Rapid Quantitation of Potato Glycoalkaloids by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

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The potential for application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to the quantification of potato glycoalkaloids was investigated. A MALDI-TOF MS method was developed for the analysis of α-chaconine and α-solanine using tomatine as an internal standard. Quantitative analysis of a number of potato cultivars by MALDI-TOF MS and high-performance liquid chromatography (HPLC) showed high correlation ($R^2 = 0.98$) between the two methods. The MALDI-TOF MS method developed provides a very rapid analysis, requires very little sample preparation, and is suitable for routine glycoalkaloid analysis.

Keywords: *MALDI; chaconine; solanine; tomatine*

INTRODUCTION

The potato tuber (*Solanum tuberosum* (*S. tuberosum*)) is a very common and valuable food source due to its high yield per acre and nutrient levels (Maga, 1980). Although the potato contains many important nutritional factors, the plant and tubers also contain toxic glycoalkaloids (GAs) which pose a threat to human health.

GA toxicity is well-documented (Maga, 1980; Morgan and Coxon, 1987), and a number of cases of poisoning to varying degrees, some resulting in death, have been reported (Morris and Lee, 1984). The concentration of GAs in the tuber is dependent on many pre- and postharvest factors, including light exposure, temperature stress, tuber damage, and genetic factors. GAs are not appreciably destroyed by cooking, baking, or frying (Jadhav et al., 1981). Commercial cultivars commonly contain between 20 and 150 μg/g of total GA for unpeeled tuber (Slanina, 1990). An upper level of 200 μg/g of total GA (Groen et al., 1993) has been recognized, which represents only a 4−5-fold safety factor between the average GA level and a potentially toxic dose; therefore, GAs are considered by some to be the most serious toxic components of the human diet (Hall, 1992).

From a human health standpoint, it is desirable for breeders to eliminate GAs in potatoes; however, this is not the case for several reasons. At low levels, GAs are a component of potato flavor (Ross et al., 1978), and confer disease and pest resistance to the potato plant (Morgan et al., 1983; Fewell et al., 1993, 1994). GA production is also reliant on a number of different genes (Sandford and Sinden, 1972). Another reason for the lack of attention to GA levels is the difficulty and expense required for routine analysis of the thousands of crosses performed annually.

A number of different techniques exist for the analysis of GAs in potato material (van Gelder, 1991; Coxon, 1984). The simplest methods, including colorimetric, gravimetric, and titrimetric techniques, lack the required specificity and suffer from contamination by other potato components. The most common methods rely on expensive and time-consuming chromatographic techniques such as gas chromatography (GC) or high-performance liquid chromatography (HPLC). GC analysis requires extensive derivitization or hydrolysis of the GA to obtain the more volatile alkaloid. Because HPLC methods rely on UV detection in the 200−208 nm region (Saito et al., 1990; Bushway et al., 1986; Friedman and Dao, 1992), extensive sample cleanup is required. GAs which lack a double bond for UV absorption, such as tomatine, are poorly detected. The use of pulsed amperometric detection of the sugar moiety of GAs has been used to improve detection (Friedman et al., 1994).

A newer method of analysis is the use of immunoassays (Morgan et al., 1983; Barbour et al., 1991; Phlak and Sporns, 1992, 1994; Stanker et al., 1994). Immunoassays rely on the specificity of antibodies to eliminate the problems of extensive purification and extraction of samples, although the assays developed are unable to differentiate between α-chaconine and α-solanine. Immunoassays are also rapid and inexpensive to perform. Recently a fluorescence polarization immunoassay was developed, to improve on the variability inherently associated with solid phase immunoassays (Thomson and Sporns, 1995).

Recent advances in mass spectrometry (MS) techniques have allowed for quantitative analysis. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) is a relatively new MS technique which is gaining popularity as instrumentation improves (Siuzdak, 1994; Harvey, 1994; Beavis, 1992).

The main areas of research involving MALDI-TOF MS are related to mass determinations of large biomolecules such as proteins, DNA, oligosaccharides, and protein digests (Gusev et al., 1995; Siuzdak, 1994; Fenselau, 1995). MALDI-TOF MS can readily be used with compounds of up to 350 kDa (Stahl et al., 1991). Analysis can be performed with picomole quantities of sample with reported sensitivity in the femtomole range (Juhasz and Costello, 1992; Gusev et al., 1995). While the use of MALDI-TOF MS with high molecular weight compounds is of great value, it also has potential for quantification of both high and low molecular weight compounds (Gusev et al., 1995).

MALDI-TOF uses low molecular weight UV-absorbing compounds to act as a matrix vehicle for desorption and ionization of molecules of interest. The sample of interest is cocrystallized with excess matrix and then pulsed with an UV laser to desorb and ionize the matrix. The matrix absorbs energy from the laser and transfers

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it to the analyte. This soft ionization method provides intact molecules in the gas phase, principally as cations (Harvey, 1994). Typically, the MALDI is linked to a TOF instrument for mass determination. TOF analysis works well in conjunction with MALDI as it does not have an upper limit for high m/z ions and works well with the pulsed laser used in MALDI (Siuzdak, 1994). Both the matrix and the sample will appear on the spectrum; therefore, matrices must be of low molecular weight or the sample must be of sufficient mass to prevent overlap with the matrix peaks.

Given the speed associated with MALDI-TOF MS analysis, coupled with its ability to produce intact molecules, this study was undertaken to investigate the potential of applying MALDI-TOF MS to GA analysis. An analytical method was developed using MALDI-TOF MS and compared against HPLC.

EXPERIMENTAL PROCEDURES

Materials. All water used was purified using a Milli-Q system (Millipore Corp., Bedford, MA). α-Chaconine, α-solanine, and α-tomatine were obtained from Sigma Chemical Co. (St. Louis, MO). 2,4,6-Trihydroxyacetophenone (THA) was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other reagents were reagent grade or better.

Potato samples A−D were donated by Dr. N. R. Knowles, Department of Agricultural, Food, and Nutritional Science, University of Alberta. Samples A and B were *S. tuberosum* cv. Russet Burbank stored for 8 and 20 months, respectively. Samples C and D were *S. tuberosum* cv. Shepedy and Yukon Gold, respectively, both stored for 8 months. All samples were stored in the dark at 4 °C and 95% relative humidity. Potato samples E and F were commercially obtained *S. tuberosum* tubers. Sample E was peeled, while sample F was unpeeled. Samples were cut into 1 cm^3 pieces, freeze-dried, and ground sufficiently to pass through a 20-mesh screen. Samples were stored at 4 °C until needed.

Apparatus. Potato samples were freeze-dried using a Virtis Pilot Scale Freeze Drier (Virtis Co. Inc., Gardiner, NY). Freeze-dried samples were ground using a Braun Model KSM2 coffee grinder (Braun Canada Ltd., Mississauga, ON) to pass through a 0.85 mm screen. Standard curve fitting was done with Microsoft Excel 5.0 Solver (Microsoft, Redmond, WA).

Extraction of GAs for HPLC. A modified method of Saito et al. (1990) was used for extraction. A 1 g sample of freeze-dried potato was shaken vigorously with 1 mL of water for 1 min; this was followed by the addition of 20 mL of methanol and shaking for 2 min. The mixture was vacuum-filtered through Whatman No. 1 filter paper. The vial and filter paper were washed with 2 × 10 mL of methanol. The filtrate was diluted to 50 mL with methanol. A 5 mL aliquot was then mixed with 8 mL of water and added to a Sep-Pak cartridge which had been conditioned with 10 mL of methanol and 10 mL of water. The cartridge was washed with 5 mL of 40% methanol followed by elution of the glycoalkaloids with 15 mL of methanol. The sample was concentrated and taken up in 1 mL of methanol.

HPLC. The extracted sample was injected through a 20 μL loop onto a 300 × 3.9 mm μBondapak NH_2 (Bio-Rad Laboratories Ltd., Mississauga, ON) column at 25 °C. The mobile phase was acetonitrile:20 mM potassium dihydrogen phosphate (75:25 (v/v)) delivered at a flow rate of 1.0 mL/min using a Beckman Model 110A/332 pump (Beckman Instruments Inc., Fullerton, CA). A Bio-Rad UV monitor, Model 1305 (Bio-Rad Laboratories Ltd.), set at 208 nm was used for detection. Output was monitored using a Hewlett-Packard 3390A integrator (Hewlett-Packard, Avondale, PA). Peak heights were compared against a linear standard curve using similarly prepared standards at concentrations ranging from 0 to 100 mM. All samples were extracted in triplicate, and each extraction was analyzed in triplicate.

MALDI-TOF. A 100 μM solution of tomatine in water:methanol (50:50 (v/v)) was used for all extractions. Freeze-dried potato (400 mg) was shaken vigorously with 10 mL of extraction solution for 1 min in a 20 mL vial. Samples containing very high levels of GAs were treated similarly, except only 200 mg of tissue was used. The sample was then placed on a Junior Orbit Shaker (Lab-Line Instruments, Inc., Melrose Park, IL) for 1 h at 200 rpm. Approximately 5 mL of the solution was poured into a 14 × 100 mm test tube and centrifuged at 1500 rpm for 5 min. Supernatant (1 mL) was transferred to a microcentrifuge tube and stored at −20 °C until analyzed.

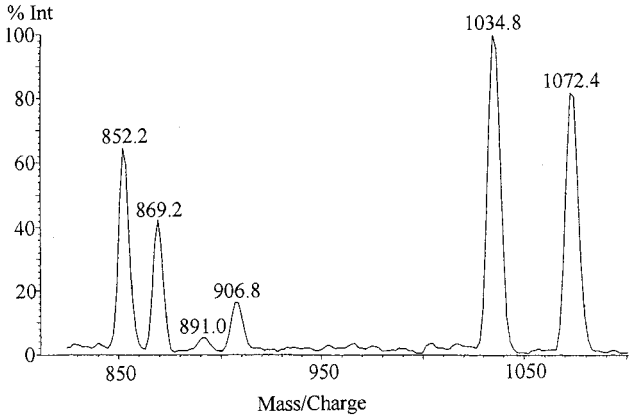

Figure 1. MALDI-TOF MS analysis of Russet Burbank potato after 8 months storage. Peaks, from left to right, are α-chaconine, α-solanine, α-chaconine + potassium, α-solanine + potassium, tomatine, and tomatine + potassium.

MALDI testing was performed using a Kompact MALDI I (Kratos Analytical, Ramsey, NJ) using a 337 nm laser with a maximum output of 6 mW. The equipment was operated using a Sun SPARCstation with Kompact 4.0.0 software with SunOS (Release 5.30). Samples were tested using a 20 sample stainless steel probe. The sample (0.5 μL) was placed on the probe and allowed to air-dry. A saturated solution (1 μL) of THA in methanol:water (50:50 (v/v)) was added on top of the sample and allowed to air-dry. The samples were scanned with a power setting of 80 and positive high detection and averaged over 100 shots. Peak heights relative to the internal standard tomatine were compared to a standard curve prepared with spiked potato tissue samples (200 mg) in 10 mL of extraction solvent up to a concentration of 55 μg/mL of α-solanine or α-chaconine.

RESULTS AND DISCUSSION

GAs are well-suited to analysis by MALDI-TOF MS as they have very similar chemical properties, but differ enough in molecular weight to be resolved, and are difficult to analyze by more conventional methods. The mass resolution of present commercial MALDI-TOF instruments ranges from 250 to 500 (Siuzdak, 1994), allowing for resolution of glycoalkaloids differing by as little as 4 Da. Furthermore, GA molecular weights are sufficiently high such that matrix peaks do not interfere with the analysis.

Prior to using MALDI-TOF MS as an analytical technique, a matrix in which the sample of interest desorbs and ionizes well must be found. Compounds of similar structure will typically produce similar results with a given matrix. A number of common matrices were tested, including sinapic acid, dihydroxybenzoic acid, α-cyanohydroxycinnamic acid, 4-hydrazinobenzoic acid, and THA. THA was found to produce the highest peaks with the greatest degree of base line separation. Suggested sample amounts for MALDI-TOF MS are 1−10 pmol on the probe (Gusev et al., 1995; Rideout et al., 1993). GAs were tested at 1, 10, and 100 ng/spot (approximately 1, 10, and 100 pmol) and performed well at all three concentrations. Consequently, subsequent

Table 1. Comparison of GA Analysis by MALDI-TOF MS and HPLC[a]

	MALDI			HPLC		
sample	α-chaconine	α-solanine	total GA	α-chaconine	α-solanine	total GA
A	13.20 (2.76)	8.52 (0.58)	21.72 (3.32)	13.30 (0.67)	11.87 (0.45)	25.17 (1.12)
B	23.44 (1.43)	12.92 (1.95)	36.36 (3.17)	19.36 (0.16)	14.66 (0.06)	34.02 (0.22)
C	5.98 (0.31)	3.19 (0.20)	9.18 (0.50)	8.08 (1.53)	5.00 (0.98)	13.08 (2.51)
D	4.63 (0.79)	2.99 (0.24)	7.62 (0.93)	6.59 (1.34)	4.12 (0.90)	10.71 (2.24)
E	0.85 (0.06)	0.76 (0.24)	1.62 (0.29)	0.85 (0.25)	0.35 (0.34)	1.20 (0.59)
F	1.96 (0.1)	0.98 (0.13)	2.94 (0.20)	1.75 (0.11)	0.43 (0.12)	2.18 (0.23)

[a] mg/100 g fresh weight basis (assumed 20% moisture); values in parentheses are standard deviations for triplicate extractions of the sample.

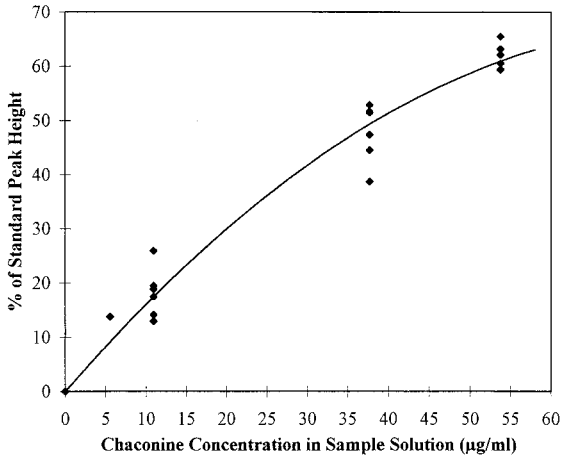

Figure 2. Standard curve for α-chaconine using MALDI-TOF MS.

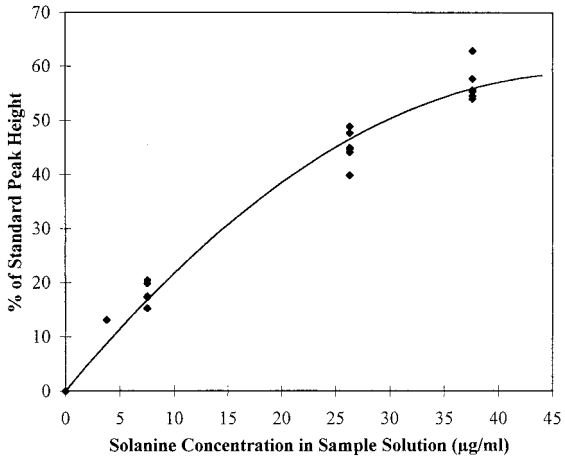

Figure 3. Standard curve for α-solanine using MALDI-TOF MS.

samples and standards were analyzed with less than 10 ng/spot.

A high degree of variability is associated with MALDI-TOF MS, both from run to run and from spot to spot. Therefore, tomatine was used as an internal standard in the analysis. Tomatine is not found in commercial potato varieties, although it occurs in some wild varieties (Gregory et al., 1981). When pure chaconine, solanine, and tomatine samples are analyzed using MALDI-TOF MS with only the extraction solution, each produces a single peak. Upon analysis of potato sample, a potassium peak appears for each GA (Figure 1). This peak is 39.1 mass units, the molecular weight of a potassium ion, higher than the actual molecular weight of the glycoalkaloid. Calculations were always carried out on the sum of the two peak heights. In the middle of the study it was learned that the tomatine was only about 80% pure, the other 20% containing a double bond (Bushway et al., 1994). While this presents a problem for HPLC analysis, the slight difference in mass (2 mass units) of the major impurity merely leads to some peak broadening for samples and did not preclude the use of the commercially available tomatine without purification.

A standard curve was produced in a range that would encompass the range of GA concentration in potato tissue up to 25 mg/100 g. Potato tissue (200 mg) was added to the standards, as in the absence of potato tissue relative peak heights were much larger than when potato tissue was present. Consequently, a useful standard curve could not be generated in the absence of potato tissue. Measurements were made on two separate days with triplicate spots and analysis in triplicate on each spot to eliminate spotting and equipment variation. A second-order polynomial curve through zero was then fit to the data (Figures 2 and 3). The second-order curve fit, rather than a linear model, can be explained by the fact that the presence of solanine or chaconine resulted in greater ionization of the tomatine as well. It is speculated that this ionization pattern may be due to the presence of the double bonds in the chaconine and solanine, allowing for better absorption of energy and a resulting increased energy transfer to tomatine.

The extraction method used when potatoes are analyzed for GAs is an important consideration. Since many extraction solvents used for GAs contain acid (Coxon, 1984), initial extraction solvents containing 5% acetic acid were evaluated; however, concentration of the acid during drying resulted in hydrolysis of the sugars from the GAs. While 100% methanol is a commonly used extraction solvent, a water:methanol mixture is preferred as it provides a medium which is easily applied to the MALDI probe. Testing of the extraction over time indicated that the majority of the GAs appeared after only a few minutes of extraction, but 60 min were required for complete equilibrium.

Results of MALDI-TOF MS and HPLC analyses are given in Table 1. Both analyses produced very similar results ($R^2 = 0.98$) and similar standard deviations and compared favorably to HPLC results of Saito et al. (1990). It is interesting to note that samples A, C, and D, which were commercial cultivars stored for only 8 months, had relatively high GA levels (21.72, 9.18, and 7.62 mg/100 g, respectively). Sample A had levels above the recommended allowance for GAs. None of these samples showed excessive greening or sprouting before analysis, which is often used in industry as an indicator for high GA levels. This demonstrates the need for testing of potatoes, rather than reliance on secondary indicators. Another benefit of MALDI is the ability to detect other glycoalkaloids which may be present such as β-chaconine. Other glycoalkaloids could be identified by their molecular weight, and once a suitable standard curve is established, could also be quantified.

The greatest advantage of MALDI-TOF MS is its speed of analysis. Even when triplicate extractions and triplicate analyses/extraction was considered, MALDI-TOF MS analysis was still much faster than HPLC. Each HPLC test required 10−12 min, or nearly 2 h/sample, for triplicate analysis on triplicate extractions. MALDI-TOF MS permitted triplicate analysis on an extraction in under 6 min, for a total of 20 min/sample, assuming triplicate extraction. Moreover, HPLC requires extensive cleanup of the sample prior to analysis, which is very labor intensive and represents an additional 30−40 min per sample (with triplicate extractions). This contrasts sharply to the MALDI-TOF MS method in which 10−15 samples could be prepared in the same time period. The major disadvantage to MALDI-TOF MS is the associated capital cost. However, MALDI-TOF MS is a very recently developed technology, and, with increased application, it is anticipated that mass production of instruments will reduce their cost (Siuzdak, 1994).

This study has demonstrated the applicability of MALDI-TOF MS for very rapid routine GA analysis with quantification. We are continuing the study of MALDI-TOF MS to expand its use to the detection and quantification of all potato glycoalkaloids. Our laboratory is pursuing this goal and the potential for use of this novel technique in other areas of food analysis.

ABBREVIATIONS USED

GA, glycoalkaloid; HPLC, high-performance liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; TOF, time-of-flight.

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