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Profiling the chlorogenic acids of sweet potato (*Ipomoea batatas*) from China

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Abstract

The leaves, stem and root of sweet potato cultivated in China have been analysed qualitatively for chlorogenic acids by structure-diagnostic LC–MS^3. Chlorogenic acids were not detected in the root. Caffeoylquinic acids were quantitatively the major subgroup of chlorogenic acids detected in the stem and the only subgroup detected in the leaves. This subgroup was dominated by 5-caffeoylquinic acid. The stem also contained three feruloylquinic acids, 3,5- and 4,5-dicaffeoylquinic acid, and small amounts of at least four caffeoyl-feruloylquinic acids. This is the first report of feruloylquinic and caffeoyl-feruloylquinic acids from sweet potato. Two chemically unrelated and coeluting substances with the same molecular mass ($M_r = 530$) extracted from the Chinese sweet potato interfered with the characterisation of the caffeoyl-feruloylquinic acids. At least five caffeoyl-feruloylquinic acids were detected in the peel of sweet potato cultivated in Tanzania that lacked these interfering substances.
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Keywords: Caffeoylquinic acids; Caffeoyl-feruloylquinic acids; Chlorogenic acids; Dicaffeoylquinic acids; Feruloylquinic acids; *Ipomoea batatas*; Sweet potato

1. Introduction

Sweet potato, *Ipomoea batatas* (L.), is an herbaceous perennial grown in more than one hundred countries including barren mountain areas in China. This important staple food crop has attracted attention because of its content of potentially bioactive phytochemicals, including chlorogenic acids. Sweet potato has long been known to contain chlorogenic acids (Uritani & Miyano, 1955) and several caffeoylquinic and dicaffeoylquinic acids, and at least one tricaffeoylquinic acid have been reported in the leaves of Japanese plants (Islam et al., 2002; Kurata, Adachi, Yamakawa, & Yoshimoto, 2007; Takenaka, Nanayama, Isobe, & Murata, 2006), but there have been no studies on plants grown in China.

Chlorogenic acids have interesting properties *in vitro*, such as inhibition of Na^+-dependent D-glucose uptake in rat intestinal brush border membrane vesicles (Welsch, Lachance, & Wasserman, 1989) and coffee beverage rich in chlorogenic acids has been shown to modify gastrointestinal hormone secretion and glucose tolerance in humans (Johnston, Clifford, & Morgan, 2003) although the mechanism(s) has not been fully elucidated.

Chlorogenic acids are a large family of esters formed between quinic acid and one to four residues of certain *trans* cinnamic acids, most commonly caffeic, *p*-coumaric and ferulic: sinapic and dimethoxycinnamic acid also occur and in some plant species various aliphatic acids may replace one or more of the *trans* cinnamic acid residues (Clifford, 2000, 2003; Clifford, Knight, Surucu, & Kuhnert, 2006). In the IUPAC system (−)-quinic acid is defined as 1L-1(OH),3,4/5-tetrahydroxycyclohexane carboxylic acid and that nomenclature is used throughout this paper (IUPAC, 1976). Chlorogenic acid analysis in plant extracts

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is made difficult by the lack of authentic standards and by the difficulty of discriminating between positional and geometric isomers (Clifford, 2003). These difficulties have been partially overcome by the development of structure-diagnostic LC–MS^n procedures that can define a chlorogenic acid by its molecular ion and discriminate between positional isomers by their patterns of fragmentation (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford, Knight, & Kuhnert, 2005; Clifford et al., 2006; Clifford, Marks, Knight, & Kuhnert, 2006; Clifford, Wu, Kirkpatrick, & Kuhnert, 2007; Clifford, Wu, & Kuhnert, 2006; Clifford, Zheng, & Kuhnert, 2006) without the need for isolation and purification of individual compounds. In this paper we report the application of these analytical methods to sweet potato stem and leaves that are popular as vegetables in China.

2. Material and methods

2.1. Materials

The purple sweet potato (*Ipomoea batatas* L.) was harvested from the dedicated sweet potato cultivation plots at Hunan Agricultural University in July 2004. All other reagents used were good quality products from normal commercial sources.

2.2. Methods

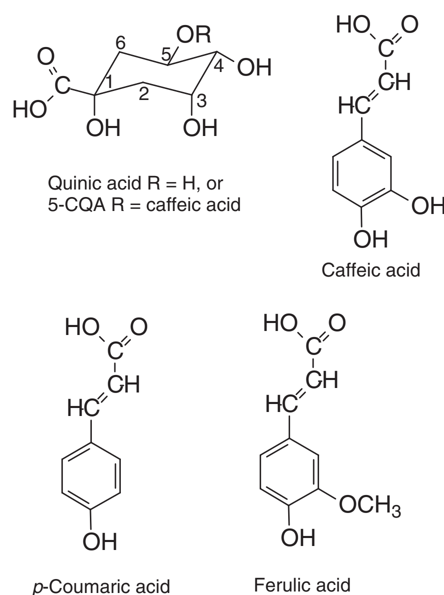
2.2.1. Extraction

Sweet potato stem, leaves or root (500 mg) was extracted (3 × 40 ml, 20 min each) with 70% v/v aqueous methanol using an HT-1043 solid–liquid continuous extraction system (Tecator, Bristol, UK). The solvent cups containing the extract were allowed to cool for a few minutes and filtered via Whatman No. 1 filter paper into a 100 ml volumetric flask and made up to volume with 70% aqueous methanol. An aliquot (10 ml) was treated with Carrez reagents (0.5 ml reagent A plus 0.5 ml reagent B), mixed by inversion and vortexing at least five times for 20 s at 1-min intervals, and centrifuged (2000*g*, 20 min). An aliquot of supernatant (7 ml) was transferred to a glass-tube and evaporated to dryness under nitrogen at 60 °C. The residue was dissolved in 200 µl methanol and transferred with washing (4 × 200 µl) into a volumetric flask (5 ml), made up to volume with water, centrifuged at (13,400*g*, 10 min), syringe filtered (0.45 µm), stored at −12 °C until required, thawed at room temperature, and used directly for LC–MS^n.

2.2.2. LC–MS^n

The LC equipment comprised a Surveyor MS Pump, autosampler with a 20 µl loop, and a PDA detector with a light-pipe flow cell (recording at 325, 280 and 254 nm, and scanning from 240 nm to 600 nm). This was interfaced with an LCQ Deca XP Plus mass spectrometer fitted with an ESI source (Thermo Finnigan, San Jose, CA, USA) and operating in data-dependent MS^n mode to obtain fragment ion m/z. As required, more sensitive targeted MS^n experiments were used to seek compounds with a particular molecular ion, for example, m/z 353, 367, 515, 529 and 677 for caffeoylquinic, feruloylquinic, dicaffeoylquinic, caffeoylferuloylquinic and tricaffeoylquinic acids, respectively. MS operating conditions (negative ion) had been optimized using 5-caffeoylquinic acid (Sigma Chemical Company, Poole, Dorset, UK) with a collision energy of 35%, ionization voltage of 3.5 kV, capillary temperature 350 °C, sheath gas flow rate of 65 arbitrary units, and auxiliary gas flow rate of 10 arbitrary units (Clifford et al., 2003).

Chlorogenic acids separation was achieved on a 150 × 3 mm column containing Luna 5 µm phenylhexyl packing (Phenonemex, Macclesfield, UK). Solvent A was water:acetonitrile:glacial acetic acid (980:20:5 v/v, pH



Name	Number	R_1	R_3	R_4	R_5
3-*O*-caffeoylquinic acid	1	H	C	H	H
5-*O*-caffeoylquinic acid	2	H	H	H	C
4-*O*-caffeoylquinic acid	3	H	H	C	H
3-*O*-feruloylquinic acid	4	H	F	H	H
5-*O*-feruloylquinic acid	5	H	H	H	F
4-*O*-feruloylquinic acid	6	H	H	F	H
3,4-di-*O*-caffeoylquinic acid	7	H	C	C	H
3,5-di-*O*-caffeoylquinic acid	8	H	C	H	C
4,5-di-*O*-caffeoylquinic acid	9	H	H	C	C
3-*O*-feruloyl, 4-*O*-caffeoylquinic acid	10	H	F	C	H
3-*O*-caffeoy, 4-*O*-feruloylquinic acid	11	H	C	F	H
3-*O*-feruloyl, 5-*O*-caffeoylquinic acid	12	H	F	H	C
3-*O*-caffeoy, 5-*O*-feruloylquinic acid	13	H	C	H	F
4-*O*-feruloyl, 5-*O*-caffeoylquinic acid	14	H	H	F	C
4-*O*-caffeoy, 5-*O*-feruloylquinic acid	15	H	H	C	F

C = caffeic acid; F = ferulic acid

Fig. 1. The structure of chlorogenic acids found in sweet potato (*Ipomea batatas*).

2.68); solvent B was acetonitrile:glacial acetic acid (1000:5 v/v). Solvents were delivered at a total flow rate of 300 μl min^{-1}. The gradient profile was 4% B to 33% B linearly in 90 min, a linear increase to 100% B at 95 min, followed by 5 min isocratic, a return to 4% B at 105 min, and 5 min isocratic to re-equilibrate (Clifford et al., 2003).

3. Results and discussion

All data for chlorogenic acids presented in this manuscript use the recommended IUPAC numbering system (IUPAC, 1976) and specimen structures are presented in Fig. 1. Where necessary, previously published data

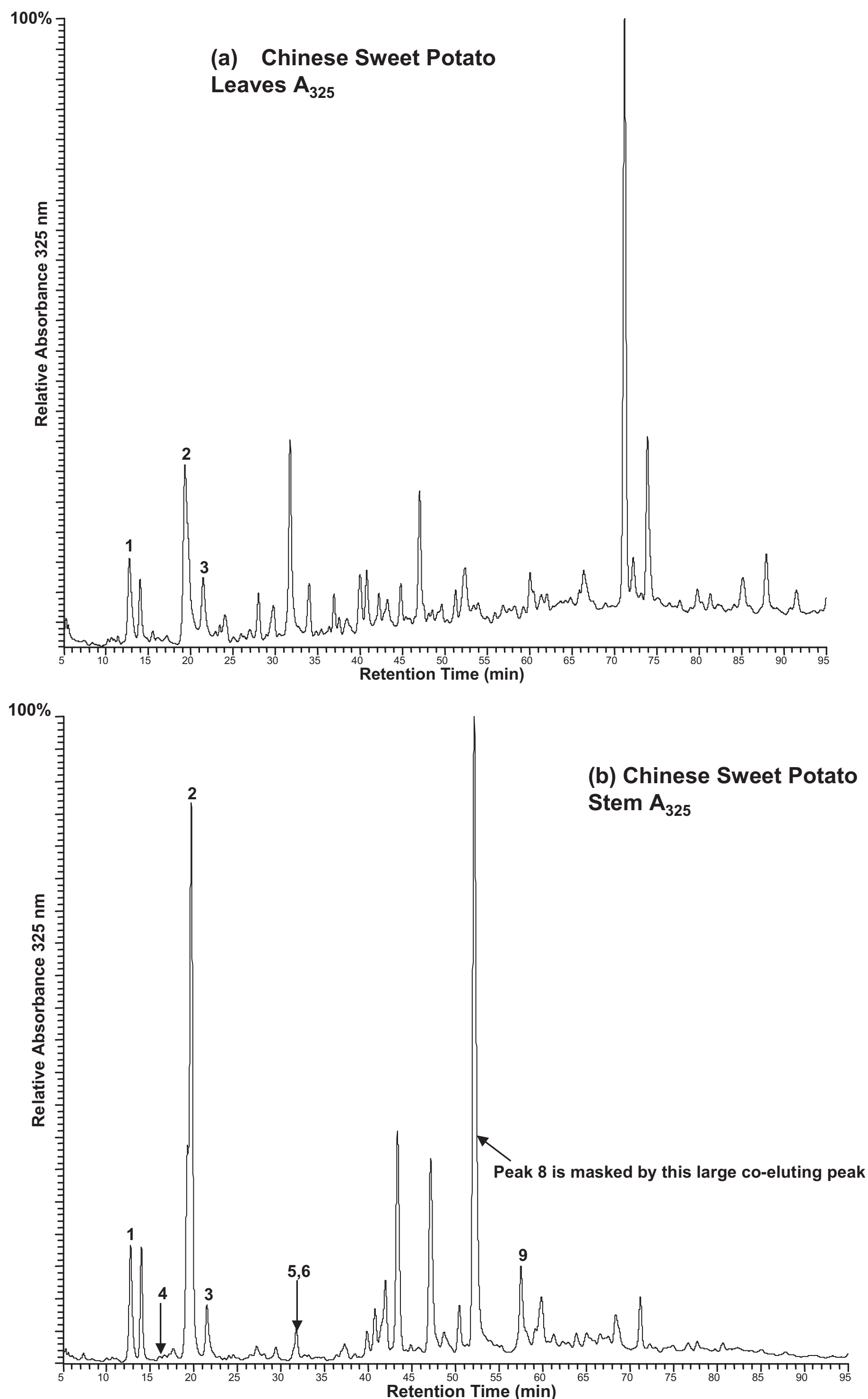


Fig. 2. Chromatogram recorded at 325 nm of an extract of sweet potato (*Ipomea batatas*). stem and leaves. For peak identification see Fig. 1.

(Islam et al., 2002; Kojima & Kondo, 1985; Uritani & Miyano, 1955; Yoshimoto et al., 2002), have been amended to ensure consistency and avoid ambiguity. Peak assignments have been made on the basis of the structure-diagnostic hierarchical keys previously developed, supported by examination of the UV spectrum and retention time relative to 5-caffeoylquinic acid, and by comparison with data for an extract of green coffee beans analysed contemporaneously (Clifford et al., 2003, 2005, 2006).

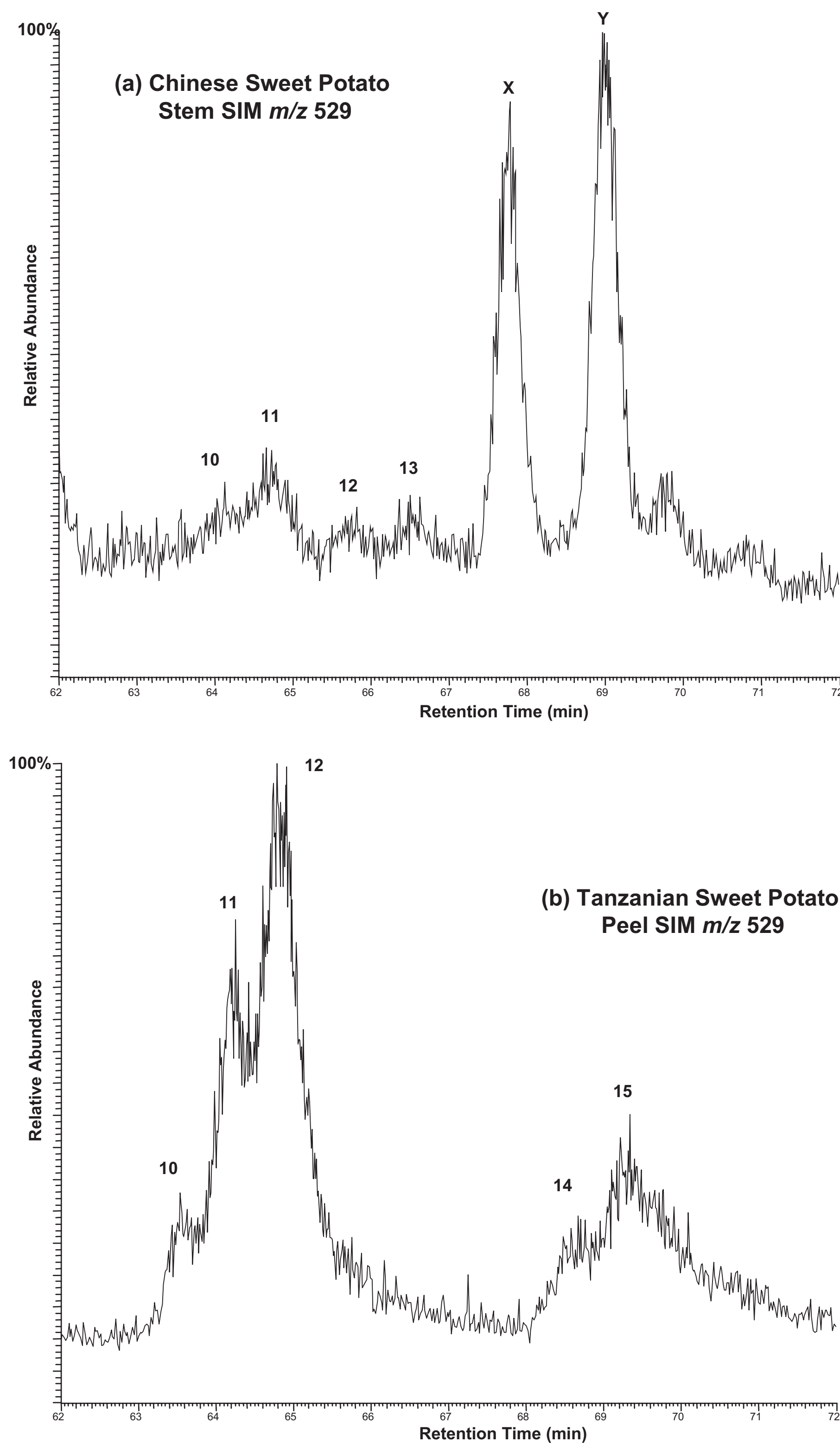


Fig. 3. SIM m/z 529 (62–72 min) for extracts of (a) Chinese sweet potato stem; (b) Tanzanian sweet potato skin; and (c) green robusta coffee beans. For peak identification see text and Fig. 1.

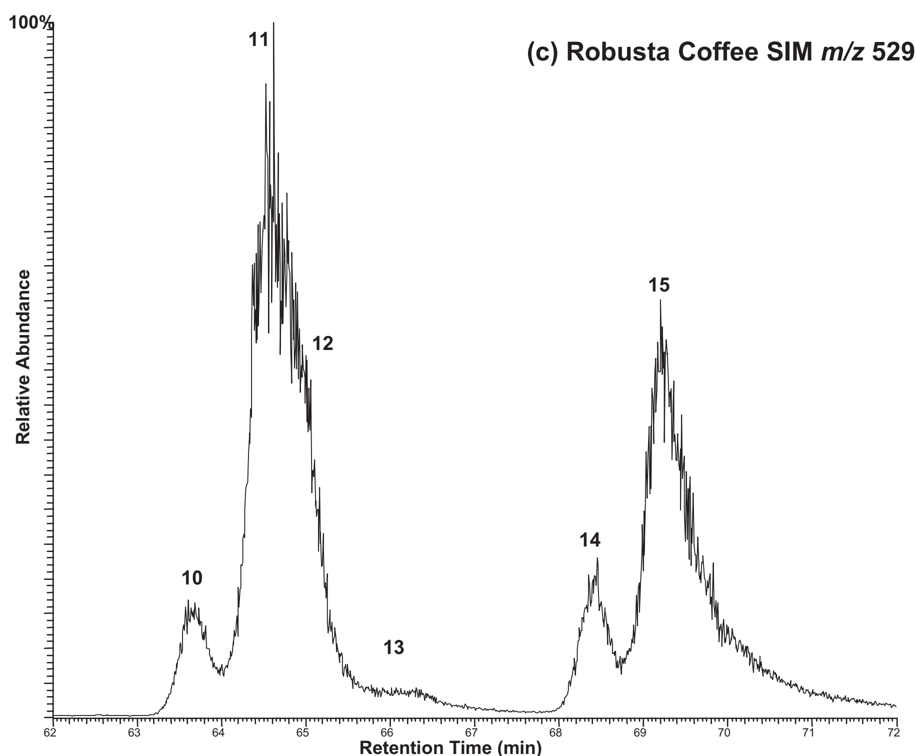


Fig. 3 (*continued*)

Chlorogenic acids were not detected in sweet potato root. Representative chromatograms of stem and leaf are presented in Fig. 2. Mass spectral data have been published previously (Clifford, 2003, 2005). Three parent ions at *m*/*z* 353 were detected in the extracts of the sweet potato stem and leaves. These were characterized by their retention time and MS^2 and MS^3 fragmentation as the widely occurring 3-acyl, 4-acyl and 5-acyl isomers of caffeoylquinic acid, with 5-caffeoylquinic acid dominant. These compounds have previously been reported in sweet potato leaf from Japan (Islam et al., 2002), but not in the stem so far as we are aware. Similarly, the analogous isomers of feruloylquinic acid were detected for the first time from this source but only in the stem and there at a low concentration relative to the caffeoylquinic acids.

Two parent ions observed at *m*/*z* 515 were characterized as 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid but 3,5-dicaffeoylquinic acid was masked in the UV by a co-eluting component that was not further investigated. The failure to detect 3,4-dicaffeoylquinic acid was unexpected, but a comparison with a green coffee extract analysed contemporaneously confirmed that it would have been resolved if it were present. We have previously observed 3,4-dicaffeoylquinic acid in plants cultivated in Tanzania (Nandutu, 2004), and it has previously been reported in sweet potato from Japan (Islam et al., 2002). The stem extract prepared from the Chinese sweet potato was found to generate several parent ions at *m*/*z* 529 (Fig. 3a) and initial observations suggested that two late-eluting isomers dominated this subgroup of putative caffeoyl-feruloylquinic acids. However, although fragment targeted MS^3 (m/z $529 + 353$ and $529 + 367$) established the presence of at least four caffeoyl-fruloylquinic acids (10–13) these two large peaks (X and Y) were not caffeoyl-feruloylquinic acids despite producing a parent ion at *m*/*z* 529. Fig. 3b presents the equivalent SIM trace (*m*/*z* 529) obtained with an extract of Tanzanian sweet potato peel that clearly shows at least five peaks, all confirmed as caffeoyl-feruloylquinic acids by targeted MS^3. A contemporaneous trace for an extract of green robusta coffee beans (Fig. 3c), the source in which the caffeoyl-feruloylquinic acids were first recognized (Clifford et al., 2003; Iwahashi, Morishita, Osaka, & Kido, 1985; Morishita, Iwahashi, & Kido, 1986; van der Stegen & van Duijn, 1981), is provided for comparison. These caffeoyl-feruloylquinic acids have since been found in Aster and Achillea (Asteraceae) (Clifford et al., 2006; Radulovic, Stojanovic, & Asakawa, 2006), black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) (Kammerer, Carle, & Schieber, 2004), and Maté (Bravo, Goya, & Lecumberri, 2007), suggesting that they are much more widespread than has been realised. However, as illustrated by the data obtained for Chinese sweet potato, it is essential to have fragmentation data and not to rely solely on the molecular ion when seeking these substances in plant extracts.

A specific search for tricaffeoylquinic acids (*m*/*z* 677) was unsuccessful although we have previously observed 3,4,5-tricaffeoylquinic acid in samples grown in Tanzania (Nandutu, 2004) and it has been reported in plants from Japan (Islam et al., 2002).

4. Conclusions

Sweet potato contains a range of chlorogenic acids, dominated by 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, and including small amounts of the less common feruloylquinic acids and the little studied caffeoyl-feruloylquinic acids. Two chemically unrelated co-eluting substances having the same mass as the caffeoyl-feruloylquinic acids ($M_r = 530$) were detected in sweet potato from China using targeted LC–MS^3 but absent from material grown in Tanzania. This observation illustrates the importance of examining the fragmentation behaviour in order to confirm a structural assignment based on the observed molecular ion. The profile of chlorogenic acids in sweet potato may vary with country of origin.

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