Glycoalkaloid Content and Anthocyanin Stability to Alkaline Treatment of Red-Fleshed Potato Extracts

L .E. Rodriguez-Saona, R. E. Wrolstad and C. Pereira

ABSTRACT

Red-fleshed potato (*Solanum tuberosum*) breeding clones were screened for steroidal glycoalkaloid (SGA) content. SGAs and anthocyanins (ACN) were characterized by HPLC and Mass Spectroscopy (MS). Total SGA ranged from 2.0 to 36.3 mg/100g tuber. Two cultivars with highest anthocyanin content, showed low levels of SGAs. The effect of alkaline treatments on SGA precipitation, anthocyanin content and profile was studied by adjusting the pH of aqueous extracts from 7.6 to 11. Complete removal of SGAs from colored extracts was achieved at pH > 9.2, however it caused substantial anthocyanin loss. Best results were obtained at pH 8.0 with 30% monomeric anthocyanin degradation and 90% SGA precipitation.

Key Words: glycoalkaloids, anthocyanins, alkaline precipitation, red-fleshed potato

INTRODUCTION

STEROIDAL GLYCOALKALOIDS (SGAS) ARE NITROGEN CONTAINING compounds which have the C_{27} skeleton of cholestane and are produced following the steroid biosynthesis pathway (Valkonen et al., 1996; Friedman and McDonald, 1997). Alkaloids are present in many species of the family *Solanaceae,* including cultivated and wild potatoes (*Solanum* spp.) and tomatoes (*Lycopersicon* spp.).

At least 90 structurally different steroidal alkaloids have been isolated and characterized in over 300 *Solanum* species (Friedman and McDonald, 1997). The major SGAs in commercial potatoes (*Solanum tuberosum*) are α-solanine and α-chaconine, which are glycosylated derivatives of the aglycone solanidine (Stanker et al., 1994). Wild *Solanum* species used in potato breeding to introduce desired traits (frost, disease and pest resistance) may result in increased concentrations of SGAs or incorporation of new alkaloids (Van Gelder et al., 1989). Shih and Kuc (1974) reported that several alkaloids existing in potatoes may have emerged through hybridization of such species as *S. chacoense* and *S. demissum.* SGA's tend to accumulate in plant organs with high metabolic activities such as flowers, sprouts, unripe berries, and young leaves (Jadhav et al., 1981; Van Gelder, 1991; Valkonen et al., 1996).

SGAs are natural toxins that probably evolved as protective compounds in response to tissue invasion. SGAs have antimicrobial, insecticidal and fungicidal properties which provide resistance against several insect pests and herbivores (Roddick et al., 1990; Tingey, 1984). However, they also have pharmacological and toxicological effects on humans (Jadhav et al., 1981, 1997; Van Gelder, 1991). Toxicity of SGAs is due to their significant anticholinesterase activity and disruption of cell membranes (Morris and Lee, 1984; Roddick and Rijnenberg, 1987). A safety level of SGAs in potatoes was established at 200 mg/kg for acute toxicity, but the level does not account for possible sub-acute or chronic effects. An upper limit of 60 to 70 mg/kg has been proposed for cultivars to be selected for human consumption (Van Gelder, 1991; Valkonen et al., 1996).

There is potential for the use of red-fleshed potato anthocyanin extracts as a food coloring alternative to artificial dyes (Rodriguez et al., 1998). However the levels of SGAs in pigment extracts are of concern since they might be concentrated with the anthocyanins. SGAs are fairly heat-stable compounds, slightly affected by steaming, boiling, baking, frying, cooking and microwaving of potatoes (Van Gelder, 1991; Friedman and McDonald, 1997). Since SGAs will precipitate under basic conditions, this property has been used in purification of alkaloids (Friedman and Dao, 1992). Stability of anthocyanins is affected by basic pH, which increases their rate of decomposition (Francis, 1989; Wrolstad, 1999).

In order to produce a red anthocyanin colorant from potatoes, information is needed on alkaloid content in the tuber and in the anthocyanin containing extract. Methods are needed to separate the alkaloids from the colored extracts with minimum anthocyanin degradation. The objectives of this study were to identify and quantify the SGAs (α-solanine and α-chaconine), and to determine the ACN/SGA ratio of different red-fleshed potato breeding clones. An additional objective was to evaluate the effects of alkaline treatment on stability of SGAs and pigments of an anthocyanin containing extract .

MATERIALS & METHODS

Plant material

Red fleshed potato (*Solanum tuberosum*) tubers were supplied by the USDA Agriculture Research Service at Prosser, WA (18 breeding clones) during fall 1996 and 1997. The Oregon State University Crop Science Department also provided 6 breeding clones for two consecutive plantings (Fall 1996 and 1997) at 2 locations (Corvallis and Klamath Falls).

The breeding clones supplied by the USDA Agriculture Research Service (1996) were grown in greenhouse pots and were smaller in size (average wt 38 g/tuber) than field-grown tubers from the OSU Crop Science Department (average wt 125 g/tuber). Fresh tubers were washed with cold water upon arrival to eliminate extraneous matter and refrigerated at 4°C until analyzed.

Pigment and SGA extraction

Potato tubers (ca 50 g) were cut in slices (ca 2 cm thick), blanched at 100°C for 10 min, blended with 100 mL acetone and filtered through a Buchner funnel. The filter cake residue was re-extracted with aqueous acetone (30:70 v/v) until clear. Filtrates were combined, shaken in a separatory funnel with chloroform (2:1 chloroform:acetone) and stored overnight at 1°C. The aqueous portion (top) was collected and placed on a Büchi rotovapor at 40°C until all residual acetone was evaporated. The aqueous extract was made to a known volume with distilled water and analyzed for SGAs following the HPLC procedure described by Friedman and Dao (1992). Due to the limited sample size of the fall 1996 tubers from Prosser (WA), measurements were

Authors Rodriguez-Saona and Wrolstad are affiliated with the Dept. of Food Science & Technology, Oregon State Univ., 100 Wiegand Hall, Corvallis, OR 97331. Author Pereira is affiliated with the Dept. of Statistics, Oregon State Univ., Kidder Hall 64, Corvallis, OR 97331. Address inquiries to Dr. R.E. Wrolstad.

made in duplicate and average values are reported. For all other tuber samples we replicated the extractions.

Anthocyanin partial purification

The aqueous extract was passed through a C-18 mini-column (high load C-18 tube), 20 mL capacity and 5g sorbant weight (Alltech Assoc., Inc., IL), previously activated with methanol followed by 0.01% aqueous HCl (Giusti and Wrolstad, 1996). Anthocyanins and phenolic acids were adsorbed onto the mini-column; sugars and acids were eluted with 2 volumes of 0.01% aqueous HCl and anthocyanins were recovered with methanol containing 0.01% HCl (v/v). The methanol was evaporated using a Büchi rotovapor at 40°C and the pigments were dissolved in deionized water with 0.01% HCl.

Effect of alkaline treatments on anthocyanin and SGA content

Three model juices were prepared using purified potato anthocyanin extracts that contained SGAs. The juices were prepared by diluting different potato anthocyanin extracts in distilled water to an initial monomeric anthocyanin content of 37 mg/100mL. The pH of the juices was adjusted to 2.3 with HCl.

Juice A. Potato juice (250 mL) was divided into 3 aliquots (60 mL each) and the pH was increased with $Na_3PO_4 \cdot 12H_2O$ to the desired pH value (from 7.6 to 11). A total of 9 alkaline treatments and a control (pH 2.3) were tested. Five randomly chosen alkaline treatments and the control (pH 2.3) were tested. five randomly chosen alkaline treatments and the control were evaluated for each aliquot. Treatments were arranged in increasing pH order and at the selected pH level a 10 mL portion was recovered and placed in a centrifuge tube.

Juice B. Potato juice (350 mL) was divided into 3 aliquots (100 mL each), the first 2 samples were treated with $Na_3PO_4 \cdot 12H_2O$ and the third was treated with KOH to evaluate the effect of different bases (weak vs strong). Nine treatments (pH from 7.6 to 11) and a control were evaluated, the juices were increased to the desired pH and an aliquot (10 mL) was taken and placed in a centrifuge tube. All pH treatments were replicated between samples.

Juice C. Potato juice (160 mL) was divided into 2 aliquots (80 mL each), and treated with KOH. Seven treatments and a control were evaluated, the juices were increased to the desired pH value (from 7.6 to 11), a portion (10 mL) was taken and placed in a centrifuge tube. All pH treatments were replicated between samples.

After alkaline treatment the solutions were placed in a water bath (40°C) for 30 min and then centrifuged at 10,000 rpm for 45 min. The supernatant was collected and phoshoric acid (0.5 mL) was added immediately to acidify the solution. The pellet contained the precipitated SGAs.

Statistical analysis. Each sample was repeatedly measured as the pH was changed (repeated measures). Because there was evidence of random sample effects and due to imbalance of the data (different pH levels were studied for each sample), analyses were conducted with the general mixed model procedure in SAS (The *SAS System for Windows, Release 6.12*, SAS Institute, Inc., 1997). P-values reported are from an unbalanced split plot model with sample as the whole plot unit and pH as the subplot unit. There was low power for detecting departures from the Huynt-Feldt assumptions for repeated measures, so only p-values <0.01 was considered significant.

Alkaloid precipitation

Solutions (250 mL each) containing three concentrations of SGA (15, 30 and 60 mg/100 mL) at initial pH 2.3 were prepared using precipitated SGAs from potato tubers. Solutions were divided into 4 samples for each alkaloid concentration and adjusted to different pH values (7.0, 7.5, 8.0, and 8.5) with KOH. after alkaline treatment the solutions were treated as previously described.

Statistical analysis. Each sample was repeatedly measured as the pH was changed (repeated measures). After finding no evidence of violation of the Huynh-Feldt assumptions (sphericity test $p>0.5$), the data were analyzed using a split-plot model. Alkaloid concentration was the whole plot factor applied to samples arranged in a completely randomized design with pH as the split plot factor. Because there was no evidence of sample effects ($p>0.5$), polynomial regressions were conducted without sample as a factor in the model. Analyses were conducted with the GLM procedure in SAS (The *SAS system for Windows, release 6.12,* SAS Institute, Inc., 1997).

Monomeric anthocyanin content

Monomeric anthocyanin content was determined using a pH-differential method (Wrolstad et al., 1982). A Shimadzu 300 UV spectrophotometer and 1 cm pathlength cells were used for spectral measurements at 420, 510 and 700 nm. Pigment content was calculated as Pg-3-glu, using an extinction coefficient of 31,600 L $cm^{-1}mol^{-1}$ and molecular weight (MW) of 433.2g mol^{-1} (Wrolstad, 1976).

Analytical HPLC system

Apparatus. An analytical High Performance Liquid Chromatograph (HPLC) Perkin-Elmer Series 300, equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett-Packard HPLC^{2D} ChemStation software were used.

Columns and mobile phase. System I. ODS C-18 column (5 micron), 250 × 4.6 mm i.d. (Poly LC Inc., Columbia, MD), fitted with a 10 × 4.6 mm i.d. spherisorb ODS-2 microguard column (Alltech, Deerfield, IL). Solvent A: 100% HPLC grade acetonitrile, B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. System II: Spherisorb ODS-2 column (5 micron) 250 × 4.6 mm i.e., fitted with a 10 × 4.6 mm i.d. Spherisorb ODS-2 micro guard column (Alltech, Deerfield, IL). Solvent A: 50% acetonitrile with 5 mM sodium lauryl sulfate and 5 mM sodium sulfate decahydrate. The pH was adjusted to 4.5 with 1% sulfuric acid. All analytical systems were run at a flow rate of 1 mL/min and with an injection volume of 50 μL. Solvents and samples were filtered through a 0.45 μm filter type HA (Millipore Corp., Bedford, MA).

Analysis conditions. Potato anthocyanins were separated using System I. The program used a linear gradient from 0 to 30%A in 30 min. Simultaneous detection at 520, 320 and 280 nm was used and spectra were collected for all peaks. Potato SGAs were separated isocratically (100%A) using System II. The signal was monitored at 200 nm. SGAs were quantified using a standard curve at 4 concentrations (0.5, 0.25, 0.125 and 0.0625 mg/mL of α-solanine and α-chaconine (Sigma Chemical Co., St. Louis, MO).

Semi-preparative HPLC system

Apparatus. A High performance Liquid Chromatograph (HPLC) Perkin-Elmer Series 400 and a Semi-Preparative Dynamax Rainin Model SD-300 Liquid Chromatograph, equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett-Packard HPLC^{2D} ChemStation software were used.

Columns and mobile phase. Microsorb C-18 reversed phase column (5 micron) 250 × 21.4 mm, fitted with a 50 × 21.4 mm i.d. guard column (Rainin Instrument Co., Inc., Emeryville, CA). Solvents: same as System I. Flow rate: 20 mL/min. Injection volume: 1 mL. Solvents and samples were filtered through a 0.45 μm filter type HA (Millipore Corp., Bedford, MA).

Isolation of purified pigments

Semi-preparative HPLC system was used. The program followed a linear gradient from 10 to 18A in 10 min. Simultaneous detection was done at 520, 320 and 280 nm. Purified anthocyanins were concentrated with a C-18 Sep-Pak cartridge and re-dissolved in distilled water.

Alkaline hydrolysis of anthocyanins

Semi-preparative isolated anthocyanins were saponified using 10

mL of 10% aqueous KOH for 15 min as described by Giusti and Wrolstad (1996).

Mass spectroscopy (MS)

Low-resolution MS was done using electrospray MS. The instrument was a Perkin-Elmer SCIEX API III+ Mass spectrometer, equipped with an Ion Spray source (ISV=4700, orifice voltage of 80) and loop injection. Partially purified potato anthocyanin extract and semi-preparative HPLC isolated anthocyanins were injected.

RESULTS & DISCUSSION

Steroidal glycoalkaloid content in red-fleshed potatoes

The isolation of SGAs from potatoes was based on the fact that the major alkaloids are soluble in slightly acidified water (Jadhav et al., 1981). More than 20 different extraction systems including solvents such as ethanol, methanol, chloroform/methanol, water/acetic acid, tetrahydrofuran (THF)/water/acetonitrile and other combinations have been described (Friedman and McDonald, 1995, 1997).

In a previous investigation on use of red-fleshed potatoes as a potential source of natural colorants (Rodriguez-Saona et al., 1998) we found that anthocyanin isolation by acetone extraction with subsequent chloroform partition also extracted the SGAs from potato tubers. Further isolation of SGAs by solid-phase extraction using a Sep-Pak C-18 column showed reduced amounts of SGAs (in some cases more than 50% loss) when compared to direct HPLC injection of the acidified aqueous extract. The lower recoveries with the Sep-Pak C-18 column might be due to the competition for binding sites between anthocyanin pigments, phenolic acids and SGAs in potato tubers, resulting in underestimation of the alkaloid content.

The major SGAs in red-fleshed potato tubers, identified by HPLC (Fig. 1a) and ESMS (Fig. 1b), were α-solanine and α-chaconine. The presence of solanidine (aglycon), solanidine with a hexose and solanidine with hexose and rhamnose were also detected by ESMS (Fig. 1b). These alkaloids most likely had been produced from fragmentation of α-solanine and α-chaconine, since the alkaloid aglycones are rarely found in potatoes (Friedman and McDonald, 1977). However, we cannot disregard their presence in trace amounts in potato tubers since ESMS is a more sensitive technique to detect SGAs. The presence of β and γ forms of glycoalkaloids (stepwise cleavage of the sugar moiety) had been reported in potato tubers (Friedman and Dao, 1992; Morris and Petermann, 1985) especially during prolonged storage (Friedman and McDonald, 1997) due to enzymatic hydrolysis.

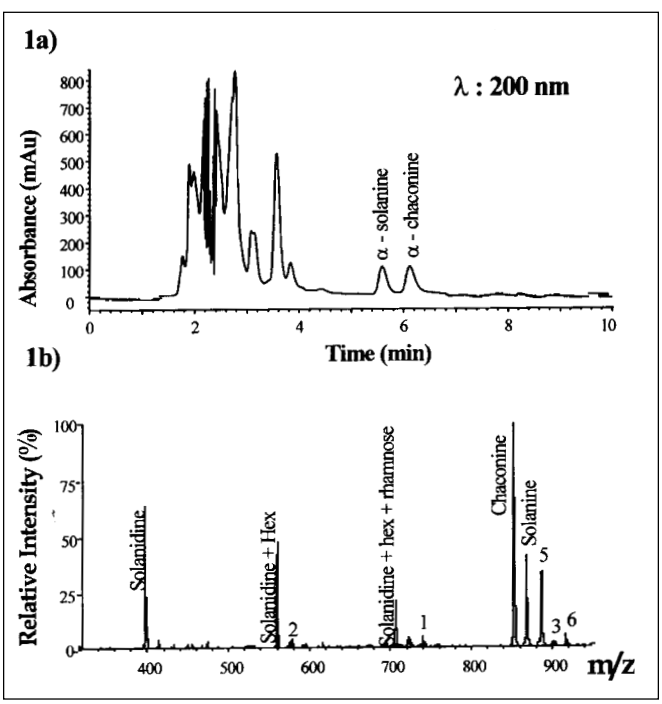

Fig. 1—(a) HPLC separation of steroidal glycoalkaloids in red-fleshed potatoes. Spherisorb ODS-2, 250 × 4.6 mm i.d. column. Solvent: 50% acetonitrile with 5 mM sodium lauryl sulfate and 5 mM sodium sulfate decahydrate, pH 4.5. Isocratic conditions. Flow rate: 1 mL/min and Injection volume: 50 μL. (b) Mass Spectroscopy of potato pigment extract. The molecular ions correspond to alkaloids and anthocyanins detected.

Table 1—Alkaloid content in different red-fleshed potato breeding clones

Source[a]	Clones	Solanine (mg/100g tuber)	Chaconine (mg/100g tuber)	ACNS/ Alkaloid Ratio
Prosser (WA) 1996	NDOP5847-1	3.4	6.7	3.1
Prosser (WA) 1996	NDOP5847-2	3.5	6.1	0.5
Prosser (WA) 1996	NDOP5847-3	5.5	10.7	0.7
Prosser (WA) 1996	NDOP5538-1	9.1	0.5	0.6
Prosser (WA) 1996	NDOP5538-2	3.7	8.1	0.5
Prosser (WA) 1996	NDOP5538-3	11.4	9.1	0.4
Prosser (WA) 1996	NDOP5538-4	3.4	6.9	0.9
Prosser (WA) 1996	NDOP5538-5	7.4	10.7	0.5
Prosser (WA) 1996	NDOP5538-6	5.8	16.5	0.4
Prosser (WA) 1996	NDOP6639-7	18.8	21.2	0.3
Prosser (WA) 1996	NDOP5538-8	3.7	4.9	1.1
Prosser (WA) 1996	NDOP5538-9	4.2	11.5	0.7
Prosser (WA) 1996	NDOP5538-10	6.6	10.1	0.7
Prosser (WA) 1996	NDOP5538-11	6.7	13.7	0.7
Prosser (WA) 1996	NDOP5538-12	16.1	20.2	0.5
Prosser (WA) 1996	NDOP5538-13	3.9	8.2	1.1
Prosser (WA) 1996	NDOP5589-1	3.0	7.3	0.8
Prosser (WA) 1996	NDOP5589-2	3.7	8.1	0.9
Prosser (WA) 1997	NDOP5847-1	1.4	3.1	8.7
Corvallis (OR) 1996	NDO4069-4	1.6	5.1	5.2
Corvallis (OR) 1997	NDO4069-4	4.6	10.9	2.1
Klamath (OR) 1996	NDO4069-4	2.1	5.4	4.8
Corvallis (OR) 1996	NDO5849-1	0.7	1.3	10.7
Corvallis (OR) 1997	NDO5849-1	1.0	1.3	6.0
Corvallis (OR) 1996	NDO5538-2	1.5	3.5	2.6
Corvallis (OR) 1997	NDO5538-2	1.5	4.6	1.8
Corvallis (OR) 1996	NDO6236-1	1.4	2.4	3.4
Corvallis (OR) 1997	NDO6236-1	4.3	8.2	1.1
Corvallis (OR) 1996	NDO6236-2	1.9	2.6	2.4
Corvallis (OR) 1997	NDO6236-2	3.3	7.2	1.4
Corvallis (OR) 1996	NDO4239	3.4	2.9	1.4
Corvallis (OR) 1997	NDO4239	1.0	1.3	5.4
Corvallis (OR) 1997	All Blue	10.4	16.8	0.7

[a]Potatoes were grown in the field except for those obtained from Prosser (WA) 1996, which were grown under greenhouse conditions.

Red-fleshed potato breeding clones (24) were evaluated for steroidal glycoalkaloid (SGA) content (Table 1). The total SGA content varied among the clones, ranging from 2.0 to 36.3 mg/100g tuber fresh weight (fw) for NDO5849-1 and NDOP5538-12 clones, respectively. All red-fleshed clones provided by the OSU Crop Science Dept. (Corvallis, OR) showed the lowest SGA levels (average 4.7±2.0 mg/100g tuber fw) while those tubers provided by USDA Agricultural Research Service (Prosser, WA) in the fall of 1996 showed a higher SGA content (average 17.2±8.7 mg/100g tuber fw). Immature tubers tend to accumulate higher levels of SGAs than fully mature tubers. Upon exposure to light immature tubers are more likely to develop excessive SGA levels (Van Gelder, 1991; Friedman and McDonald, 1997). The variability in SGAs levels could be attributed to inherited differences of potato cultivars or to environmental factors during growth and storage. Besides location, the growing conditions of the potato tubers were different, with tubers supplied by USDA Agricultural Research Service (Prosser, WA) in 1996 being grown in a greenhouse. Growing potatoes under greenhouse conditions stresses the plant, resulting in relative higher SGAs than cultivars grown in the field. Breeding clone NDOP5847-1, provided by USDA in the fall of 1997, was grown in the field and showed significantly lower SGAs than previous greenhouse plantings (Table 1). Furthermore, those tubers grown under typical field conditions showed a marked increase in ACN/SGA ratio (Table 1), which was mainly due to lower levels of glycoalkaloids.

Some OSU cultivars harvested during fall of 1997 (NDO4069-4, NDO6236-1 and NDO6236-2) showed increased SGA levels as compared to those from the 1996 planting. Red-fleshed potato plants showed high susceptibility to herbicide treatment and most potato plants died or were significantly damaged and stressed. NDO4069-4 showed the highest susceptibility, with a few plants producing small tubers (average wt 20g). Imposition of certain environmental conditions such as weather, light exposure, mechanical damage, sprouting, humidity or storage conditions will induce biogenesis of SGAs in potato tubers (Salunkhe and Wu, 1979; Friedman and McDonald, 1997.

The alkaloid ratio (α-chaconine to α-solanine) varied widely among potato breeding clones, ranging from 0.8 to 3.2. Friedman and Dao (1992) reported alkaloid ratios with an overall alkaloid ratio of 1.9±0.6. The accumulation of α-chaconine in potato tubers could be attributed to cultivar differences due to germplasm, developmental stages or environmental/stress factors. α-Chaconine accumulates in the potato plant because of its severe toxicity (strong cell disruption and anticholinesterase effects). It has been hypothesized that the production of a mixture of glycoalkaloids (α-chaconine and α-solanine) by the potato tuber results in synergistic effects leading to increased toxicity (Roddick et al., 1990; Friedman and McDonald, 1997). By producing both SGAs the plant can convert α-chaconine to the non-lytic β_2-chaconine during development stages without suppressing protection against pathogens, since β_2-chaconine and α-solanine are acetylcholinesterase inhibitors. As the plant matures, it converts the β_2-chaconine to the α-form in one energy efficient step (Friedman and McDonald, 1997).

Rodriguez-Saona et al. (1998) reported the monomeric anthocyanin content of these red-fleshed potato breeding clones. The ratio of anthocyanin/SGAs (ACN/DGA) was calculated for the potato clones (Table 1). The ratio of ACN/SGA ranged from 0.3 to 10.7 for NDOP5538-7 and ND5849-1, respectively. The potato clones with highest anthocyanin content (NDC4069-4 and NDOP5847-1) showed relatively low SGAs content and high ACN/SGA ratios which enhance their potential use as natural colorants.

The SGA concentrations in tuber peels and flesh were evaluated for cultivar NDOP5847-1. Potato peels were 15 times higher in SGA concentration as tuber flesh. It has been reported that the majority of SGAs in commercial potatoes are in the peels, concentrated in a 1.5-2.0 mm layer under the skin with the eye-zones containing the highest concentrations (Kozukue et al., 1987; Uppal, 1987; Wunsch and Munzert, 1994). The total SGAs levels were 44.2±4.7 mg/100g fw peels and 3.1±0.4 mg/100g fw flesh.

The alkaloid ratio (α-chaconine to α-solanine) was on average 2.4, very close to the ratio found for NDOP5847-1 whole tuber (2.2). When the SGA concentration was expressed in terms of total tuber wt, the alkaloid content in the tuber flesh (2.9±0.5 mg/100g was higher than in peels (2.0±0.2 mg/100g) since peels represented only 5% of total tuber wt.

Effect of alkaline treatment on red-fleshed potato juice extract

Purification of glycoalkaloids by precipitation with ammonium hydroxide (ca pH 10) is a common procedure because all SGAs except leptines are only sparingly soluble in water at pH 7 or above (Friedman and McDonald, 1997; Friedman and Dao, 1992). We evaluated the effect of different alkaline treatments, ranging from pH 7.6 to 11, on the extent of alkaloid precipitation and anthocyanin degradation in a red potato juice extract.

The potato juice extracts were standardized according to the monomeric anthocyanin content, and since we used different red-fleshed potato breeding clones for the juices, the initial SGA contents were different. The SGAs precipitated (Fig. 2) as a quadratic function of pH ($p<0.01$), and no detectable amounts of SGAs were found at pH>9.5. Juice A was different ($p<0.01$) from the other two (Juices B and C) in total alkaloid response to pH. The alkaloid content in juice A started much higher and descended more sharply than in juices B and C (difference in linear terms, $p<0.01$). There was no evidence ($p>0.1$ for all effects) of differences between juices B and C, nor between alkali agents (K_3PO_4 vs KOH) within juices B and C.

SGA precipitation in model solution was compared at different alkaloid concentrations (Fig. 3). The pH treatment ($p<0.01$) and alkaloid concentration ($p<0.01$) explained 98% of the variability in total alkaloid content ($R^2=0.98$). The higher the initial concentration of alkaloids the larger the extent of precipitation. Limited alkaloid precipitation was achieved at pH 7 (34% for C1 and 10% for C2 and C3), increased precipitation was obtained at higher pH (89%, 79% and 75% for C1, C2 and C3 respectively, at pH 8.0), and between 85% (C3) to 95% (C1 and C2) precipitation at pH 8.5. There was clear

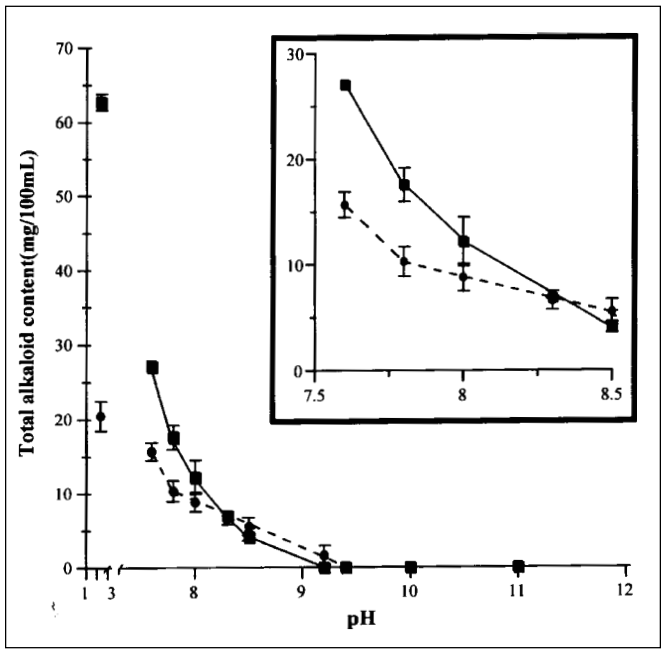

Fig. 2—Effect of alkaline treatment on precipitation of steroidal glycoalkaloids in anthocyanin/SGA containing extracts. Connecting points are not fitted regression lines.

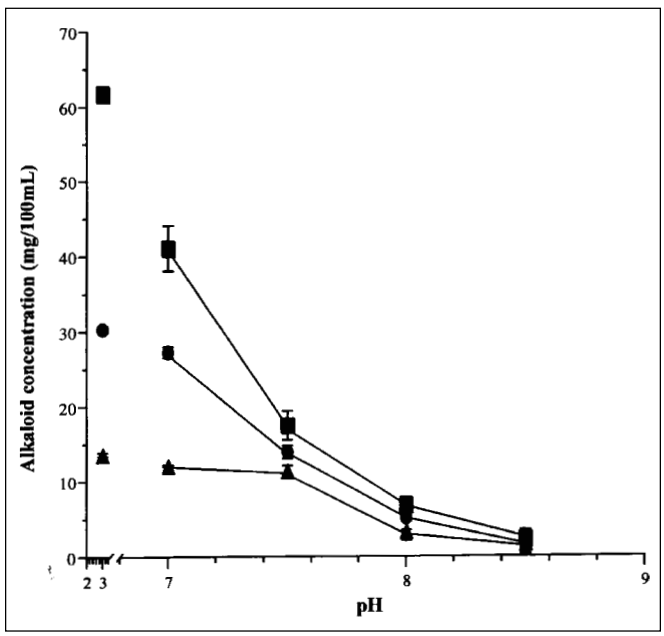

Fig. 3—Effect of alkaline treatments and initial alkaloid concentration on extent of SGA precipitation using model systems. Connecting points are not fitted regression lines. Regression equations for the pH ranges between 7.0 and 8.5 are presented.

evidence of highly significant main effects (alkaloid concentration and pH) and interactions ($p<0.01$ in each case). The multivariate model showed different polynomial trends for different solutions (Fig. 3). Solutions C1 and C2 showed quadratic trends; while solution C3 showed a cubic trend ($p<0.01$) as evidenced by the curve shape showing no change in SGA levels, a pronounced decrease and then a leveling off.

The percent precipitation of glycoalkaloids was different for the treatments, but the final SGA concentrations were similar at high pH (8.0 and 8.5) and no significant difference in SGAs content ($p=0.72$) was observed at pH 8.5. The results suggest that the critical factor in SGA precipitation was the alkaline pH and not the initial alkaloid concentration. The limited solubility of the alkaloids at basic pH (>8.0) might determine the final alkaloid concentration more than the initial SGA concentration of the juice extracts.

Anthocyanins undergo color changes with pH, displaying more intense red coloration (flavylium form) and higher stability under acidic conditions (pH<3). At increasing pH, the anthocyanin-containing extracts fade to colorless (carbinol pseudo base and chalcone) form before changing to purple or blue (quinonidal base) at pH>6 (Brouillard, 1982, 1983; Jackman et al., 1987). The basic conditions (pH>7.5) caused to some extent an irreversible degradation of the anthocyanins in the potato juice extract, resulting in a decrease in monomeric anthocyanin content and an increase in polymeric color (Fig. 4) after returning to acidic conditions (pH<2).

The monomeric anthocyanin content of potato juice extracts decreased with alkaline treatment. The extent of pigment degradation depended on the alkaline agent ($p<0.01$) used to modify pH (Fig. 5). A mean pigment degradation of 25% and 35% was obtained at pH 7.6 and 8.3, respectively; the pigment loss was significantly increased between pH 8.5 and 10 (42% to 75% loss) and then tended to stabilize at pH>10. Alkaline treatment at pH>9.0 resulted in substantial degradation (60% to 80%) of anthocyanins.

A data subset using pH levels from 7.6 to 8.5 was evaluated (Fig. 5 inset) in order to obtain a nearly balanced design. There was strong evidence of an agent effect ($p<0.01$) and no evidence of a juice effect after adjusting for agents ($p=0.17$). The statistical model showed that the decrease in monomeric anthocyanin was essentially linear in pH over that range. There was evidence that $Na_3PO_4 \cdot 12H_2O$ had a more negative slope (fig. 5), therefore showed an overall higher pigment degradation than KOH treatment. In these pH ranges, there was no evidence of quadratic or cubic main effects and interactions ($p=0.28$). Other advantages of the use of KOH (strong base) were that pH adjustment of the pigment extracts was faster, required less alkali and did not result in precipitation after further concentration.

The anthocyanin pigments in red-fleshed potatoes have been previously characterized (Harborne, 1960; Sachse, 1973; Lewis, 1996; Rodriguez-Saona et al., 1998) with the major anthocyanin being pelargonidin-3-rutinoside-5-glucoside (Pg-3-rut-5-glu) acylated with *p*-coumaric acid, representing more than 70% of the total area (Rodriguez-Saona et al., 1998). Peak assignment was done as reported by Rodriguez-Saona et al. (1998). The anthocyanin profile changed with pH treatment (Fig. 6). All anthocyanin pigments decreased in area except for 4 and X, that increased, especially at pH>9.0. Peaks 4 and X were isolated by semi-preparative HPLC and analyzed by ESMS and HPLC (alkaline hydrolysis) and were identified as pelargonidin derivatives acylated with *p*-coumaric acid, with the same molecular mass as peak 5 (M^+ 886.2). Presumably, the alkaline treatment resulted in geometric and/or positional isomers of the major anthocyanin (peak 5). The presence of *cis* and *trans* isomers of *p-coumaroyl* containing anthocyanins obtained from flowers of *Hyacinthus orientalis* has been reported by Hosokawa et al. (1995). Also, alkaline treatment could result in the esterification of *p-coumaric* at different positions of the anthocyanin molecule. Nagels et al, (1980) synthesized different *caffeoylquinic acid* isomers using heat and a saturated $NaHCO_3$ solution.

Saponification is a common method to determine acylating groups attached to anthocyanins, using saturated conditions of KOH for 8–15 min at 25°C to cleave the ester bond (Giusti and Wrolstad, 1996; Hong and Wrolstad, 1990). We hypothesized an increase in the area of Pg-3-rut-5-glu (peak 1) would occur after alkaline treatment due to saponification of the acylating cinnamic acids. However, its area remained unaltered or slightly decreased after the treatments. This suggested that the extent of the alkaline reaction (ca 90 min), temperature (40°C), or concentration of alkali used to adjust pH were responsible for anthocyanin polymerization (Fig. 4 and Fig. 6). Severe pigment degradation was observed at pH>9.5, resulting in up to 90% destruction of pg-3-rut-5-glu acylated with *p*-coumaric acid.

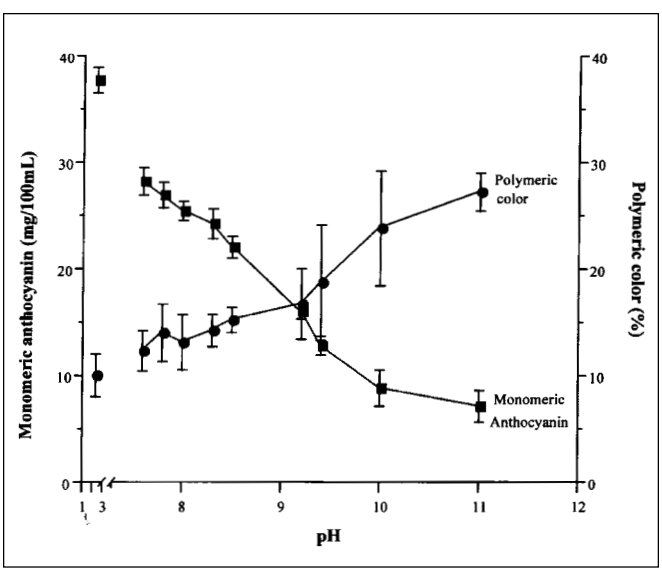

Fig. 4—Effect of the alkaline treatment on monomeric anthocyanin and polymeric content of anthocyanin/SGA containing extracts. Connecting points and not fitted regression lines. Data show average values for different juices.

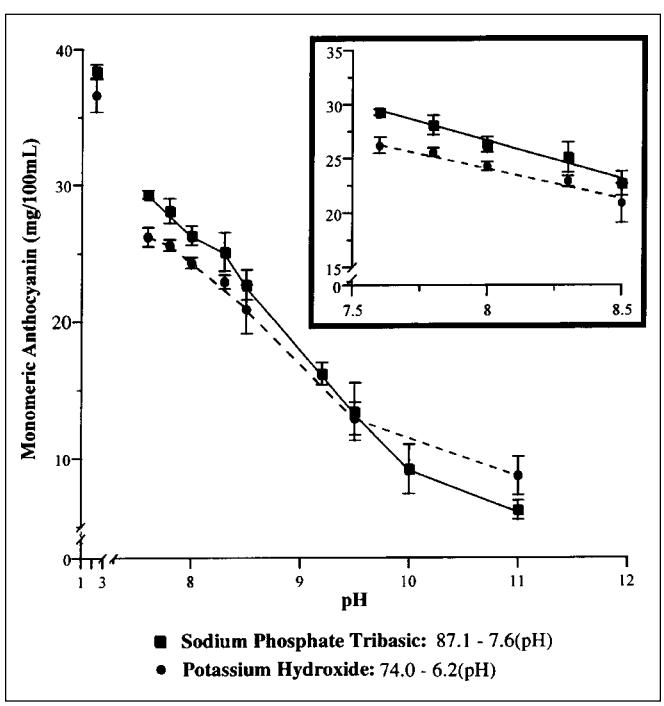

Fig. 5—Effect of alkaline treatment and agent ($Na_3PO_4 \cdot 12H_2O$ and KOH) on monomeric anthocyanin content of anthocyanin/SGA containing extracts. Connecting points are not fitted regression lines. Regression equations for the pH ranges between 7.6 and 11 are presented.

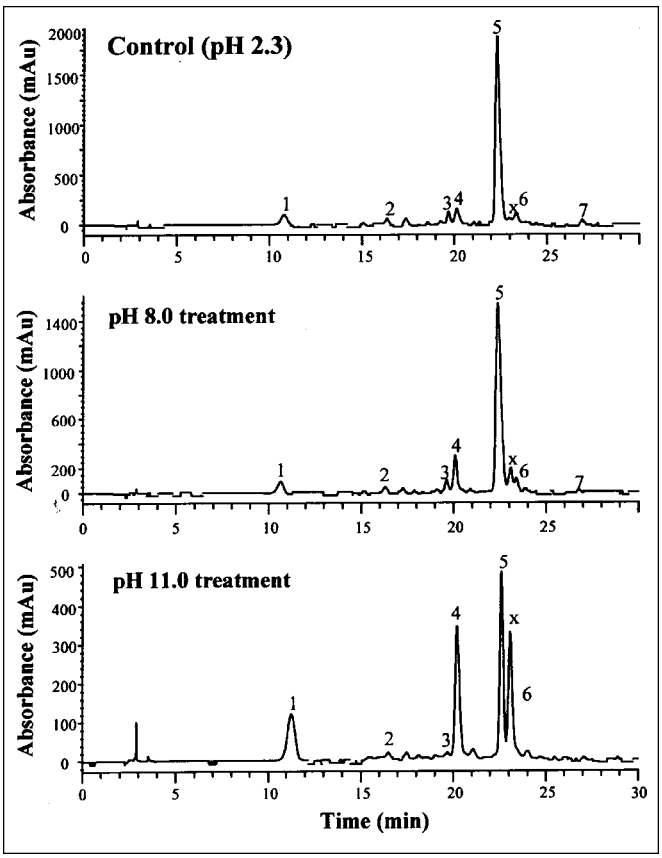

Fig. 6—Effect of alkaline treatments on anthocyanin profile of red-fleshed potato extracts ODS C-18, 250 × 4.6 mm i.d. column. Solvent A: 100% acetonitrile, B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. Linear gradient from 0 to 30% A in 30 min. Flow rate: 1 mL/min and Injection volume: 50 μL.

CONCLUSIONS

THE STEROIDAL GLYCOALKALOID LEVELS DETERMINED IN DIFFERENT red-fleches potato breeding clones ranged from 2.0 to 36.3 mg/100g fw tuber. Tubers grown in a greenhouse showed higher SGAs than field grown tubers. Potato clones with highest anthocyanin content (NDC4069-4 and NDOP5847-1) showed relatively low SGA content and high ACN/SGA ratios, which would enhance their potential use as natural colorants. The major SGAs, α-solanine and α-chaconine, were readily soluble in water and were concentrated along with anthocyanins. Alkaline treatment was effective for precipitating SGAs and separating them from anthocyanins, but resulted in significant pigment degradation at pH>9.0. The agent used to modify pH had an effect on monomeric anthocyanin content. We recommend treatment of the anthocyanin-containing juices with KOH to a final pH of 8.0, which precipitated up to 90% of the alkaloids without severe anthocyanin degradation (ca 30%) or changes in pigment profile.

REFERENCES

Brouillard, R. 1983. The *in vivo* expression of anthocyanin colour in plants. Phytochemistry 22: 1311-1323.

Brouillard, R. 1982. Chemical structure of anthocyanins. In *Anthocyanins as Food colors*. P. Markakis (Ed.), p. 1-40. Academic Press, New York.

Bushway, R.J., Savage, S.A. and Ferguson, B.S. 1987. Inhibition of acetyl cholinesterase by solanaceous glycoalkaloids and alkaloids. Am. Potato J. 64: 409-413.

Fitzpatrick, T.J., Herb, S.F., Osman, S.F. and McDermott, J.A. 1977. Potato glycoalkaloids: Increases and variations of ratios in aged slices over prolonged storage. am. Potato J. 54: 539-544.

Francis, F.J. 1989. Food colorants: Anthocyanins. Crit. Rev. Food Sci. & Nutr. 28(4): 273-314.

Friedman, M. and Dao, L. 1992. Distribution of glycoalkaloids in potato plants and commercial potato products. J. Agric. Food Chem. 40: 419-423.

Friedman, M. and McDonald, G.M. 1995. Extraction efficiency of various solvents for glycoalkaloid determination in potatoes and potato products. Am. Potato J. 72: 66A.

Friedman, M. and McDonald, G.M. 1997. Potato glycoalkaloids: Chemistry, analysis, safety, and plant physiology. Crit. Rev. Plant Sci. 16(1): 55-132.

Giusti, M.M. and Wrolstad, R.E. 1996. Characterization of red radish anthocyanins. J. Food Sci. 61: 322-326.

Harborne, J.B.1960. Plant polyphenols. 1. Anthocyanin production in the cultivated potato. Biochem. J. 74: 262-269.

Hong, V. and Wrolstad, R.E. 1990. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. J. Agric. Food chem. 38: 708-715.

Hosokawa, K., Fukunaga, Y., Fukushi, E., and Kawabata, J. 1995. Acylated anthocyanins from red *Hyacinthus orientalis.* Phytochemistry 39: 1437-1441.

Jackman, R., Yada, R. and Tung, M. 1987. A review: Separation and chemical properties of anthocyanins used for their qualitative and quantitative analysis. J. Food Biochem. 11: 279-308.

Jadhav, S.J., Sharma, R.D. and Salunkhe, D.K. 1981. Naturally occurring toxic alkaloids in food. Crit. Rev. Toxicol. 9: 21-104.

Jadhav, S.J., Lutz, S.E., Mazza, G. and Salunkhe, D.K. 1997. Potto glycoalkaloids: Chemical, analytical and biochemical properties. In *Antinutrients and Phytochemicals in Food*, F. Shahidi (Ed.), p. 94-114. ACS Symposium Series 662, American Chemical Society, Washington, DC.

Kozukue, N., Kozukue, E. and Mizuno, S. 1987. Glycoalkaloids in potato plants and tubers. HortScience 22: 294-296.

Lewis, C.E. 1996. Biochemistry and regulation of anthocyanin synthesis in potato and other tuber-bearing *Solanum* species. Ph.D. thesis, Dept. of Plant & Plant Microbial Science, Univ. of Canterbury, Christchurch, New Zealand.

Linnemann, A.R., van Es, A. and Hartmans, K.J. 1985. Changes in the content of L-ascorbic acid, glucose, fructose, sucrose and total glycoalkaloids in potatoes (cv Bintji) stored at 7, 16 and 28°C. Potato Res. 28: 271-278.

Morris, S.C. and Lee, T.H. 1984. The toxicity and teratogenicity of Solanaceae glycoalkaloids, particularly those of the potato (*Solanum tuberosum*). Food Technol. Australia 36: 118-124.

Morris, S.C. and Petermann, J.B. 1985. Genetic and environmental effects on levels of glycoalkaloids in cultivars of potato (*Solanum tuberosum*). Food Chem. 18: 271-282.

Nagels, L., Van Dongen, W., DeBrucker, J. and DePooter, H. 1980. High-performance liquid chromatographic separation of naturally occurring esters of phenolic acids. J. Chrom. 187: 181-187.

Olsson, K. and Roslund, C. 1994. Changes in glycoalkaloid content of potato during long-term storage. In *Abstracts from the Joint Meeting of the Agronomy and Utilization Sections of the European Association of Potato Research*, Villa Real, Portugal, June 24-30, p. 41.

Roddick, J.G. and Rijnenberg, A.L. 1987. Synergistic interaction between the potato glycoalkaloids -solanine and -chaconine in relation to lysis of phospholipid/sterol liposomes. Phytochemistry 26: 1325-1328.

Roddick, J.G., Rijnenberg, A.L. and Weissenberg, M. 1990. Membrane disrupting properties of the steroidal glycoalkaloids solasonine and solamargine. Phytochemistry 29: 1513-1518.

Rodriguez-Saona, L.E., Giusti, M.M. and Wrolstad, R.E. 1998. Anthocyanin pigment composition of red-fleshed potatoes. J. Food Sci. 63: 458-465.

Sasche, J. 1973. Anthocyane in den kartoffelsorten Urgenta and Desiree. Z. Lebensm. Unters. Forsch. 153: 294-300.

Salunkhe, D.K. and Wu, M.T. 1979. Control of postharvest glycoalkaloids formation in potato tubers. J. Food Prot. 42: 519-525.

Shih, M.J. and Kuc, J. 1974. - and ß-solamarine in Kennebec *Solanum tuberosum* leaves and aged tuber slices. Phytochemistry 13: 997-1000.

Stanker, L.H., Kamps-Holtzapple, C. and Friedman, M. 1994. Development and characterization of monoclonal antibodies that differentiate between potato and tomato glycoalkaloids and aglycons. J. Agric. Food Chem. 42: 2360-2366.

Tingey, W.M. 1984. Glycoalkaloids as pest resistance factors. Am. Potato J. 61: 157-167.

Uppal, D.S. 1987. Varietal and environmental effects on the glycoalkaloid content of potato (*Solanum tuberosum* L.). Plant Foods Hum. Nutr. 37: 333-340.

Valkonen, J., Keskitalo, M., Vasara, T. and Pietila, L. 1996. Potato glycoalkaloids: A burden or a blessing? Crit. Rev. Plant Sci. 15(1): 1-20.

Van Gelder, W.M.J. 1991. Chemistry, toxicology, and occurrence of steroidal glycoalkaloids: Potential contaminants of the potato (*Solanum tuberosum* L.). In *Poisonous Plant Contamination of Edible Plants.* Abbel-Fattah M. Rizk (Ed.), p. 117-156. CRC Press, Boca Raton, FL.

Van Gelder, W.M.J., Tuinstra, L.G.M., Van der Greef, J. and Scheffer, J.J.C. 1989. Characterization of novel steroidal alkaloids from solanum species by combined gas chromatography-mass spectrometry. Implications for potato breeding. J. Chrom. 482: 13-22.

Wrolstad, R.E. 1996. Color and pigment analyses in fruit products. Oregon State Univ. Agric. Exp. Stn. Bull. 624. Oregon State Univ., Corvallis, OR.

Wrolstad, R.E. 1999. Colorants. In *Food Chemistry: Principles and Applications*, G.L. Christen (Ed.). AFNC Press, Cutten, CA. (In press)

Wrolstad, R.E., Culbertson, J.D., Cornwell, C., and Mattick, L.R. 1982. Detection of adulteration in blackberry juice concentrate and wines. J. Assoc. Off. Anal. Chem. 65: 1417-1423

Wu, M.T. and Salunkhe, D.K. 1976. Changes in glycoalkaloid content following mechanical injuries to potato tubers. J. Am. Soc. Hort. Sci. 101: 329-331.

Wünsch, A. and Munsert, M. 1994. Effect of storage and cultivar on the distribution of glycoalkaloids in potato tuber. Potato Res. 37: 3-10.

Ms received 5/9/98; revised 11/19/98; accepted 12/7/98.

Technical paper #11327 from the Oregon Agricultural Experimental Station.

This work was supported by Grant 94-37500-0808 from the USDA, NRI Competitive Grants Program and the Hermiston-Food Science Cooperative Project on Vegetable Quality.

We thank A. Mosley and B. Charlton from the OSU Crop Science Dept., C. Brown from the USDA Irrigated Agriculture Research & Extension Center at Prosser (WA), and G. Reed from the Hermiston Research & agricultural Extension Center 9OR) for supplying red-fleshed potatoes and supporting the project. We thank D.A. Griffin of the Dept. of Agricultural Chemistry, OSU, for Mass Spectroscopy analyses, and R.W. Durst and M.M. Giusti for technical assistance.