.9	11	0.04
Red pear cherry	6.5	1.3	0.01
Yellow pear cherry	10.3	4.5	0.05
Yellow cherry	11.4	9.7	0.11
Tomatillos	34.0	0.5	0.02
Green Zebra	66.9	0.6	0.04
Standard	123	0.3	0.04
Large yellow	227	1.1	0.24
Unripe green			
Small immature	3.4	548	1.86
Medium immature	17.1	169	2.88
Large immature	37.9	10	0.39
Pickled	80.0	28	2.20
Mature	127	16	2.04

Adapted from [96,97,101].

Table 10
Tomatine content of processed tomato products (in mg/kg)

Sample	Fresh	Dehydrated
Stewed red tomatoes	11	20
Tomato juice	28	49
Tomato red sauce	57	50
Fried green tomatoes	11	44
Microwaved green tomatoes	12	134
Tomato ketchup	25	–
Pickled green tomatoes, A	28	353
Pickled green tomatoes, B	72	989

Adapted from [96,97].

Table 11
Comparison of tomatine content of dehydrated tomatoes and tomato products determined by HPLC and ELISA (in mg/kg)

Sample	HPLC	ELISA
Manteca red tomato	10	11.3
Manteca green tomato	308	312
Breaker tomato	77	75
Large immature tomato	397	386
Mature green tomatoes	144	135
Tomato roots	376	377
Tomatillos	6	6.1
Canned tomato sauce	64	57
Pickled tomatoes	121	114

Adapted from [10,51].

Table 12
Tomatine content of field-grown standard and transgenic tomatoes at different stages of maturity (in mg/100 g of fresh weight)

Tomato variety	Immature green	Mature green	Breaker	Red
Standard, parent	35	6.8	3.5	0.7
Standard, transgenic	12	15	6.2	1.1
Cherry, commercial	210	55	14	2.5
Cherry, transgenic	280	52	27	3.9
Cherry, transgenic	190	58	21	2.6

Adapted from [96,97].

2.6. Calystegine A_2 and B_2 content of potatoes

In addition to glycoalkaloids, potatoes also contain two water-soluble, biologically active (glycosidase-inhibiting) nortropane alkaloids, calystegine A_3 and B_2, whose structures resemble those of atropine, discussed below (Fig. 3) [73]. It was of interest to assess variations in the levels of both glycoalkaloids and calystegines in the different potato cultivars in order to examine possible compositional and biosynthetic relationships between the two classes of secondary metabolites in potatoes. Table 5 shows the calystegine A_3 and B_2 content of the same potato flesh and peel samples mentioned above, determined by GC–MS. The results showed that, as is the case with glycoalkaloids, the calystegine content varied widely among the different potato cultivars and is mostly present in the peel. The dietary significance of calystegine-containing potatoes is not known.

2.7. Chlorogenic acid and protease inhibitors in potatoes

The polyphenolic compound chlorogenic acid (Fig. 3), glycoalkaloids, and protein inhibitors of carboxypeptidase, chymotrypsin, and trypsin present in potatoes may act as so-called anti-feeding agents of the potato plant. HPLC and UV spectroscopy (Fig. 5) were used to measure chlorogenic acid, the most abundant potato polyphenol, in fresh and processed potatoes [78,83,84]. The observation that chlorogenic acid underwent a light-dependent change in alcoholic extracts of potatoes is of analytical interest. Moreover, because chlorogenic acid is not stable at high pH, it may not be active as an antioxidant in alkaline solutions [85].

Additional observations included the following: (a) the chlorogenic acid content of leaves, roots, sprouts, stems, and tubers paralleled their glycoalkaloid content (Table 6); (b) boiling and baking destroyed chlorogenic acid in potatoes, whereas microwaving was less destructive; (c) commercial potato products (chips, fries, skins) contained no chlorogenic acid; and (d) exposure of potatoes to light increased their content of chlorogenic and glycoalkaloids but not protease-inhibitors. Light also induced an increase in the chlorogenic acid content of potato tubers [86].

Dehydrated White Rose potatoes contained the following amounts of protease inhibitors (in units/g): trypsin, 1020;

chymotrypsin, 370; and carboxypeptidase A, 112. Dehydrated potatoes contain ∼25% and fresh potatoes ∼6% of the corresponding values for soybeans [87]. It is not known whether potato protease inhibitors can prevent cancer, as appears to be the case for the Bowman–Birk inhibitor (BBI) of soybeans [88].

The reviews by Robbins [89] on analytical methods of phenolic acids in foods, by Friedman [84] on the chemistry and plant physiology of chlorogenic acid in potatoes, and by Schoeffs [90] on methods of analysis of chlorophyll and carotenoids in foods (see Fig. 5) complement the analytical data discussed in this brief overview.

3. Tomato glycoalkaloids

3.1. General aspects

Tomatoes, a major food source for humans, accumulate a variety of secondary metabolites including phenolic compounds, phytoalexins, protease inhibitors, carotenoids, lycopenes, and glycoalkaloids [10]. These metabolites protect against adverse effects of many predators including fungi, bacteria, viruses, and insects. Since tomato glycoalkaloids are reported to be involved in host-plant resistance and to have a variety of pharmacological and nutritional properties in animals and humans, better understanding is needed of the role of these tomato compounds both in the plant and in the diet. As mentioned earlier, reported beneficial effects of α-tomatine in the human diet include lowering cholesterol and triglycerols, enhancing the immune system, inhibiting the growth of cancer cells, and protecting against virulent bacteria. The development of transgenic tomatoes has also stimulated interest in determining whether their glycoalkaloid content differs significantly from that found in standard varieties during different stages of fruit maturity.

Detection of tomato glycoalkaloids is therefore of interest. Methods used to analyze tomatine include GC, GC–MS, and HPLC, reviewed in [10]. Reported HPLC methods for the analysis of tomatine relevant to the theme of this paper are described in [91–102].

To improve analytical methods for tomato glycoalkaloids, extensive studies were carried out on different parts of the tomato plant, commercial red tomatoes, field grown standard and transgenic tomatoes at different stages of maturity, and processed tomato products including juice, ketchup, salsa, sauce, and sun dried tomatoes. The following discussion coves our experimenal findings on the analysis of tomato glycoalkaloids, assessment of the results, and relevant studies by other investigators.

3.2. Dehydrotomatine and α-tomatine

The glycoalkaloid known as tomatine consists of a mixture of two glycoalkaloids, α-tomatine and dehydrotomatine (Fig. 2) [96–99,102]. Both compounds are present in all parts of the tomato plant. Immature green tomatoes contain up to 500 mg α-tomatine/kg of fresh fruit weight. Tomatine is largely degraded as the tomato ripens, to levels of about 5 mg/kg of fresh fruit weight in red tomatoes (Tables 7–11). Microwaving and frying did not affect tomatine levels of foods.

Consumers of green tomatoes, high-tomatine red tomatoes, and tomato products such as pickled green and green fried tomatoes consume significant amounts of tomatine. The compositions of green and red tomatoes are similar except that the former contain chlorophyll but no lycopene and about 100 times more tomatine than the latter [101]. A variant indigenous to Peru produces tomato fruit with a very high α-tomatine content, in the range 500–5000 mg/kg of dry weight. The Peruvians seem to enjoy eating these bitter high-tomatine tomatoes, which are consumed without apparent acute toxic effects. Thus tomatine seems to be much safer for humans than are potato glycoalkaloids.

3.3. Transgenic tomatoes

Use of gene manipulation to introduce desirable traits into tomatoes, such as tolerance to stress and pesticides and resistance to phytopathogens raises questions about the tomatine content of the transgenic tomatoes. A priori, it cannot be predicted whether or not the genes encoding the formation of enzymes involved in tomatine synthesis are metabolically linked to the manipulated genes. The α-tomatine and dehydrotomatine content in a variety of field grown tomatoes supplied by several companies, including transgenic, slow-ripening tomatoes at different stages of maturity were analyzed by HPLC. The tomatine levels of the transgenic tomatoes were not different from those seen in the standard varieties grown under the same field conditions (Table 12).

3.4. Tomatine hydrolysis products

Studies were carried out to optimize the acid hydrolysis of the tetrasaccharide side chain of α-tomatine to products with three, two, one, and zero sugar groups (Fig. 2) [100]. This was accomplished by following the time course for hydrolysis in 1N HCl at 100 °C, isolating the hydrolysis products by chromatography on an aluminum oxide column, and determining the number and nature of hydrolysis products, including free sugars, with the aid of TLC and GC–MS of alditol acetate sugar derivatives. A 20 min hydrolysis time was useful for the formation of a mixture of the monosaccharide δ-tomatine; the disaccharide γ-tomatine; and the trisaccharide β_1-tomatine. Efforts to isolate the other possible trisaccharide, β_2-tomatine, were unsuccessful, apparently because its xylose moiety is degraded during the hydrolysis. A 70 min exposure achieved complete hydrolysis of α-tomatine to tomatidine. α-Tomatine was stable to hydrolysis at 37 °C, suggesting that it may be stable at acid pH values of the gut of insects, animals, and humans. Both tomatine and hydrolysis products inhibited the growth of human cancer cells [36].

3.5. Inheritance of tomato glycoalkaloids

To demonstrate the transfer of useful traits from wild species, glycoalkaloid profiles and other characteristics were determined using potato tubers of somatic hybrids whose progenies were the cultivated potato *Solanum tuberosum*, containing α-chaconine and α-solanine, and the wild type *Solanum acuale*, containing α-tomatine and demissine [103]. TLC, HPLC, and GC–MS studies revealed that all somatic hybrids contained all four glycoalkaloids derived from the fusion parents. The total glycoalkaloid levels of most hybrids were intermediate between those of their parents. The data suggest that glycoalkaloids, including tomatine, can be passed to progenies during breeding programs designed to develop improved potatoes as well as tomatoes.

3.6. Assessment of HPLC methods for dehydrotomatine and α-tomatine

To place our findings in proper perspective, it is relevant to briefly examine reported analytical studies of dehydrotomatine and α-tomatine. The observation that commercial tomatine consists of a mixture of the known tomato glycoalkaloid α-tomatine and a new glycoalkaloid dehydrotomatine [96,104] stimulated interest in determining the distribution of these two glycoalkaloids in tomato fruit and other parts of the tomato plant. Friedman and Levin [99] appear to have been first to analyze the two glycoalkaloids in tomatoes, tomato plant parts, and processed tomato products by HPLC with pulse amperometric detection (PAD), or UV detection at 200 nm. Dehydrotomatine and α-tomatine were found to have the same concentration response by PAD and very different responses by UV detection. The lower detection limit by UV was ~5 μg and that by PAD was ~0.1 μg. Leonardi et al. [105] used our method to measure the dehydrotomatine and α-tomatine content of several Italian tomato varieties and of a tomato salad. Bacigalupo et al. [106] measured dehydrotomatine and α-tomatine in green tomato fruits and in tomato leaves by time-resolved fluorescence spectroscopy using a europium chelator entrapped in liposomes. The results obtained were comparable to those by HPLC using our method. Kuronen et al. [107] devised an HPLC method to separate commercial tomatine into dehydrotomatine and α-tomatine and commercial tomatidine into peaks associated with tomatidenol and pure tomatidine. Väänänen et al. [108] used HPLC with UV detection to evaluate the recovery of dehydrotomatine and α-tomatine to wild potato *Solanum brevidens* leaf material on different SPE sorbents.

A study on the influence of several parameters on retention times of potato glycoalkaloids [73] was adapted to tomatoes. These parameters were manipulated in order to further optimize the separation of dehydrotomatine and α-tomatine by HPLC with UV detection at 208 nm [102]. The new data show that (a) the column packing and mobile phase used afforded better separation of the two glycoalkaloids than those obtained in previous studies (~4 min versus ~2 min); and (b) the lowest concentration that can be measured by the new separation–detection method (0.39 μg for dehydrotomatine and 0.94 μg for α-tomatine) is several-fold lower than for an earlier HPLC-UV method. However, the HPLC-PAD method, with a still lower detection limit than any of the HPLC-UV methods, is more suitable for analyzing the very low tomatine levels in red tomatoes. The observed distribution of the two glycoalkaloids in the various vegetative tomato plant parts should facilitate future studies designed to define their respective roles in host-plant resistance, and during postharvest processing and storing of tomatoes, as well as their respective roles in animal and human nutrition and health.

3.7. Atropine and scopolamine content of jimson weed seeds

The anticholinergic toxic manifestation of seeds produced by the *Solanum* plant *Datura stramonium* (jimson weed) is associated with the alkaloids atropine and scopolamine present in these seeds [109]. Bulk commercial grain, such as soybeans and wheat, may occasionally be contaminated by nongrain impurities including jimson-weed seeds that coexist with the crop to be harvested. Contamination of soybean and wheat grain shipments with various potentially toxic weed seeds should be avoided. Grain and animal feed contamination as well as reported substance abuse by adolescents and young adults suggest the need for reliable methods for analyzing biologically active components of the seeds. To meet this need and to develop a better understanding of the possible role of the seeds in the diet, an HPLC procedure was developed for analysis of the atropine and scopolamine content of the seeds obtained from a variety of sources. Fig. 3 shows the structures of the two alkaloids and Fig. 5 illustrates the separation of the two alkaloids in a seed extract on an HPLC column.

Alkaloid content of seeds grown in different parts of the United States ranged from 1.69 to 2.71 mg/g for atropine and 0.36–0.69 mg/g for scopolamine. Experiments with jimson-weed-seed-fortified wheat flour showed that atropine and scopolamine largely survive bread-baking conditions. GC–MS revealed that in addition to atropine and scopolamine, a jimson-weed-seed extract contained three additional tropane-like alkaloids. Oral toxicity studies revealed that body weight gain was significantly reduced in rats fed jimson weed seeds at 0.5% of the diet [110,111].

These and additional compositional, nutritional, and toxicological studies of jimson weed [112–115] and other seeds containing biologically active compounds such as morning glory (*Ipomoea* spp.) [116,117], sicklepod (*Cassia obtuasifolia*) [118–122], velvet leaf (*Abutilon neophrasti*) [121,123] seeds, and of nightshade (*Solanum nigrum*) berries [124,125] demonstrate the importance of these contaminants for food safety and human health. They also facilitate setting standards for toxic weed seeds and berries

in wheat, soybeans, and other commodities, thus enhancing the quality and safety of the diet.

In conclusion, the described methods, in which chromatographic procedures play a seminal role, lead to improvement in the precision and reliability of food ingredient analysis for quality control and safety of final products for the benefit of growers, researchers, processors, and consumers. These analytical studies have also facilitated concurrent studies of toxicities and of beneficial effects mentioned earlier as well as investigations of the biosynthesis of potato glycoalkaloids [126–130].

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