High-Performance Liquid Chromatographic Determination of the Glycoalkaloids α-Solanine and α-Chaconine in 12 Commercial Varieties of Mexican Potato

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The glycoalkaloid content in 12 commercial varieties of Mexican potatoes was measured by HPLC in both the peel and the flesh of the potato. The principal glycoalkaloids α-solanine and α-chaconine were present in higher concentration in the peel than in the flesh of all varieties. The main alkaloid in the peel of the potatoes was α-chaconine and comprised about 65−71% of the total glycoalkaloids. The high concentration of α-chaconine in peel, which is more toxic than α-solanine, gives more protection to the tuber against predators. The total alkaloids in the peel of Alpha, Juanita, Michoacan, Norteña, Rosita, and Tollocan varieties were higher than the limit recommended for food safety. However, the peel represents less than 10% of the total tuber in most of the varieties. The total alkaloids contained in the peel of Atzimba, Lopez, Marciana, Montsama, Murca, and Puebla was lower than the limits recommended for food safety. The glycoalkaloid content in the boiled peeled potatoes was less than 9 mg/100 g but in Alpha, Montsama, and Puebla varieties, both glycoalkaloids were absent. According to the results, the consumption of the 12 commercial varieties of Mexican potatoes does not represent any danger to human health.

Keywords: *Potato; Solanum tuberosum; glycoalkaloids; α-solanine; α-chaconine; HPLC analysis*

INTRODUCTION

Potato and tomato are the two most important species in the family Solanaceae. They are native of the American Continent but at present they have extended all over the world. *Solanum tuberosum* is an important plant from the nutritional, agricultural, and toxicological point of view (Macrae et al., 1987). It is consumed by millions of people every day. Its nutritional value and protein quality are superior to those of cereals and it is considered the second crop in total protein production after soybean (Van der Zaag, 1983).

The presence of glycoalkaloids as plant secondary metabolites has been found in all varieties in high or low concentration that has to be controlled or revised constantly (Hellenas et al., 1995). In previous work, it was determined the nutritional and toxicological composition of wild Mexican potatoes, which showed high glycoalkaloid concentration (Sotelo et al., 1998). This finding stimulated the authors to analyze the 12 varieties representative of potatoes production in Mexico from their glycoalkaloid content.

Tubers of the common potato (*S. tuberosum*) have alkaloids derived biosynthetically from the cholestan ring. These steroidal alkaloids are usually found in plant tissue as glycosides, (Osman, 1983). The principal glycoalkaloids in potato tubers are α-solanine and α-chaconine. Both are glycosylated forms of the steroidal alkaloid solanidine. Solanine and chaconine are chemically similar and only differ in the carbohydrate moiety. In α-solanine, the carbohydrate moiety is composed of galactose, glucose, and rhamnose (*β*-solatriose), while in α-chaconine there are glucose and two moieties of rhamnose (*β*-chacotriose). (Figure 1). Together, α-solanine and α-chaconine comprise approximately 95% of total potato glycoalkaloids (TGA) (Olsson, 1989; Roddick et al., 1988).

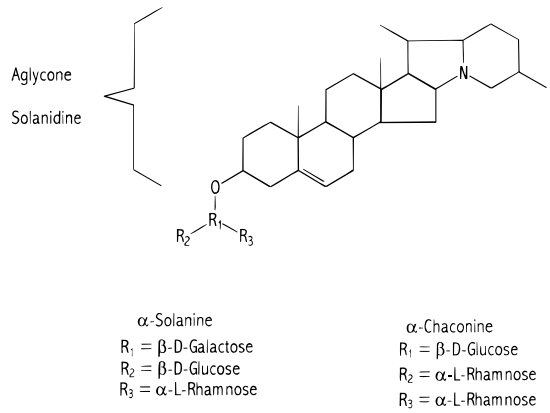

Figure 1. Structure of the solanidine alkaloids: α-solanine and α-chaconine.

Potato tubers typically contain about 20−60 mg of TGA 100 g^{-1} freeze-dried matter, (Griffiths et al., 1994) equivalent to 4−12 mg of TGA 100 g^{-1} fresh weight. At these concentrations, glycoalkaloids are considered to enhance potato flavor. However, at concentrations above 20 mg 100 g^{-1} in fresh weight, they give a bitter taste and can cause gastroenteric symptoms, coma, and even death. The toxicity may be due to adverse effects on the central nervous system and disruption of cell membranes, which adversely affects the digestive system and general body metabolism. The toxic dose is considered

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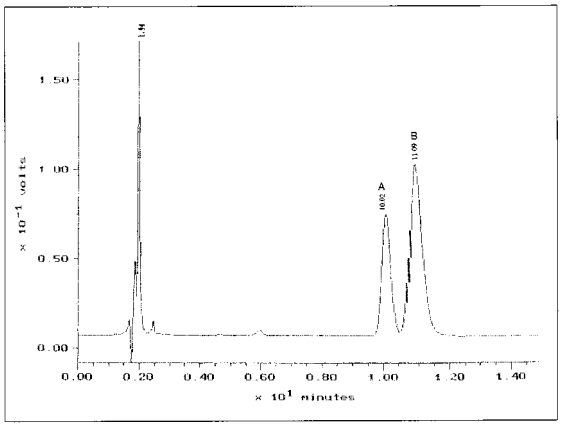

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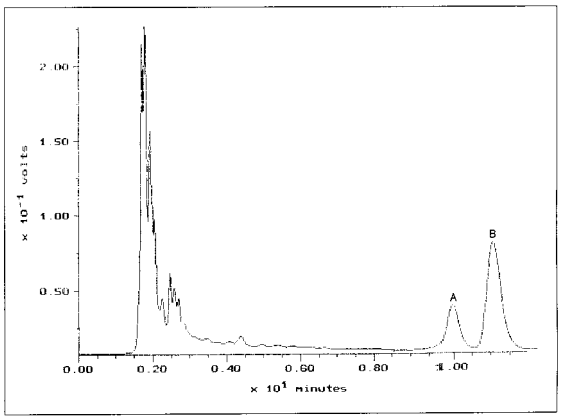

(II)

Figure 2. HPLC chromatograms. μBonda-Pak C18 column; mobile phase, acetonitrile:phosphate buffer, 0.05 M (30:70 v/v), pH 6.50; flow rate, 1.5 mL/min; UV detection, 200 nm and 0.05 AUFS. (I) Standard of α-solanine (A) and α-chaconine (B). (II) α potato flesh extract added with α-solanine (A) and α-chaconine (B) standards before the extraction.

to be approximately 2−5 mg/kg body mass (Morris and Lee, 1984).

Glycoalkaloids, like many secondary metabolites, are thought to function in chemical defense of the plant, acting as nonspecific protectors or repellents against potential pest predators (Osman, 1980; Roddick, 1989). The inhibitory effects of glycoalkaloids on both fungal and insect pests of the potato indicate that their evolutionary significance is as natural pesticides (Jadhav et al., 1991). The concentration of tuber glycoalkaloids increases in response to a number of factors, such as injury, fungal attack, poor growing conditions, climate, and the most important, inadequate storage conditions. Light exposure is probably the most important commercial factor influencing the TGA content in potato tubers (Dao and Friedman, 1994; Edwards and Cobb, 1996).

Glycoalkaloids are not destroyed by typical food processing, like boiling cooking, baking, frying, and microwaving. For these reasons, it is very important to monitor the concentration of potato tuber glycoalkaloids destined for human consumption (Bushway and Ponnampalam, 1981).

This investigation was conducted to measure the α-solanine and α-chaconine content in 12 major commercial varieties of potato, as they usually are prepared and consumed in Mexico.

Table 1. Moisture Content in Peel and Flesh of 12 Commercial Varieties of Mexican Potatoes (g/100 g of sample)[a] and the Ratio Peel to Total Potato Weight (*R*)

variety	peel	flesh	*R*
Alpha	81.98	84.11	0.05
Atzimba	80.75	85.19	0.07
Juanita	84.81	87.43	0.07
López	82.00	84.13	0.05
Marciana	84.41	87.56	0.08
Michoacán	87.32	86.60	0.06
Montsama	83.48	82.41	0.05
Murca	88.77	84.79	0.07
Norteña	83.33	85.07	0.09
Puebla	80.57	87.80	0.10
Rosita	83.50	86.96	0.08
Tollocan	82.15	82.18	0.05

[a] The values are average of three determinations.

MATERIALS AND METHODS

Materials. Solvents were of HPLC spectroquality grade. The solvents used for the extraction were of analytic grade (J. T. Baker Chemical and Merck, R.A). Glycoalkaloids standards were α-solanine and α-chaconine (99% pure) (Sigma Chemical Co., St. Louis, MO). All water was purified using a Milli-Q system (Millipore Corp., Bedford, MA).

Potato Samples. Tubers of 12 commercial potato varieties were obtained from the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP). The tubers were stored in boxes open to sunlight and analyzed 2 months after harvest date, since it is the time when the potatoes are usually sold in the markets for their consumption. Ten tubers of uniform size of each variety without greening or fungal infection were cleaned and peeled with a domestic potato cutter, and the ratio of peels to total weight was recorded. The moisture content in peels and peeled potatoes was determined according to the AOAC method (1990). The peeled potatoes were boiled in water for 60 min and then fractioned into small pieces and dried at 50−55 °C for 14 h. The dried samples of peeled potatoes were milled, and the peels were freeze-dried (freeze-dryer model 5, Labconco Co., Kansas City, MO). Both dried samples were milled and stored in a desiccator in the dark at room temperature until used.

The residual water where the peeled potatoes were cooked was evaporated and alkaloids were investigated in the residue following the Abisch and Reichstein technique (1960), using seven different reagents to detect the presence of these compounds.

Alkaloids Extraction. Samples of 500 mg of dried peel or 1500 mg of dried flesh with 20 mL of 5% aqueous acetic acid were placed in a 50 mL Erlenmeyer flask and stirred for 30 min. The sample was then vacuum filtered through a Whatman no. 42 filter paper, and the residue was reextracted three times with the same solution for 30 min each time. The four filtrates were combined and transferred to a 125 mL separating funnel. The pH was adjusted to 11 with ammonium hydroxide, and the alkaline extract was partitioned four times with 10 mL of water saturated butanol. The combined butanol extracts were evaporated almost to dryness, and the residue was removed from the flash with three portions of 1 mL each of methanol and placed in a vial with a Pasteur pipet. The extract was evaporated to dryness, and the residue was redissolved in 1 mL of methanol and analyzed. In those cases where the concentration of glycoalkaloid was very high, a smaller sample was taken for the extractions.

All samples were extracted in triplicate, and each extraction was analyzed in triplicate.

The extraction technique used was based on a modification of the technique of Dao and Friedman (1996). Extraction time was reduced to 2 h from 6 h.

High-Performance Liquid Chromatography (HPLC). The α-solanine and α-chaconine glycoalkaloids were separated and quantified using a Waters Chromatographic system, Waters 510 pump, 486 variable wavelength UV−vis detector

Table 2. Glycoalkaloids, α-Solanine, and α-Chaconine Present in Peel of Commercial Varieties of Mexican Potato[a]

variety	α-solanine (mg/100 g dry basis)	α-chaconine (mg/100 g dry basis)	total glycoalkaloids (mg/100 g dry basis)[b]	total glycoalkaloids (mg/100 g fresh weight basis)[b]
Alpha	42.35 ± 1.23	96.16± 0.65	138.51 ± 1.11	24.52
Atzimba	22.39 ± 0.21	43.92 ± 0.62	66.31 ± 0.75	12.77
Juanita	75.01 ± 0.29	145.43 ± 0.58	220.44 ± 0.70	33.48
López	30.07 ± 0.07	49.68 ± 0.49	79.75 ± 0.50	14.35
Marciana	40.92 ± 0.06	75.44 ± 0.08	116.36 ± 0.10	18.13
Michoacán	114.42 ± 0.29	220.54 ± 0.85	334.96 ± 0.99	42.46
Montsama	6.70 ± 0.06	10.83 ± 0.00	17.63 ± 0.07	2.90
Murca	18.47 ± 0.45	45.40 ± 0.43	63.87 ± 0.72	7.17
Norteña	161.49 ± 1.07	388.26 ± 0.92	549.74 ± 1.74	91.63
Puebla	29.52 ± 0.79	41.14 ± 0.79	70.66 ± 1.58	13.73
Rosita	106.48 ± 0.62	201.09 ± 0.46	307.57 ± 1.04	50.75
Tollocan	74.18 ± 0.64	165.91 ± 0.32	240.10 ± 0.90	42.84

[a] Values are expressed as mean ± standard deviation ($n = 9$). [b] Total glycoalkaloids = sum of α-solanine and α-chaconine.

Table 3. Glycoalkaloids, α-Solanine and α-Chaconine Present in the Flesh of Commercial Varieties of Mexican Potato[a]

variety	α-solanine (mg/100 g dry basis)	α-chaconine (mg/100 g dry basis)	total glycoalkaloids (mg/100 g dry basis)[b]	total glycoalkaloids (mg/100 g fresh weight basis)[b]
Alpha	nd[b]	nd	nd	nd
Atzimba	20.94 ± 0.58	4.34 ± 0.04	25.29 ± 0.54	3.74
Juanita	13.30 ± 0.19	14.66 ± 0.25	27.97 ± 0.14	3.51
López	5.13 ± 0.07	5.01 ± 0.09	10.14 ± 0.03	1.61
Marciana	4.87 ± 0.03	1.29 ± 0.01	6.16 ± 0.05	0.77
Michoacán	6.43 ± 0.03	9.66 ± 0.01	16.09 ± 0.03	2.16
Montsama	nd	nd	nd	nd
Murca	2.80 ± 0.04	1.33 ± 0.05	4.14 ± 0.06	0.63
Norteña	6.39 ± 0.03	9.27 ± 0.03	15.66 ± 0.02	2.34
Puebla	nd	nd	nd	nd
Rosita	33.18 ± 0.11	31.02 ± 0.24	64.20 ± 0.21	8.37
Tollocan	6.87 ± 0.11	3.17 ± 0.06	10.04 ± 0.09	1.79

[a] Values are expressed as mean ± standard deviation ($n = 9$). [b] nd = not detected.

controlled with a personal computer and SIM (interface) using Maxime 820 software. The column was a 3.9 × 300 mm-reversed phase μBonda-Pak C_{18} (Millipore Corp., Milford, MA). The mobile phase was acetonitrile-0.05 M monobasic ammonium phosphate buffer (30:70 v/v), at pH. 6.5. Solvent flow rate was 1.5 mL/min and the UV absorbance detector was set at 200 nm with a sensitivity of 0.05 AUFS. The size of the injection samples was 20 μL.

Validity Study. This study was made because some changes were done to the original method. The validity was tested with glycoalkaloid standards and for a recovery study, different amounts of α-solanine (0.12 and 0.24 mg in triplicate) and α-chaconine (0.15 and 0.30 mg in triplicate) were added to 1500 mg of dried peeled Alpha potato. The samples were thoroughly mixed, extracted as above, and analyzed by HPLC.

The retention times for α-solanine and α-chaconine were approximately 10 and 11 min, respectively (Figure 2). The resolution factor (α) of α-solanine versus α-chaconine was calculated to be 1.10. The detector peak area response was linear over the range of 0.012−0.24 mg/mL for α-solanine and of 0.015−0.30 mg/mL for α-chaconine. The limit of quantitation was estimated to 0.009 mg/mL for each substance. The limit of detection was 0.0024 mg/mL for α-solanine and of 0.003 mg/mL for α-chaconine. The mean recoveries obtained from triplicate samples were 99.7 ± 0.47**%** for α-solanine and of 99.6 ± 0.54**%** for α-chaconine. The repeatability of the method was determined analyzing six samples of Alpha potato to which glycoalkaloids standards were added and then mixed, extracted, and analyzed by HPLC. The repeatability for α-solanine and α-chaconine were 0.37 and 0.10%, respectively, expressed as coefficient of variation (CV). The stability of glycoalkaloids extracts under refrigerated conditions was monitored for 4 weeks. The statistic analyses (ANDEVA) showed that glycoalkaloids extracts were stable during 4 weeks of storage. For these analyses, a probability of $P > 0.05$ was accepted as significant. The results of this validity study show that the method used is efficient and useful for glycoalkaloid analysis.

RESULTS AND DISCUSSION

Glycoalkaloid Content in Commercial Varieties of Mexican Potato. The moisture content of the fresh peel and flesh of the potatoes studied and the ratio of peel to total potato weight are shown in Table 1.

The α-solanine and α-chaconine content in peel and flesh of 12 commercial varieties of potato most commonly consumed in Mexico-expressed in dried basis is shown in Table 2 and Table 3, respectively. The results obtained show that all potato varieties studied contain α-solanine and α-chaconine in their peel, while not all of the varieties contain them in their flesh, such as Alpha, Montsama, and Puebla varieties.

The principal glycoalkaloid present in the peel of potatoes was α-chaconine, this comprises approximately 65−71% of the total glycoalkaloids. The high α-chaconine concentration in peel has a physiological significance since this alkaloid, is more toxic than α-solanine (Dao and Friedman, 1994; Friedman et al., 1992). High concentrations of α-chaconine give major protection and resistance to the tubers against potential pest predators.

The total glycoalkaloid concentration in the peel of Alpha, Juanita, Michoacan, Norteña, Rosita, and Tollocan varieties was higher than the limit recommended for food safety (20 mg/100 g fresh sample) (Osman, 1983). This author found that the peel only comprises approximately 10% of the total tuber; however, in the present study, the range of peel percentage to the total potato weight in the varieties studies was 5−10% (Table 1).

The total glycoalkaloids in the peel of Atzimba, Lopez, Marciana, Montsama, Murca, and Puebla varieties was lower than the limits recommended for food safety.

In the flesh, (boiled peeled potato) α-solanine and α-chaconine content was irregular. α-Solanine was the

main glycoalkaloid in Atzimba, Marciana, Murca, and Tollocan, while α-chaconine was present mostly in Michoacan and Norteña. In Juanita, Lopez, and Rosita, α-solanine and α-chaconine were in equal proportion (50:50), while in Alpha, Montsama, and Puebla, α-solanine and α-chaconine were not detected. The glycoalkaloids were not detected in the broth of the boiled peeled potatoes.

All the peeled potato varieties contained low levels of total glycoalkaloids, 0.63−3.74 mg/100 g fresh flesh and Rosita had the highest level with 8.37 mg/100 g peeled potato in fresh basis. These levels were lower than the limits recommended for food safety.

In all the varieties the highest amount of total glycoalkaloids was in the peel of the potato but the contribution of the glycoalkaloids in the peel to the flesh was very low and for this reason, it should not be a problem for the consumers of the potato with peel.

In summary, our study demonstrates that the in-home preparation and consumption of the 12 commercial varieties of Mexican potatoes in good condition (without greening or fungal infection), does not represent any health risk to human.

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