



Rapid Determination of Phenolic Compounds in Extracts of the Leaves of *Ipomoea batatas* (L.) Lam. by HPLC-DAD-ESI-MS

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Ipomoea batatas (L.) Lam. (Sweet potato) is not only used as staple food, but also is a traditional herbal drug in China for the treatment of various disease. For analyze the component of the extraction, we have developed a chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) method to characterize th fraction of ethyl acetate, which gave prominent [M-H]⁻ ions by electrospray ionization mentored in the negative ion mode. A total of ten phenolic compounds were identified or tentatively characterized based on the UV spectra and Tandem mass spectra of the HPLC peaks. The tandem mass spectrometric fragmentation behaviour of caffeoylquinic acid derivatives and flavonoids have been investigated extensively.

Key Words: Sweet potatoes, Phenols, HPLC-DAD-ESI-MS.

INTRODUCTION

The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant which belongs to the family Convolvulaceae. It represents an important crop in tropical, subtropical and warm temperature regions with its agronomic and nutritional advantages¹. The storage root of sweet potato are used as staple food, raw material for alcohol production and animal feed, while in China, the leaves of sweet potato are a common side dish in cuisine and used as a traditional medicine for a long history. They poach the leaves and drink the water to cure various diseases such as the inflammation of the throat, ascites, diarrhea and astriction.

Sweet potato has long attracted attention for its content of potentially bioactive phytochemicals, including secondary metabolic products. It has been known to contain chlorogenic acid, several caffeoylquinic acids, dicaffeoylquinic acids and flavonoids²⁻⁴. These phytochemicals have been claimed to possess many pharmacological properties such as antioxidant^{5,6}, anticancer, antiviral and antimicrobial effects⁷, antimutagenicity antidiabetes; anti HIV⁸, but there have been few studies on the leaves of the plant grown in China to expound the material basement of the activity.

In this paper, a rapid method for determination of the phenolic compounds in the *Ipomoea batatas* Lam. was described by HPLC/DAD/ESI-MSⁿ (High-performance liquid chromatography with diode-array detection in parallel with

electrospray ionization and ion trap Tandem mass spectrometry). The compounds were identified or tentatively characterized, including flavonoids and quinic acid derivatives and the ESI-MS fragmentation behaviour of these compounds was also investigated.

EXPERIMENTAL

The dry leaves of sweet potato were purchased from local market near Yancheng City in China. All solvents used for extraction were of analytical grade (Hanbon Sci & Tech, Jiangsu, China), methanol used for HPLC were of chromatographic grade (Hambon Sci & Tech, Jiangsu, China) and water used was distilled water. Acetic acid used were of analytical grade (Nanjing Chemical Reagent Co. Ltd.)

Extraction and isolation: One hundred grams of dry leaves of sweet potato was extracted with 1 L 95 % ethanol and then evaporated to form a syrup. The syrup was dissolved in 300 mL of water by sonication and partitioned with chloroform and ethyl acetate of equal volume three times successively, the ethyl acetate solution was vacuum evaporated at 65 °C. About 2.5 g residue of ethyl acetate was obtained. The residues was dissolved with methanol and filtered through a 0.45 µm filter for HPLC analysis.

HPLC condition: The analysis were performed using an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, a diode-array

detector (DAD), an autosampler and a column compartment. The sample was separated on Welch XB-C₁₈ (250 mm × 4.6 mm, 5 μm). The mobile phase consisted of water containing 0.1 % (V/V) acetic acid (A) and methanol (B) with a gradient from 25 % (V/V) B to 35 % (V/V) B in the first 15 min, then from 40-45 % over 20 min and then to 50 % (V/V) B over 10 min. The mobile phase flow rate was 1.0 mL/min, and the column temperature was set at 25 °C. The DAD was set to monitor at 255 nm and the on-line UV spectra were recorded in the range 190-400 nm.

Mass spectrometry: For HPLC/MS analysis, Agilent 1100 Series LC/MSD Trap SL was connected to the HPLC instrument via an ESI interface. Ultrahigh-purity helium (He) was used as the collision gas purity nitrogen (N₂) as the nebulizing gas. The ionization parameters in the negative ion mode were as follows, dry temp: 350, Nebulizer: 45.00 psi, dry gas: 10.00 L/min, HV Capillary: 3000 V. for full-scan MS analysis, the spectra were recorded in the range m/z 50-1000. Data-dependent acquisition was used so that the two most abundant ions in each MS scan were selected in turn and subjected to tandem mass spectrometry analysis. The collision energy for CID was adjusted to 35 % of maximum.

RESULTS AND DISCUSSION

A detailed study of the fragmentation pattern of the caffeates and flavonoids from sweet potato was made employing triple quadrupole mass analyzers. In this way, the mass fragmentation behaviour could be analyzed in terms of the potential applied. Most constituents displayed a similar spectral behaviour with two maximum absorption peaks at 220-250 and 320-330 nm and a shoulder at 290-300 nm (Table-1). They were characterized as hydroxycinnamic acid derivatives⁹. Some peaks corresponding to flavones (three absorption peaks around 250, 280 and 350 nm) were also observed. HPLC/MS was utilized for further characterization of individual substances. During HPLC/MS analysis, a key step was to determine the molecular weight of each component analyzed. Usually, the most abundant peak in a full MS spectrum was assigned as [M-H]⁻ and if adduct ions ([M-H + HCOOH]⁻) and dimers could be

observed, this assignment was more solid. The typical chromatogram of an extract of the leaves of sweet potato is shown in Fig. 1, while Table-1 shows the retention times, UV absorptions, MS and MS² spectral data and identification results for the peaks in the chromatogram.

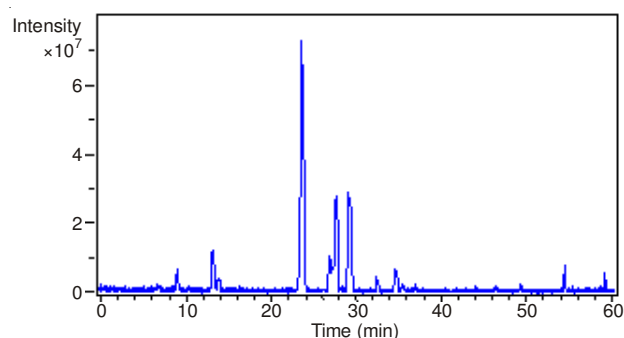


Fig. 1. LC-negative ion ESI-MS total ion current (TIC) profile

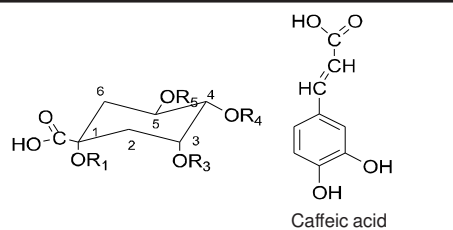
Two parent ions at m/z 353 were detected at the elution time 9.3 and 10.5 min they were characterized by UV¹⁰ and mass spectra as caffeoylquinic acid. They yielded a [M-M]⁻ ion at m/z 173, indicating the sequential loss of water. They were 3-acyl, 4-acyl or 5-acyl isomers of caffeoylquinic acid, with 5-caffeoylquinic acid dominant¹¹ and its chemical structures was shown in Fig. 2.

Compound 3 yield a [M-H]⁻ at m/z 179, it only loses the neutral molecule CO₂, giving the anion of m/z 135, because the hemolytic cleavage of benzylic bond it relatively rare with an electrospray ionization source. It was identified as caffeic acid¹².

Four peaks at m/z 515 were characterized as di-O-caffeoylquinic acids (CQAs) isomers. they all shown the loss of 162 u, the caffeoyl group, to provide a very clear ion at m/z 353. A second loss of 162 u produces the m/z 191 ion, which represents the intact quinic acid function. The loss of two neutral species with a mass of 162 u identifies the ester functions as being the same and to be caffeic acid. Because the presence of mixed ester group will, therefore, be indicated by the sequential loss of one acidic group, followed by expulsion

TABLE-1
CHROMATOGRAPHIC, UV AND SPECTRAL CHARACTERISTICS OF
PHENOLIC COMPOUNDS FROM CHINESE SWEET POTATO LEAVES

Comp. No.	Retention time (min)	UV λ _{max} (nm)	[M-H] (m/z)	HPLC-ESI-MS ^a m/z	Identification
1	9.3			MS ² [353]:191,179	Caffeoylquinic acid
2	10.5	220,302sh,328	353	MS ³ [191]:173	
3	13.1	217,297	179	MS ² [179]:135	Caffeic acid
4	23.1	217,242,297,326	515	MS ² [515]:353(100),179(10),191(1),255(10)	1,3-diCQA
5	24.0			MS ³ [353]:191(100),173(79),179(40),135(17)	1,4-diCQA
				MS ² [515]:353(100),179(1),173(12),300(9),335(9)	
6	28.9			MS ³ [353]:191(100),173(20),179(35),135(1)	3,4-diCQA
				MS ² [515]:353(100),179(8),255(2),173(7)	
7	29.7			MS ³ [353]:179(100),173(61),179(8),190(23)	3,5-diCQA
				MS ² [515]:353(100),173(5),335(4)	
8	27.0	254,295,355	463	MS ² [463]:301,302	Quercetin-3-glucoside
				MS ³ [301]:255,107,151,179,271	
9	32.0	350,255	447	MS ² [447]:285,284,255	Kaempferol-3-O-glucoside
				MS ³ [285]:257,107,155,227,285	
10	46.2	255,370	301	MS ² [301]:151	Quercetin



Name	R ₁	R ₃	R ₄	R ₅
5-CQA	H	H	H	Caf
1,3-diCQA	Caf	Caf	H	H
1,4-diCQA	Caf	H	Caf	H
3,4-diCQA	H	Caf	Caf	H
3,5-diCQA	H	Caf	H	Caf

Caf = Caffeic acid
CQA = Caffeoylquinic acid

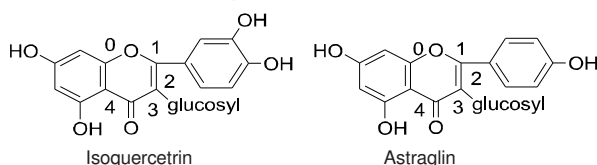


Fig. 2. Main chemical structures of compounds identified from the leaves of sweet potato

of the second functionality and the spectrum would contain two ions in the mid-range of the spectrum, one for each of the acidic groups. The m/z 191 ion also give rise to peaks at m/z 173 by the loss of molecules of water. The peaks at m/z 179 ion, the acid of the ester with an intact carboxyl group, which carries a negative charge easily; if a mixed ester were being analyzed, two different ions could be observed which would identify the mass of each acid functionality. In the present case, both ester are identical and only one ion is observed. The fragments at m/z 135 are formed by the loss of CO_2 from the carboxylic acids, such processes are known to occur in aromatic carboxylic acids¹³. For diCQA, the ease of removal of the caffeoyl residue during fragmentation is $1 \approx 5 > 3 > 4$, which was one of the reasons for the diversity of the intense of the peaks in mass spectra. The four isomers was discriminated by using the hierarchical key established by Clifford and co-workers¹⁴ and comparison of retention time, elution order on reversed-phase packing¹⁵. They were identified as 1,3-diCQA, 1,4-diCQA, 3,4-diCQA, 3,5-diCQA directly.

The peak at m/z 463 $[\text{M}-1]^-$, m/z 447 $[\text{M}-1]^-$, were identified as isoquercitrin (quercetin-3-glucoside) and astragalin (kaempferol-3-O-glucoside). And the peak at m/z 301 $[\text{M}-1]^-$ was identified as quercetin based on UV spectra and MS fragmentation behaviour.

The presence of an glucoside was supported by loss of 162 u ($[\text{M}-\text{H}-162]^-$) from the parent ion m/z at 463 $[\text{M}-1]^-$ and 447 $[\text{M}-1]^-$. The anion at m/z 301 produces the ion at m/z 151 and 107 through two RDA reactions as depicted in Fig. 3. the C-ring cleavage products ions can be used to confirm the nature of structure of A- and B-rings. The cleavage of bond 3 and 4 of the C-ring explains the loss of CO ($m = 28$) which had been observed in both isoquercitrin and astragalin. It is interesting that, at the same time, it would loss two proton or H_2O , ion m/z at 285 and 301 yielded the $[\text{M}-\text{CO}]^-$ at m/z 257, $[\text{M}-\text{CO}-\text{H}_2\text{O}]^-$ at m/z 255, $[\text{M}-\text{CO}-2\text{H}]^-$ at m/z 271.

The C-ring cleavage pathway was various, the cleavage of bond 2 and 4 of C-ring explains the m/z 226 ion derived

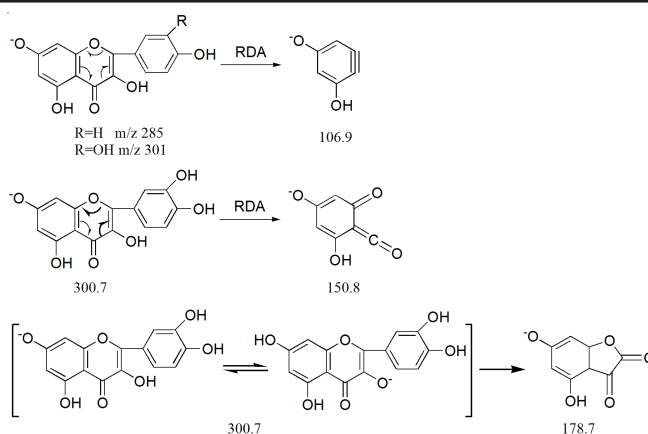


Fig. 3. Proposed MS fragmentation pathway for the $[\text{M}-\text{H}-162]^-$ ions of compounds 8, 9 and 10

from m/z 285 in the spectrum of astragalin. The cleavage of bond 1 and 2 of C-ring of ion m/z 301 from the ion 179 in the spectrum of isoquercitrin (Fig. 3).

Conclusion

Based on the above MS fragmentation rules, ten polyphenolic compounds were identified or characterized from the leaves of Chinese sweet potato in one LC-MSⁿ run. In this study, a HPLC-DAD-ESI-MS/MS method was applied and a good example was made for the rapid identification of the constituents in plant extracts. Phenolic compounds are ubiquitous bioactive compounds and a diverse group of secondary metabolites universally present in higher plant, such as caffeic acid and caffeoylquinic acid derivatives. The findings in association with the treatment of the inflammation of the throat, ascites, diarrhea, astriction which were kept for a long time could be extended and do a great help to food technologists and enhance the importance of this food stuff in the human diet.

REFERENCES

- K.D. Sarwan, P.S. Hari and D. Seema, Compendium of Transgenic Crop Plants: Sweet Potato, Wiley Online Library (2009).
- M.S. Islam, M. Yoshimoto, S. Yahara, S. Okuno, K. Ishiguro and O. Yamakawa, *J. Agric. Food. Chem.*, **50**, 3718 (2002).
- M. Takenaka, K. Nanayama, S. Isobe and M. Murata, *Biosci. Biotechnol. Biochem.*, **70**, 172 (2006).
- W. Zheng and M.N. Clifford, *Food Chem.*, **106**, 147 (2008).
- J.-K. Jung, S.-U. Lee, N. Kozukue, C.E. Levin and M. Friedman, *J. Food Compos. Anal.*, **24**, 29 (2011).
- S. Kaviarasan, A.S. Sivakumar, A. Barik, A. Kunwar, G.H. Naik and K.I. Priyadarsini, *J. Food. Biochem.*, **35**, 596 (2011).
- M.T. Pochapski, E.C. Fosquiera, L.A. Esmerino, E.B. Santos, P.V. Fargo, F.A. Santos and F.C. Groppo, *Pharmacog. Mag.*, **7**, 165 (2011).
- S. Islam, *J. Food Sci.*, **71**, R13 (2006).
- D. Kammerer, R. Carle and A. Schieber, *Rapid Commun. Mass Spectrom.*, **18**, 1331 (2004).
- V. Ossipov, K. Nurmi, J. Loponen, E. Haukioja and K. Pihlaja, *J. Chromatogr. A*, **721**, 59 (1996).
- E.C. Tatsis, S. Boeren, V. Exarchou, A.N. Trognis, J. Vervoort and I.P. Gerotheranassis, *Phytochemistry*, **68**, 383 (2007).
- A. Ribas-Agustí, M. Gratacós-Cubarsí, S. Sárraga, J.A. García-Regueiro and M. Castellari, *Phytochem. Anal.*, **22**, 555 (2011).
- H. Budzikiewicz, C. Djerassi and D.H. Williams, *Mass Spectrometry of Organic Compounds*, Holden-Day: San Francisco, p. 219 (1967).
- M.N. Clifford, S. Knight and N. Kuhnert, *J. Agric. Food. Chem.*, **53**, 3821 (2005).
- Y. Zhang, P. Shi, H. Qu and Y. Cheng, *Rapid Commun. Mass Spectrom.*, **21**, 2971 (2007).