Chlorogenic Acid Content of Fresh and Processed Potatoes Determined by Ultraviolet Spectrophotometry

Lan Dao and Mendel Friedman*

Food Safety Research Unit, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710

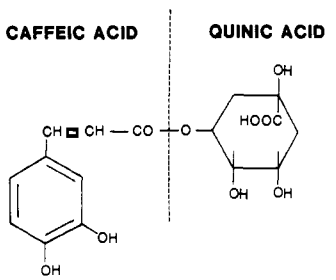
Chlorogenic acid is reported to be involved in preharvest defenses of the potato plant against fungi and insects, in postharvest browning, and in after-cooking blackening, all of which affect quality and safety. As part of a program of potato improvement, HPLC and ultraviolet spectroscopy were evaluated to measure the chlorogenic content of commercial and experimental potatoes, of parts of the potato plant, and of processed potato products. HPLC analysis appears to be less satisfactory because chlorogenic acid undergoes a time- and light-dependent change in the methanolic and ethanolic extracts of potatoes used. The decrease of the chlorogenic acid peak on chromatograms was accompanied by a corresponding increase of a new peak. Use of ultraviolet spectrophotometry to estimate chlorogenic acid by contrast appears to be reproducible. Recoveries of spiked samples measured by UV were higher than those measured by HPLC. Thus, our results suggest that the UV method may have advantages over HPLC. Seven varieties of potatoes contained from 10 to 19 mg of chlorogenic acid/100 g of fresh weight. The experimental potato plant NDA 1725 contained 754 mg/100 g of fresh weight for sprouts, 224 mg/100 g for leaves, 26 mg/100 g for roots, and 17 mg/100 g for tubers. The relative concentrations paralleled those of the glycoalkaloids α-chaconine and α-solanine. Oven-baked potatoes contained 0% of the original amount of chlorogenic acid, boiled potatoes 35%, and microwaved potatoes 55%. Commercially processed french-fried potatoes, mashed potato flakes, and potato skins contained no chlorogenic acid. The absence of chlorogenic acid was confirmed by ultraviolet spectrophotometry and thin-layer chromatography. The significance of these findings for plant physiology, food quality, and food safety is discussed.

INTRODUCTION

Chlorogenic acid (3-*O*-caffeoylquinic acid, see structure below), constitutes about 90% of the total phenolic compounds of potato tubers (Mondy and Gosselin, 1988; Malmberg and Theander, 1985). The compound appears to be responsible for bluish-gray discoloration of boiled or steamed potatoes following exposure to air. This so-called "after-cooking blackening or darkening" is perceived by many consumers as undesirable (Swain, 1962). The blackening appears to be due to the formation of a reduced ferrous (Fe II) ion–chlorogenic acid complex in the potato. Following exposure to oxygen in the air, the colorless ferrous complex is oxidized to a dark ferric (Fe III) complex (Hughes et al., 1962; Schwimmer, 1981).

Although the after-cooking blackening in potatoes may produce undesirable appearance and taste, it is apparently not associated with nutritional damage. However, the reported participation of chlorogenic acid in enzymatic browning reactions can lead to such damage (Barbeau and Kinsella, 1985; Dryden and Satterlee, 1978; Hurrell and Finot, 1984; Molnar-Perl and Friedman, 1990). Thus, chlorogenic acid is oxidized by polyphenol oxidase to a highly reactive *o*-quinone intermediate which then could interact with NH_2 groups of lysine, SH groups of cysteine, SCH_3 groups of methionine, and indole rings of tryptophan in nucleophilic addition and in polymerization reactions, the so-called browning reactions. These transformations destroy essential amino acids, impair digestibility and nutritional quality, and may also result in the formation of toxic compounds (Friedman, 1991, 1992).

These considerations and reports that chlorogenic acid may also be involved in defenses against insects and phytopathogens in plants (Elliger et al., 1981; Deshpande et al., 1984; Sinden et al., 1988) and that it damages chromosomes in in vitro tests (Stich and Rosin, 1984; Stich et al., 1981; Friedman and Smith, 1984) stimulated interest to measure chlorogenic acid and related phenolic compounds in fruits and vegetables, including potatoes (Buescher et al., 1975; Clifford and Wight, 1976; Griffiths et al., 1992; Malmberg and Theander, 1985; Pomenta and Burns, 1971; Ramamurthy et al., 1992; Sioud and Luh, 1966; Torres et al., 1987; Trugo and Macrae, 1984; Van Buren et al., 1973; Walter and Purcell, 1979; Walter et al., 1979).



CHLOROGENIC ACID

In previous papers, we reported on an improved ultraviolet spectroscopy method to measure the chlorogenic acid content of morning glory (*Ipomoea* spp.) toxic weed seeds, both untreated and following exposure to autoclaving and conventional and microwave baking (Friedman and Dao, 1990; Friedman et al., 1989). Because of an unexpected problem we encountered with the high-performance liquid chromatographic (HPLC) method, the main objective of this study was to evaluate the effec-

* Author to whom correspondence should be addressed [telephone (510) 559-5615; fax (510) 559-5777].

tiveness of the ultraviolet method for measuring chlorogenic acid in commercial and experimental potato varieties, in parts of the potato plant, and in home and commercially processed potato products.

EXPERIMENTAL PROCEDURES

Materials. All solvents were of HPLC or spectroquality grade. Chlorogenic acid was obtained from Aldrich Chemical Co., Milwaukee, WI. NDA 1725 experimental potato plants were grown in a greenhouse and harvested 2.5 months after planting. Other experimental potato varieties were obtained from the Potato Breeding Program, University of Idaho, Aberdeen, ID, and from the Simplot Co., Caldwell, ID. Large and small Idaho Russet potatoes, small red potatoes, potato skins, french-fried potatoes, and mashed potato flakes were purchased in a local store or restaurant.

The high-glycoalkaloid NDA 1725 potato plant was selected for detailed studies because we aim to modify it genetically to reduce its glycoalkaloid content (Stapleton et al., 1991, 1992). This potato plant was cut up into tubers, roots, and leaves. The roots and tubers were cleaned with water and dried with a paper towel. Sprouts were harvested from these tubers after about 4 months of storage.

The NDA 1725 potato tubers were subjected to three types of home processing: (a) boiling in water for 30 min; (b) baking in an oven at 212 °C for 45 min; and (c) microwaving at 218 °C for 30 min.

All potato samples except the mashed potato flakes were cut into small cubes which were placed into freeze-drying jars and then frozen in liquid nitrogen. The frozen samples were lyophilized, and the dried samples were then ground in a Wiley mill to pass a 40-mesh screen. The powders were stored in a refrigerator.

Preparation of Extracts. The freeze-dried potato powders were first defatted by extracting with hexane in a Soxhlet extractor for 16 h. A sample of defatted powder (200 mg) was then refluxed for 6 h with 20 mL of ethanol instead of Soxhlet extracting, as previously described for morning glory seeds (Friedman et al., 1989). The refluxed sample was then filtered through a 0.45-μm membrane and the filtrate adjusted to a volume of 20 mL with ethanol.

Spiking Experiments. To determine the effectiveness of the ultraviolet method, 200-mg samples of NDA 1725 potato and 100-mg samples of leaf and sprout powders were each spiked with various amounts of chlorogenic acid prior to extraction.

Ultraviolet Spectrophotometry. The instrument used was a Perkin-Elmer Lamda 6 UV–vis spectrophotometer equipped with an Epson Equity III computer. The UV spectrum, 250–400 nm, was determined from the ethyl alcohol extract after suitable dilution with ethanol. The concentration of chlorogenic acid was calculated from the absorption maximum at 325–328 nm from a standard curve prepared from authentic chlorogenic acid. The molar extinction coefficient (ϵ) of chlorogenic acid was determined as 18 130 ± 242 (n = 8).

Thin-Layer Chromatography. Preparative thin-layer chromatography was performed on 0.25 mm thick, 20 × 20 cm, silica gel precoated plates (Merck, Darmstadt, Germany). The extracted solution was evaporated with a stream of nitrogen and the residue dissolved in 1 mL of methanol. A 15-μL sample of this solution was then applied as a single band to the thin-layer plate, along with a chlorogenic acid standard. The plate was developed with ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:27 v/v) until the solvent front reached 13 cm. The plate was air-dried and then sprayed with natural product reagent (1% methanolic diphenylboric acid ethylamino ester) followed by 5% ethanolic poly(ethylene glycol) 4000 to visualize the spots (Wagner et al., 1984).

High-Performance Liquid Chromatography. A Beckman Model 334 liquid chromatography with a 427 integrator and a 165 UV-visible variable-wavelength detector was used. The column used was a reversed-phase Ultrasphere Octyl, 5 μm, 4.6 × 250 mm, with a precolumn (Beckman Instruments, San Ramon, CA). Fifty microliters was injected via a Beckman system organizer equipped with a 20-μL loop. The mobile phase consisted of 0.5% formic acid–methanol (8:3 v/v) operating at a flow rate of 1.3 mL/min. The ultraviolet absorbance was monitored at 325 nm. The absorbance units full scale (AUFS) was 0.10.

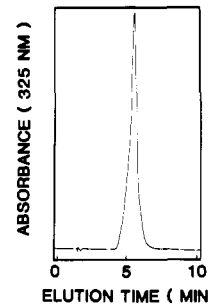


Figure 1. HPLC chromatogram of freshly prepared chlorogenic acid in methanol.

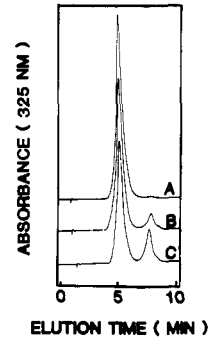


Figure 2. HPLC chromatogram of chlorogenic acid in methanol. (A) Freshly prepared; (B) after 1 day; (C) after 7 days.

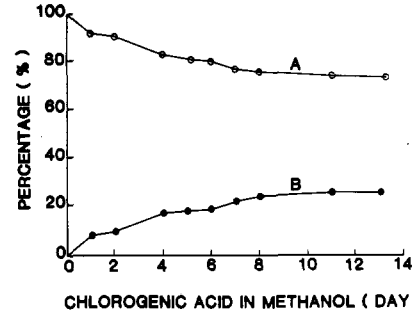


Figure 3. Rate of decrease of the HPLC peak for chlorogenic acid (A) and the appearance of a new peak (B).

RESULTS AND DISCUSSION

Chlorogenic Acid Content of Potatoes by HPLC. Figure 1 shows an HPLC chromatogram of freshly prepared authentic chlorogenic acid (100 ppm) in methanol. The retention time (Rt) of the single peak was 5.8 min. When the freshly prepared chlorogenic acid solution was left standing overnight at room temperature and then chromatographed, a second peak appeared at 8.2 min (Figure 2). However, a thin-layer chromatogram of this solution had only a single spot which coeluted with chlorogenic acid (R_f = 0.57). The same results were obtained when these experiments were carried out with ethanol rather than methanol solutions of chlorogenic acid.

The unexpected appearance of a second peak prompted us to carry out a time study on the relationship between the chlorogenic acid band and the new peak. Figure 2 shows that the chlorogenic acid peak slowly decreased with time. This decrease is accompanied by a corresponding increase in the size of the second peak. Figure 3 shows the results of a more extensive kinetic study. After 1 day, the concentration of the new compound was about 8% of the original chlorogenic acid concentration. This value gradually increased with time, reaching about 17% after 4 days,

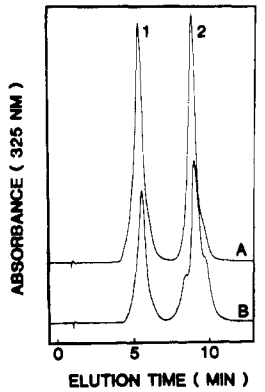


Figure 4. HPLC chromatograms of a mixture of chlorogenic acid (peak 1) and caffeic acid (peak 2). (A) Freshly prepared; (B) after 7 days.

20% after 6 days, and 29% after 14 days. The rate of increase then slows down (Figure 3). The maximum value was also obtained in 5 h after exposure of the chlorogenic acid solution to UV light in a chamber. By contrast, the new compound was not formed at all when the solution was stored in the dark.

The compound producing the second peak could be a methyl ester formed as a result of esterification of the COOH group on the quinic acid part of chlorogenic acid by the methanol solvent. A corresponding ethyl ester could form in ethanol solutions. Another possibility is that chlorogenic acid slowly hydrolyzes to caffeic and quinic acids or to methyl caffeate and quinic acid as a result of transesterification. However, a HPLC chromatogram of a 7-day-old solution of a mixture of chlorogenic and caffeic acids indicates that the new compound is not caffeic acid (Figure 4). Caffeic acid has a retention time of 9.17 min on the HPLC chromatogram and an R_f value of 0.89 on the TLC plate. Neither value corresponds to that observed with the new compound. Quinic acid did not elute at all on our HPLC chromatogram. Still another possibility is that chlorogenic acid undergoes an isomerization to isochlorogenic or neochlorogenic acid. However, when a 7-day-old ethanol solution of chlorogenic acid was spotted on a TLC plate along with an extract of morning glory seeds, none of the isomers present in the seed extract (Friedman et al., 1989) were present in the solution. This result suggests that the second HPLC peak is probably not an isomer of chlorogenic acid.

The following additional studies were carried out in an attempt to isolate the second compound. A 14-day-old methanol solution of chlorogenic acid was repeatedly injected into the HPLC system, and the two fractions were collected. Fraction 1 with a retention time of 5.8 min corresponded to the retention of chlorogenic acid. Fraction 2 had a retention time of 8.2 min. The collected fractions were evaporated under a stream of nitrogen. Each was redissolved in 500 μL of methanol and reinjected into the HPLC system. As expected, fraction 1 appeared at 5.8 min. Unexpectedly, fraction 2 did not appear at all on the HPLC chromatogram or on TLC plates. Thus, additional studies are needed to define the structure of the compound that gives rise to the second peak.

As far as we know, none of the previous HPLC studies with alcoholic extracts of chlorogenic acid reported the time-dependent formation of a new compound (Torres et al., 1987; Malmberg and Theander, 1985; Walter et al., 1979; Ramamurthy et al., 1992). The compound's formation indicates that HPLC could underestimate the true content of chlorogenic acid in the extracts. To avoid this problem, we examined the ultraviolet absorption method

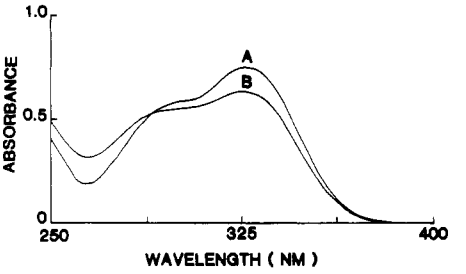


Figure 5. Ultraviolet absorption spectra of chlorogenic acid (A) and an ethanol extract of NDA 1725 potato roots (B).

Table I. Recovery of Chlorogenic Acid Added to NDA 1725 Potato Powders Prior to Extraction

chlorogenic acid added,[a] mg	recovery, % (av ± SD) UV method	HPLC method
potato tubers		
1	93.0 ± 4.9	83.9 ± 6.4
2	91.0 ● 3.6	75.0 ± 1.4
3	94.7 ● 4.3	80.9 ± 0.89
10	93.1 ± 0.3	73.4 ± 2.0
15	90.1 ● 3.7	78.9 ± 2.8
potato leaves		
10	95.5 ± 7.2	71.9 ± 0.6
15	93.5 ± 3.7	72.1 ± 1.04
potato sprouts		
10	93.1 ± 0.6	79.0 ± 0.6
15	90.2 ± 4.9	84.6 ± 3.6

[a] Potato tuber (200 mg), potato leaf (100 mg), or potato sprout (100 mg) powders plus the indicated amounts of chlorogenic acid in triplicate experiments.

Table II. Chlorogenic Acid Content in Different Potato Varieties (n = 3)

variety	chlorogenic acid, mg/100 g of fresh wt
commercial varieties	
Idaho Russet (small tubers)	9.65 ± 0.49
Idaho Russet (large tubers)	14.22 ± 0.73
small red	13.30 ± 0.69
experimental varieties	
Simplot I	13.14 ± 0.94
Simplot II	16.51 ± 0.49
NDA 1725	17.36 ± 1.19
potato 3194	18.71 ± 2.10

to measure the chlorogenic acid content in ethanolic extracts of fresh and processed potatoes.

Chlorogenic Acid Content of Potatoes by UV Spectrophotometry. Typical ultraviolet absorption spectra for chlorogenic acid and an ethanolic extract of potatoes are shown in Figure 5. These results and earlier studies (Friedman and Dao, 1990) on measuring the chlorogenic acid content of morning glory seeds provide a rational basis for estimating the chlorogenic acid content of ethanolic extracts of plant materials.

Table I shows that the recovery of chlorogenic acid added to a potato powder prior to extraction ranged from 90 to 95% when analyzed by UV compared to 71–84% when measured by HPLC. Ultraviolet spectrophotometry is thus a reliable method for measuring the chlorogenic acid content of potatoes.

Table II summarizes the chlorogenic acid content, determined by ultraviolet spectrophotometry, of three commercial and four experimental potato varieties. The values ranged from 9.7 to 18.7 mg/100 g of fresh weight. Three separate determinations for each variety gave consistent results.

Different parts of the potato plant had a much wider range of chlorogenic acid content than did the seven potato

Table III. Distribution of Chlorogenic Acid in Parts of the NDA 1725 Potato Plant (n = 3)

sample	chlorogenic acid, mg/100 g of fresh wt	glycoalkaloids,[a] mg/100 g of fresh wt
tubers	17.36 ± 1.19	15
roots	26.34 ± 0.82	86
leaves	223.53 ± 0.95	145
sprouts	754.06 ± 25.17	997

[a] Sum of α-chaconine and α-solanine (Friedman and Dao, 1992).

Table IV. Heat Stability of Chlorogenic Acid in Cooked NDA 1725 Potatoes (n = 3)

potato	chlorogenic acid, mg/g of freeze-dried wt	% loss
fresh	0.800 ● 0.05	
baked	0.000	100
boiled	0.319 ● 0.01	60.0 ± 0.12
microwaved	0.434 ± 0.02	45.7 ● 2.90

Table V. Chlorogenic Acid Content of Processed Potato Products (n = 3)

sample[a]	chlorogenic acid, mg/100 g of dried wt
potato skins I	0
potato skins II	0
french-fried potatoes I	0
french-fried potatoes II	0
mashed potato flakes I	0
mashed potato flakes II	0

[a] Samples I and II were obtained from different restaurants or stores.

varieties (Table III). The highest concentration of chlorogenic acid was found in sprouts (754 mg/100 g of fresh weight), followed by leaves (224 mg/100 g), roots (26 mg/100 g), and finally tubers (17 mg/100 g).

The glycoalkaloid content of the same potato plant, determined in an earlier study (Friedman and Dao, 1992; Table III) roughly parallels the chlorogenic acid content.

Chlorogenic Acid Content of Processed Potatoes. The stability of chlorogenic acid in potatoes subjected to three different modes of heating is shown in Table IV. The total chlorogenic acid content in NDA 1725 potatoes was reduced 46% after microwave baking, 60% after boiling, and 100% after baking in an oven. Table V shows that french-fried potatoes, mashed potato flakes, and potato skins obtained from stores and restaurants contained no chlorogenic acid. These observations confirm our earlier studies on the susceptibility of chlorogenic acid to heat, where we showed that the chlorogenic acid content decreased almost 100% in the crust fraction and about 65% in the crumb fraction of convection-baked muffins (Friedman and Dao, 1990).

Only the ethanolic extracts of microwaved and boiled potato tubers had an ultraviolet absorption maximum at about 328 nm. The oven-baked potatoes lacked a maximum absorbance. The absence of chlorogenic acid in this sample was confirmed by TLC, which showed no spot corresponding to the R_f value of 0.57 for authentic chlorogenic acid. None of the ethanolic extracts of the commercial potato products had absorption maxima, indicating an absence of chlorogenic acid.

These observations show that the nature of the heating process influences the extent of destruction of chlorogenic acid in potatoes. Oven-baking seems to be most effective, followed by boiling and then microwave-heating. Unresolved are the following questions: (a) whether heat destroys chlorogenic acid directly; (b) whether some or all of the decreases resulted from heat-catalyzed nutritionally damaging browning in which chlorogenic acid participates, as described earlier; and (c) whether some of the chlorogenic acid binds to other food components.

Significance for Plant Physiology, Nutritional Quality, and Food Safety. At least three different classes of compounds may act as so-called antifeeding agents in potatoes; i.e., they prevent phytopathogens and insects from feeding on potatoes containing them. These are (a) inhibitors of digestive enzymes, so-called trypsin or proteinase inhibitors; (b) glycoalkaloids; and (c) chlorogenic acid and related polyphenols (Brown et al., 1986; Deshpande et al., 1984; Elliger et al., 1981; Norris, 1984; Sanford et al., 1990; Sinden, 1987; Tingey, 1984). Since those parts of the potato plant that contain high levels of glycoalkaloids also have a high content of chlorogenic acid (Table III), the following questions arise:

(1) Are chlorogenic acid, glycoalkaloids, and inhibitors under the same genetic regulatory control in the plant?

(2) Do these compounds act synergistically in the defense of *Solanum* plants against attacks by fungi and insects?

(3) Will suppression of the biosynthesis of potentially toxic glycoalkaloids, one of our current objectives (Stapleton et al., 1991, 1992), result in a compensatory increase in the biosynthesis of the inhibitors and/or chlorogenic acid?

If such changes in the physiology of the potato plant do indeed occur, they may benefit both food safety and nutrition since potato proteinase inhibitors are nutritionally high-quality proteins after inactivation during cooking or baking (Pearce et al., 1984; Woolfe, 1987). Our studies on the heat-stability of chlorogenic acid (Tables IV and V; Friedman and Dao, 1990) show that this compound is also partially or completely destroyed by the usual food-processing conditions. Future compositional and nutritional studies with new potato varieties lower in glycoalkaloids should prove or disprove this hypothesis.

CONCLUSIONS

The apparent formation of a new compound from chlorogenic acid during methanolic and ethanolic extraction and storage suggests that caution needs to be exercised in the use of HPLC to estimate the chlorogenic acid content of plant materials. The use of the ultraviolet method to measure chlorogenic acid content of fresh and processed potatoes is straightforward and merits wide adoption. In applying this method, however, we assumed that the absorption maximum and the extinction coefficient of the chlorogenic acid derivative observed in HPLC are identical to those of chlorogenic acid. The validity of this assumption awaits confirmation with the pure compound, which we find difficult to characterize. It is, however, unlikely that the new compound has a much higher extinction coefficient than chlorogenic acid. For this to occur, the compound would have to have a wider conjugation of double bonds than chlorogenic acid and absorb at longer wavelengths, possibly in the yellow region of the spectrum. Visually, solutions of the new compound are colorless.

The levels of chlorogenic acid in seven potato varieties varied by only a factor of 2. In contrast, this factor was about 50 for different parts of the potato plant. The highest concentration of chlorogenic acid was in the sprouts and the lowest in the tubers. This variation was of the same order as we previously reported (Friedman and Dao, 1992) for levels of potato glycoalkaloids α-chaconine and α-solanine from the same plant parts.

Food-processing conditions such as heat induce a decrease in the chlorogenic acid content of potatoes. This decrease appears to depend on the nature of the heat used, with microwaves being least effective, boiling intermediate,

and oven-baking greatest. Although we do not know the processing history of the commercially purchased french-fried potatoes, mashed potato flakes, and potato skins, none of them contained chlorogenic acid.

These findings should facilitate further study of the participation of chlorogenic acid in enzymatic and non-enzymatic browning, after-cooking blackening, and plant defenses against phytopathogens. Such studies should catalyze the development of improved potatoes.

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