Determination of Chlorogenic Acid in Potato Tubers

Alf G. Malmberg* and Olof Theander

Chlorogenic acid has been determined in potato tubers by HPLC and GLC and the results were compared with the results of a spectrophotometric method. The HPLC analysis was performed by reversed-phase chromatography on C_{18} μBondapak column. For GLC, the chlorogenic acid or the quinic acid obtained by enzymatic hydrolysis was silylated. The methods have been applied to the peel and peeled tubers of six varieties of potato. The results establish that the chromatographic methods (HPLC or GLC) provide the most reliable determination of chlorogenic acid in potato extracts. Spectrophotometric measurements gave higher levels of the acid than HPLC and GLC analysis in a majority of the potato samples. The indirect determination by enzymatic hydrolysis and analysis of the increase in quinic acid content was not found to give reliable results.

Chlorogenic acid (3-*O*-caffeoylquinic acid) is a phenolic constituent of plants and constitutes about 90% of the total phenolic content of potato tubers. The interest in chlorogenic acid is largely due to its role in aftercooking blackening (Umaerus et al., 1979; Vertregt, 1968) and in a resistance mechanism (Kuč, 1973; Tripathi and Verma, 1975).

Numerous methods for chlorogenic acid analysis have been developed and applied during the years but only a few will be referred to here. Several methods have been based on UV absorption (Hughes et al., 1962; Amberger and Schaller, 1975) or color reactions (Zucker and Ahrens, 1958; Mesnard and Devaux, 1963). The extracts have, in many cases, been partly purified by paper (Paech and Ruckenbrod, 1953; Baruah and Swain, 1959) or column chromatography (Zucker and Ahrens, 1958) before the determination. These analyses have either been time consuming and/or nonspecific.

In the 1960's, gas chromatography (GLC) became available and offered excellent separation efficiency. A few analyses of chlorogenic acid in plant material by GLC are found in the literature (Kung et al., 1967; Wilson et al., 1968; Bombardelli et al., 1977; Sosulski et al., 1982). None of these refer to potato tubers, although Sosulski investigated the amount of chlorogenic and other phenolic acids in potato flour. For the derivatization of chlorogenic acid, different silylating agents and reaction conditions have been used (see also Pellizzari et al. (1969) and Möller and Herrmann (1982)). BSA with an acidic component as catalyst has been used most frequently.

In recent years, a series of reports on the analysis of cinnamic acid esters using high-pressure liquid chromatography (HPLC) has been published (Court, 1977; Krause and Strack, 1979; Walter et al., 1979; Nagels et al., 1980; Bergers, 1981; Möller and Herrmann, 1982; Brandl and Herrmann, 1983; Lyon and Barker, 1984). With the exception of two reports using diol columns (Nagels et al., 1980; Brandl and Herrmann, 1983) reverse-phase chromatography on C_8 or C_{18} columns with an acidic solvent system has been used.

The aim of the present work was to compare the estimation of chlorogenic acid in extracts of potato tubers by two chromatographic methods—HPLC and GLC—with a modified spectrophotometric method by Umaerus et al. (Malmberg et al., 1980). The results from extracts of the peel and the peeled tubers of six potato varieties are discussed.

Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.

EXPERIMENTAL SECTION

Plant Material. The peel and the peeled tuber of six potato varieties (Bintje, Sabina, Magnum Bonum, Ukama, Stina, and Sv 70102) were analyzed. The tubers were collected from field trials conducted by the Department of Plant Husbandry in Uppsala, Sweden.

Chemicals. All chemicals used were commercially available. The enzyme used was cellulase from *Aspergillus niger* practical grade, Type 1 (Sigma). *N,O*-Bis(trimethylsilyl)acetamide (BSA) or (methylsilyl)trifluoroacetamide (MSTFA) together with trimethylchlorosilane (Me_3SiCl) were used as silylating agents.

Extraction. Ethanol (EtOH) extracts from a previous investigation were used (Malmberg and Theander, 1984).

Hydrolysis. An aliquot of 10 mg of EtOH extract was evaporated and dissolved in 10 mL of H_2O. Samples (1 mL) were taken out for analysis before hydrolysis. Enzyme (20 mg) was added and the solution was hydrolyzed at 37 °C for 5 h. Samples (1 mL) of the hydrolyzed extract were then taken out for analysis.

Another sample (1 mL) from the enzymatic hydrolysis was treated on a column (10 × 1 cm i.d.) of Dowex 1 × 8 (Ac). The column was washed with 150 mL of H_2O and the quinic acid was eluted with 20 mL of 2 M HCOOH and used for GLC analysis.

GLC analysis was performed on a CP-sil 5 capillary column (25 m × 0.2 mm i.d., Chrompack Ltd, The Netherlands). The carrier gas flow was 0.9 mL/min (30 cm/s) of helium with a split ratio of 1:30.

Chlorogenic Acid Analysis. To an aliquot of the EtOH extract (10 mg) was added a solution containing 0.1 mg of stigmasterol (internal standard). After evaporation to dryness 0.3 mL of BSA–Me_3SiCl (10:1) was added and the residue was silylated at room temperature overnight. For GLC a temperature program from 255 to 300 °C at 2 °C/min was used with an injector and detector temperature of 320 °C.

Quinic Acid Analysis. To an aliquot of the solution (1 mL) from the enzymatic hydrolysis of the whole solution from ion exchange treatment was added a solution containing 0.01 mg of sorbitol (internal standard). After evaporation to dryness, the residue was silylated with 0.5 mL of MSTFA–EtOAc–Me_3SiCl (10:10:1) at room temperature overnight. The column temperature during the analysis was 150–190 °C programmed at a rate of 2 °C/min. Injector and detector temperature was 280 °C.

Caffeic Acid Analysis. To an aliquot of the solution (1 mL) from the enzymatic hydrolysis was added a solution containing 0.01 mg of 2-hydroxynaphthalene (internal standard). After evaporation to dryness, the sample was dissolved in 0.5 mL of 1% NaOH and applied to a SepPak C_{18} cartridge (prepacked minicolumn, Waters Associates),

0021-8561/85/1433-0549$01.50/0

Table I. Chromatographic Data for the Analysis of Chlorogenic Acid (1) and Its Hydrolysis Products, Quinic Acid (2), and Caffeic Acid (3).

no.	chromatographic method	retention time min	retention time rel I.S.	response factor	standard deviation	internal standard (I.S.)
1	HPLC	9.2	1.94	1.90[a]	0.03	2,5-dihydroxybenzoic acid
1	GLC	12.3	0.84	*b*	*b*	stigmasterol
2	GLC	13.5	1.26	0.58[c]	0.05	sorbitol
3	GLC	13.0	2.26	0.41[c]	0.18	2-hydroxynaphthalene

[a] Response factor with Sep Pak cleanup: 1.68. [b] Response factor varies with the ratio 1/I.S. The medium standard deviation at the different amounts of 1 was 0.09. [c] Response factor calculated on the amount of 1 before enzymatic hydrolysis.

which had been washed before use with 10 mL of MeOH, 10 mL of H_2O, and 10 mL of 1 M acetic acid (HOAc). The SepPak was then washed with 4 mL of 1 M HOAc and thereafter the caffeic acid and the internal standard were eluted with 2 mL of 95% EtOH followed by 5 mL of ethyl acetate (EtOAc). The two latter extracts were pooled, the solvents were removed by evaporation, and the sample was then silylated with 0.3 mL of BSA–Me_3SiCl (10:1) at room temperature overnight. The column temperature during the analysis was programmed from 120 to 250 °C at 8 °C/min and with an injector and detector temperature of 280 °C.

HPLC Analysis. An aliquot of the EtOH extract (10 mg) and a solution of 0.1 mg of 2,5-dihydroxybenzoic acid (internal standard) were mixed and evaporated. The sample was either dissolved in 0.5 mL of 5% methanol (MeOH) in buffer A (0.05 M KH_2PO_4 buffer, pH 3.50) and filtered through a Millipore filter (0.5 μm) or dissolved in 0.5 mL of buffer A for cleanup on a SepPak C_{18} cartridge before the analysis. Before use the SepPak was washed with 10 mL of MeOH, 10 mL of H_2O, and 10 mL of buffer. The sample was added to the SepPak and washed with 2 mL of buffer, before eluting the chlorogenic acid with 10 mL of MeCN–MeOH–H_2O (2:2:1). The eluate was evaporated, dissolved in 0.5 mL of 5% MeOH in buffer A, and analyzed.

A reversed-phase C_{18} μBondapak column (10 μm, 30 cm × 4 mm i.d., Waters Associates), with a short precolumn of C_{18}/Corasil Bondapak (37-50 μm), was used for the HPLC analysis. The mobile phase consisted of a linear gradient of 5–50% MeOH in buffer A applied for 20 min at a flow rate of 2 mL/min. The detector was a UV detector operating at 313 nm.

Spectrophotometric Measurements. The analyses were performed by the modified method of Umaerus and Olsson (Malmberg et al., 1980). The samples were centrifuged before the UV absorbtion was measured.

RESULTS AND DISCUSSION

Chlorogenic acid has been analyzed in the peel and the corresponding peeled potato tubers from six varieties by HPLC and GLC. The hydrolysis products of chlorogenic acid, caffeic acid, and quinic acid were also analyzed by GLC. The chromatographic data for the different methods are listed in Table I. The standard deviation was calculated from the results of at least ten reference samples.

The sample preparation for the HPLC analysis is rapid and simple. The extract together with an internal standard is dissolved in 5% MeOH in buffer, passed through a Millipore filter, and injected into the chromatograph. To protect the column, the sample may first be passed through a SepPak cartridge. This is to be recommended, but in that case the response factor had to be changed (Table I), because part of the chlorogenic acid is lost in the SepPak.

For GLC, derivatization of the acid is needed to volatilize the compound, and many different silylating agents and media described in the literature were tested. Some of the procedures (Kung et al., 1967; Wilson et al., 1968; Pellizari et al., 1969) could not be reproduced or gave a very low response in our laboratory. Other methods (Horning et al., 1967; Möller and Herrmann, 1982; Bombardelli et al., 1977) gave poor reproducibility. However, by changing the column from glass capillary to fused silica the reproducibility was increased. In order to avoid decomposition of the silylated compounds during an evaporation step and since pyridine may shorten the lifetime of the column, a silylation medium without pyridine was used in this study. It was also found that the response factor was sensitive to the amount of and ratio between chlorogenic acid and internal standard in the sample. Therefore a standard curve for the response factor was used for the quantitative calculations (Figure 1). The ratio of the peaks of the chlorogenic acid and I.S. in each sample was calculated and the response factor (*F*) was read from the standard curve. The obtained response factor was then used for the quantitative calculation. It was important for the calculations that the area of I.S. in the samples and the references was about the same.

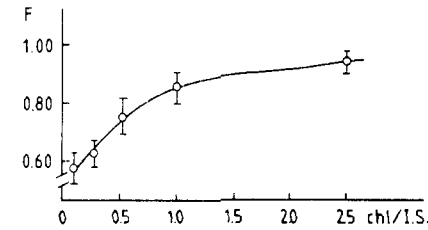

Figure 1. Standard curve for the quantitative calculations of silylated chlorogenic acid (chl). The amount of stigmasterol (I.S.) was 0.2 mg in each sample.

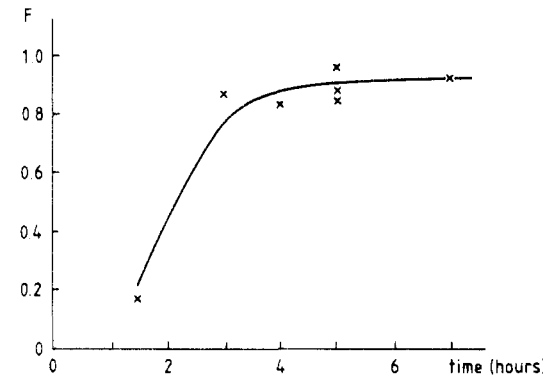

Figure 2. Enzymatic hydrolysis of chlorogenic acid at 37 °C. F denotes the ratio of μmol of quinic acid found/μmol of chlorogenic acid at the start of the hydrolysis.

An attempt to determine chlorogenic acid indirectly via enzymatic hydrolysis and analysis of quinic acid was also made. The enzymatic hydrolysis was conducted for 5 h at 37 °C and the yield reached a value of about 90% of the theoretical value (Figure 2). As quinic acid is a natural component of potato tissue, the initial levels must also be determined. A cleanup of quinic acid by ion exchange was needed after hydrolysis of peeled tuber tissue. In tissue from peel, it was possible to analyze quinic acid without

Table II. The Amount of Chlorogenic Acid (μmol/g DM) in the Peel and Peeled Tuber of Six Potato Varieties Analyzed with the Different Methods. P and T Denote Peel and Peeled Tuber, Respectively

sample	spectrophotometric measurement	HPLC	GLC	GLC of quinic acid after enzymatic hydrolysis: no ion change	GLC of quinic acid after enzymatic hydrolysis: ion change
		Bintje			
P	3.8	4.2	*a*	4.2	*a*
T	0.9	0.2	0.1	2.3	1.7
		Magnum Bonum			
P	4.7	5.3	5.1	*a*	*a*
T	0.6	1.2	0.7	2.9	*a*
		Sabina			
P	4.4	2.3	2.0	3.1	*a*
T	0.6	0.5	0.1	5.0	*a*
		Ukama			
P	4.5	0.6	*a*	0.7	*a*
T	0.7	0.1	0.1	2.5	2.4
		Stina			
P	6.5	7.1	5.1	6.0	*a*
T	0.7	0.2	0.2	1.0	0.5
		Sv 70102			
P	3.8	0.1	0.2	4.6	2.2
T	0.5	0.1	0.1	1.2	0.1

[a] Extract was used up.

cleanup. The results from the analysis of caffeic acid, the other hydrolysis product of chlorogenic acid, were found to vary too much and this approach was not pursued further.

The results of GLC and HPLC analysis of chlorogenic acid (Table II) agree rather well, except for the peel from Stina and the peeled tubers of Magnum Bonum and Sabina, where HPLC gave higher results. The results from the spectrophotometric method are higher in the peel from Sabina, Ukama, and Sv 70102 and in the peeled tuber of Bintje, Ukama, Stina, and Sv 70102. Higher results were expected in the spectrophotometric method as it is known that other components, e.g., caffeic and protocatechuic acids, may interfere (Malmberg and Theander, unpublished results). In one sample, peeled tuber of Magnum Bonum, the spectrophotometric method gave a significantly lower level than that of HPLC. It should also be noted that the isomers of chlorogenic acid are not analyzed quantitatively in this study. A mixture of the isomers, crypto- and neochlorogenic acids, (4-*O*- and 5-*O*-caffeoylquinic acid, respectively) was prepared according to Nagels et al. (1980) and chromatographed on HPLC. In our system, the crypto isomer as well as the neo isomer was eluted before chlorogenic acid as reported by Brandl and Herrmann (1983). The ratio of crypto- and neochlorogenic acid to chlorogenic acid was in agreement with the results of Brandl and Herrmann (1984). These isomers, which also separated from chlorogenic acid in GLC, contribute to the level of chlorogenic acid in the spectrophotometric method but cannot completely explain the differences between our HPLC and GLC results and the spectrophotometric values.

The results of the analysis of quinic acid differ from those derived by the other two chromatographic methods and the spectrophotometric method. In the peel, the results agree fairly well with the other chromatographic methods except for Sv 70102 where it is high and at the same level as for the spectrophotometric method. In the peeled tubers, however, it is even higher than the results of the spectrophotometric method, although ion exchange reduces the level in Sv 70102 and Stina. Presumably, quinic acid is also involved in other conjugates from which it is released by the enzymatic hydrolysis.

Our results on the determination of chlorogenic acid in potato tuber extracts with HPLC, GLC, and a spectrophotometric method indicate that the spectrophotometric method may give too high levels. For a more accurate determination of the acid, a chromatographic method (HPLC or GLC) is needed. In this study, HPLC was found to be more reproducible (Table I) and less time consuming (no derivatization) than GLC. In the GLC analysis, the reproducibility in the derivatization step was unsatisfactory and the column must be in very good condition for the analysis. An indirect determination of chlorogenic acid by hydrolysis and analysis of the quinic and/or caffeic acid released by hydrolysis was not found to be as reliable as the direct analysis.

ACKNOWLEDGMENT

We thank Gunnel Fransson for skillful technical assistance.

Registry No. Chlorogenic acid, 327-97-9; quinic acid, 77-95-2; caffeic acid, 331-39-5.

LITERATURE CITED

Amberger, A.; Schaller, K. *Potato Res.* **1975**, *18*, 161.
Baruah, P.; Swain, T. *J. Sci. Food Agric.* **1959**, *10*, 125.
Bergers, W. W. A. *Food Chem.* **1981**, *6*, 47.
Bombardelli, E.; Gabetta, B.; Martinelli, E. M. *Fitoterapia* **1977**, *48*, 143.
Brandl, W.; Herrmann, K. *J. Chromatogr.* **1983**, *260*, 447.
Brandl, W.; Herrmann, K. *Z. Lebensm.-Unters. Forsch.* **1984**, *178*, 192.
Court, W. A. *J. Chromatogr.* **1977**, *130*, 287.
Horning, M. G.; Boucher, E. A.; Moss, A. M. *J. Gas Chromatogr.* **1967**, *297*.
Hughes, J. C.; Ayers, J. E.; Swain, T. *J. Sci. Food Agric.* **1962**, *13*, 224.
Krause, J.; Strack, D. *J. Chromatogr.* **1979**, *176*, 465.
Kuč, J. A. *Teratology* **1973**, *8*, 333.
Kung, J. T.; Ryder, W. S.; Feldman, J. R. *Int. Colloq. Chem. Coffe, 3rd* **1967**, 223.
Lyon, G. D.; Barker, H. *Potato Res.* **1984**, *27*, 291.
Malmberg, A.; Theander, O. *Swed. J. Agric. Res.* **1984**, *14*, 119.
Malmberg, A.; Theander, O. unpublished results, 1984.
Malmberg, A.; von Rosen, G.; Schatz, B.-A.; Theander, O. *Swed. J. Agric. Res.* **1980**, *10*, 89.
Mesnard, P.; Devaux, G. *Bull. Soc. Chim. Fr.* **1964**, *1*, 43.
Möller, B.; Herrmann, K. *J. Chromatogr.* **1982**, *241*, 371.
Nagels, L.; Van Dongen, W.; De Brucker, J.; De Pooter, H. *J. Chromatogr.* **1980**, *187*, 181.
Paech, K.; Ruckenbrod, H. *Ber. Dtsch. Bot. Ges.* **1953**, *66*, 76.
Pellizzari, E. D.; Chuang, C-M.; Kuč, J.; Williams, E. B. *J. Chromatogr.* **1969**, *40*, 285.
Sosulski, F.; Krygier, K.; Hogge, L. *J. Agric. Food Chem.* **1982**, *30*, 337.
Tripathi, R. K.; Verma, M. N. *Indian J. Exp. Biol.* **1975**, *13*, 414.
Umaerus, M.; Jönsson, U.; Olsson, K. *Sver. Utsaedesfoeren. Tidskr.* **1979**, *89*, 175.
Vertregt, N. *Eur. Potato J.* **1968**, *11*, 226.
Walter, W. M.; Purcell, A. E.; McCollum, G. K. *J. Agric. Food Chem.* **1979**, *27*, 938.
Wilson, J. L.; Dunlap, W. J.; Wender, S. H. *J. Chromatogr.* **1968**, *35*, 329.
Zucker, M.; Ahrens, J. F. *Plant Physiol.* **1958**, 246.

Received for review September 17, 1984. Accepted February 13, 1985. This work was financially supported by the Swedish Board for Technical Development.