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Full Length Research Paper

Analysis of phenolic compounds in Ugandan sweet potato varieties (NSP, SPK AND TZ)

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Sweet potato varieties: Nsp, Tz and Spk obtained from Uganda were used in this study. Several phenolic compounds were identified on the basis of their mass spectra in full scan mode and the pattern of their fragmentation. The major compounds found were caffeoylquinic acid, dicaffeoylquinic acid, coumaroylquinic acid, feruloylquinic acid and caffeoylferuloylquinic acids. This is the first time that caffeoylferuloylquinic isomers have been identified in sweet potato samples.

Key words: Sweet potato, phenolic compounds, chlorogenic acids

INTRODUCTION

Phenolic compounds are a class of low molecular weight secondary plant metabolites found in most land plants. These compounds are of great importance for food and drink since they are responsible for their organoleptic properties; some polyphenols in food add colour like anthocyanins, which can be purple, black or red (Goda et al., 1996; Alonso et al., 2003) and this can be desirable in red wines. In addition polyphenols affect the taste of the food like the pungency of chillies and astringent taste of beers. Polyphenols are the building blocks of lignin and dietary fibre, which is known to determine the texture and nutritional value of vegetable foods.

Phenolic substances such as flavonols, cinnamic acids, coumarins and caffeic acids or chlorogenic acids are believed to have antioxidant properties, which are suggested to play an important role in protecting food, cells and any organ from oxidative degeneration (Osawa, 1999; Tikkanen et al., 1998; Wiseman et al., 2000. In model systems, antioxidants are able to scavenge free radicals and there-by prevent the free radicals from causing damage. Such properties may be important in processed foods, but whether such properties are retained when phenols are absorbed and metabolised is less clear.

Reports indicate that diets rich in phenolic compounds play a role in the prevention of various diseases associated with oxidative stress such as cancer, cardiovascular and neurodegenerative diseases (Hertog et al., 1993; Anderson et al., 1995; Manach et al., 2004; Nestle, 2004; Hang et al., 2004). In addition polyphenols, which constitute the active substances found in many medicinal plants, modulate the activity of a wide range of enzymes and cell receptors (Middleton et al., 2000).

Isolation and identification of these compounds are of interest because of the benefits they contribute. There are a considerable number of phenols that have been reported in sweet potato. Sweet potato phenolic compounds were reported by Rudkin and Nelson (1947) to consist of chlorogenic acid and other similar compounds, which were not clearly and fully identified due to the limited techniques at that time. Uritani (1955) separated three polyphenols from sweet potato, which on decomposition gave caffeic acid and quinic acid as fragments. These fragments were identified by their melting points, which is not a reliable method because other substances can have the same melting points. Thin layer chromatography was the commonly used technique in separation of the phenolics in sweet potato but this would not provide sufficient information that would lead to the identifycation and elucidation of the phenolic structures of substance separated.

In the present study, high-pressure liquid chromatography coupled with mass spectrometry (HPLC-MS^n) was used in analysing sweet potato phenolics.

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Table 1. Gradient programme.

Time (min)	Flow, µl/min	A%	B%
0	300	96	4
90	300	67	33
95	300	0	100
100	300	0	100
105	300	96	4
110	300	96	0

HPLC–MS is a powerful technique in food analysis for the identification of compounds of interest and elucidation of their structures. HPLC is used for separating and quantitation of the compound of interest (phenolics). The separation of compound /analytes are based on the interaction of individual components with stationary and mobile phases. The chromatographic separation provides information about the retention time and photodiode array (PDA) detector can determine λ max and full UV-VIS spectrum for analyses. Although this is important, it may not be sufficient to discriminate between closely related compounds. The retention times may be the same for many compounds for a particular set of HPLC conditions and accurate quantitation may be compromised by co-elution with other analytes. The separated compounds are then injected into the mass spectrometer where mass data of individual substances are provided according to their mass-charge ratio (*m/z*). These compounds can further be subjected to collision-induced dissociation leading into fragmentations. The information obtained together with their mass spectra is important in the elucidation of the structures of a compound of interest and the pattern of fragmentation may allow isomers to be distinguished (Clifford et al., 2003). The study was aimed at analysing sweet potato extracts by HPLC–MS in full scan mode for isolation and the identification of phenolic compounds.

MATERIALS AND METHODS

Materials and reagents used

The materials used in these study were freeze-dried, sun-dried, oven-dried and fresh sweet potatoes varieties; Nasport (Nsp), Spk004 (Spk) and Tanzania (Tz) from Uganda. Peels from Tz variety were used to assess the differences in quantity between the polyphenols in flesh and those in the peel.

The reagents used include: Carrez A: Zinc acetate. $2H_2O$, 21.9 g in 100 ml of milli-Q water; Carrez B: Potassium ferrocyanide, 10.6 g in 100 ml of milli-Q water; HPLC grade methanol, 70% aqueous methanol, acetonitrile and acetic acid.

Method

Sweet potato (1 g) (oven, freeze, sun-dried) or dried peel was weighed into soxhlet extraction thimbles. The phenolic compounds present in sweet potato powder (flour) were extracted into 40 ml 70% v/v aqueous methanol using a soxhlet apparatus. Three extractions were carried out to ensure full extraction of the phenolic compounds present in sweet potato. The solvents containing the extract were cooled for a few minutes, and then filtered into a 100 ml volumetric flask using Whatman No.1 filter paper. The final volume was made up to 100 ml using 70%v/v aqueous methanol. To 100 ml extract, Carrez reagent A (4 ml) was added and vortexed for 2 min and left for one minute. Carrez B reagent (4 ml) was then added and vortexed for 20 s. These reagents were added to precipitate out the polysaccharides and proteins. The mixture was centrifuged at 2000 ×*g* for 20 min. The supernatant was drawn off and stored in a cold room (5˚C) until HPLC–MS analysis.

Preparation of extracts for HPLC and MS analysis

Sweet potato phenolic extract (100 ml) was evaporated in the rotary evaporator to dryness. Methanol 10%v/v aqueous (2 ml) was added to the residue (phenolic compound). The solution was centrifuged at 13000 ×*g* to remove any particulates that would block the column. The supernatant was filtered via Durapore polyvinylidene difluoride filter (PVDF-D) (0.45 µm) (Millipore Company, UK) and 20 µl was injected into the HPLC–MS system for analysis.

HPLC–MS analysis

The HPLC–MS equipment (Thermofinnigan, San Jose, CA) comprised a Surveyor MS pump, an autosampler with a 20 µl loop and a PDA/ UV detector. For the sweet potato analysis the UV detector was set to record at channel A = 324 nm, channel B = 280 nm and Channel C = 450 nm. The detector was interfaced with an LCQ deca XP plus mass spectrometer fitted with an electro spray interface (ESI) source (Thermofinnigan) and operating in zoom scan mode for accurate determination of parent ion *m/z* and in data-dependent, MS^n mode to obtain fragment ion *m/z*. The software for the control of the equipment and the acquisition and treatment of data was Xcalibar version 1.3. The phenolic separation was achieved on a 150 × 3 mm column containing Luna 5 µ phenyl hexyl packing (Phenomenex, Macclesfield, UK). The gradient temperature control (column oven control) was 30ºC. The chromatographic conditions were: flow rate of 0.3 ml/min, sample injection volume of 20 µl and mobile phases A (2% aqueous acetonitrile, 0.5% acetic acid, pH 2.68) and B (99.5% acetonitrile, 0.5% acetic acid). The gradient program was set as shown in Table 1. The run time of 110 min was set to elute all phenolic compounds that might be present in the sweet potato.

The interface conditions were negative ionisation mode, temperature of the capillary 350ºC, an ionisation voltage 3.5 KV, gas flow rate 80 arbitrary units and auxiliary gas flow, 10 (arbitrary units). The mass detection was performed in the base peak mode, for *m/z* between 20 and 750. The number of scan events was set as 5, which was necessary to fragment the parent ion into daughter ions to give structural information.

RESULTS AND DISCUSSION

The examination of HPLC–MS chromatograms revealed the presence of several compounds, which were identified using hierarchical key (Table 2a and b) identification developed by Clifford et al. (2003). Some isomers were distinguished by comparing their retention time with similar compounds that have been previously studied under similar conditions. By means of this HPLC–MS method, several phenolic compounds were detected which includ-

Table 2a. A hierarchical key for the identification by LC–MS^n of caffeoylquinic acids and dicaffeoylquinic acids including those substituted at position 1 (M.N. Clifford, personal communication)

Criterion	Identification	Action
Parent ion *m/z* 337.5	*p*-Coumaroylquinic acids	*Go to reference (6)*
Parent ion *m/z* 353.5	Caffeoylquinic acids	*Go to 2*
Parent ion *m/z* 367.5	Feruloylquinic acids	*Go to reference (6)*
Parent ion *m/z* 515.5	Dicaffeoylquinic acids	*Go to 3*
Parent ion *m/z* 529.5	Caffeoylferuloylquinic acids	*Go to reference (6)*
MS^2 base peak *m/z* 191.5, and relatively intense (*ca* 50% base peak) secondary ion at *m/z* 179.5	3-Caffeoylquinic acid (II)	
MS^2 base peak *m/z* 173.5	4-Caffeoylquinic acid (IV)	
MS^2 base peak *m/z* 191.5, and weak or undetectable (<5% base peak) secondary ion at *m/z* 179.5	5-Caffeoylquinic acid (III) or 1-Caffeoylquinic acid (I)	Distinguish by retention time on reverse phase packing
MS^3 base peak *m/z* 173.5	4-Acyl dicaffeoylquinic acids	*Go to 4*
MS^3 base peak *m/z* 191.5	Dicaffeoylquinic acids NOT substituted at position 4	*Go to 5*
Strong (>50% base peak) MS^1 fragment ions at *m/z* 299.5 and *m/z* 203.5	1,4-Dicaffeoylquinic acid (VI)	
Weak (*ca* 15%) MS^1 fragment ion at *m/z* 335.5 and strong MS^2 (>50% base peak) fragment ion at *m/z* 179.5	3,4-Dicaffeoylquinic acid (VIII)	
MS^1 fragment ion at *m/z* 335.5 undetectable, strong MS^2 (>50% of base peak) fragment ion at *m/z* 179.5	4,5-Dicaffeoylquinic acid (X)	
MS^1 fragment ion at *m/z* 335.5 (>30% of base peak) and strong MS^2 (>50% of base peak) fragment ion at *m/z* 179.5	1,3-Dicaffeoylquinic acid (V)	
Weak MS^1 fragment ion at *m/z* 335.5 (<10% of base peak) and weak MS^2 fragment ion at *m/z* 179.5 (<10% of base peak)	1,5-Dicaffeoylquinic acid (VII)	
MS^1 fragment ion at *m/z* 335.5 undetectable, strong MS^2 fragment ion at *m/z* 179.5 (<50% of base peak)	3,5-Dicaffeoylquinic acid (IX)	

ed; three isomers of caffeoylquinic acid, three isomers of dicaffeoylquinic acid, one isomer of *p*-coumaroylquinic acid, one isomer of feruloylquinic acid, and six isomers of caffeoylferuloylquinic acid. Walter and Purcell (1979) and Thompson (1981) in their findings re-ported the presence of chlorogenic acid in sweet potato but due to the limited methods used at that time they could not identify the diff-erent chlorogenic compounds present.

p-Coumaroylquinic acid

A very weak 5-*p*-coumaroylquinic acid signal (Figure 1) was detected in the sweet potato samples at retention time *ca* 27 min and was identified based on its parent ion at *m/z* 337 and MS^2 base peak *m/z* 191 (Figure 1). The isomers 3 and 4-*p*-coumaroylquinic acids were not obser-ved in any of sweet potato samples.

Caffeoylquinic acids

Different isomers were detected in the full scan experi-ment showing *m/z* 353 at the retention times *ca* 12, 18.8 and 22 min (Figure 2) for the freeze-dried extract of Tz variety; these isomers were identified as 3-caffeoylquinic (3-CQA), 5-caffeoylquinic (5-CQA) and 4-caffeoylquinic acid (4-CQA), respectively. The three isomers were distinguished based mainly on the MS^2 fragments and MS^3 fragments. For the 3-CQA and 5-CQA, the MS^2 frag-ment base peak was *m/z* 191, deprotonated quinic acid (ii and iii) and so they could only be distinguished based on the relative intensity of the secondary ion *m/z* 179. For 5-CQA the secondary ion at *m/z* 179 is very weak (< 5%) or undetectable while 3-CQA the relative intensity of the se-condary ion at *m/z* 179 is about 50% or more.

4-CQA can be differentiated from other CQA isomers by its MS^2 fragmentation (Figure 3). 4-CQA gives an MS^2 base peak at *m/z* 173 due to loss of caffeic acid and a water molecule while 3-CQA and 5-CQA gave MS^2 at *m/z* 191 (deprotonated quinic acid) as a result of loss of caffeic acid unit. The Tz flesh variety extract had similar isomers eluted at the same retention time and were iden-tified as 4-CQA and 5-CQA. In this study CQA isomers eluted in the sequence 3, 5 and 4, which was consistent with the findings reported by Clifford et al. (2003). In freeze-dried Nsp extract one isomer 5CQA was detected

Table 2b. A hierarchical key for the identification by LC–MS^n of mono- and di-acyl-CGA not substituted at position 1 (From Clifford et al., 2003).

Criterion	Identification	Action
Parent ion *m/z* 337.5.		*p*CoQA
		Go to 2
Parent ion *m/z* 353.5.		CQA
		Go to 3
Parent ion *m/z* 367.5.		FQA
		Go to 4
Parent ion *m/z* 515.5.		DiCQA
		Go to 5
Parent ion *m/z* 529.5		CFQA
		Go to 6
MS^2 base peak *m/z* 163.5.	3-*p*CoQA	
MS^2 base peak *m/z* 173.5.	4-*p*CoQA	
MS^2 base peak *m/z* 191.5.	5-*p*CoQA	
MS^2 base peak *m/z* 191.5, and relatively intense (*ca* 50% base peak) secondary ion at *m/z* 179.5.	3-CQA	
MS^2 base peak *m/z* 173.5.	4-CQA	
MS^2 base peak *m/z* 191.5, and weak or undetectable (<5% base peak) secondary ion at *m/z* 179.5.	5-CQA	
MS^2 base peak *m/z* 193.5.	3-FQA	
MS^2 base peak *m/z* 173.5.	4-FQA	
MS^2 base peak *m/z* 191.5.	5-FQA	
MS^2 base peak *m/z* 353.5, MS^3 base peak *m/z* 173.5, and comparatively intense (*ca* 20% of base peak) secondary ion at *m/z* 335.5.	3,4-diCQA	
MS^2 base peak *m/z* 353.5 and MS^3 base peak *m/z* 191.5.	3,5-diCQA	
MS^2 base peak *m/z* 353.5, MS^3 base peak *m/z* 173.5	4,5-diCQA	
MS^3 base peak *m/z* 173.5.	*Vic*-CFQA	*Go to 7*
MS^3 base peak **not** *m/z* 173.5.	3,5CFQA	*Go to 10*
MS^2 secondary ions at *m/z* 335.5 or *m/z* 349.5 with intensities not less than *ca* 40% of base peak.	3,4CFQA	*Go to 8*
MS^2 secondary ions at *m/z* 335.5 or *m/z* 349.5 with intensities not more than *ca* 20% of base peak.	4,5CFQA	*Go to 9*
MS^2 base peak at *m/z* 367.0 and MS^3 secondary ion at *m/z* 193.5.	3C,4FQA	
MS^2 base peak at *m/z* 353.5 **or** *m/z* 367.0 with *m/z* 353.5 of near identical intensity, and MS^3 secondary ion at *m/z* 179.5.	3F,4CQA	
MS^2 base peak at *m/z* 367.0 and an intense (>50% of base peak) MS^3 secondary ion at *m/z* 193.5	4F,5CQA	
MS^2 base peak at *m/z* 353.5 and an intense (>50% of base peak) MS^3 secondary ion at *m/z* 179.5.	4C,5FQA	
MS^2 base peak at *m/z* 367.0 and MS^3 base peak at *m/z* 193.5.	3F,5CQA	
MS^2 base peak at *m/z* 353.5 and an intense (>50% of base peak) MS^3 secondary ion at *m/z* 179.5.	3C,5FQA	

at *ca* 17 min while in the oven-dried sample it was observed at *ca* 15 min.

Feruloylquinic acids

Feruloylquinic acids particularly the 5-feruloylquinic acid was detected in all the sweet potato at *ca* 32 min. This was identified based on the parent ion at *m/z* 367 and its MS^2 base peak at *m/z* 191 (Figure 3).

Dicaffeoylquinic acid (diCQA)

The dicaffeoylquinic acid isomers were located by their parent *m/z* 515 and each isomer was further distinguished by its fragmentation. The isomers identified in freeze-dried Tz peel and whole root samples were 3, 4-

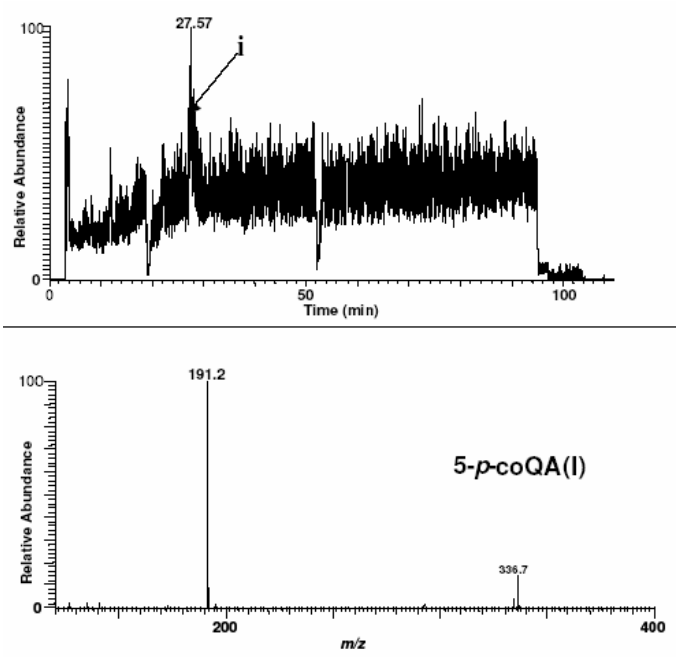

Figure 1. MS^2 spectra for *p* coumaroylquinic Acid. i indicates the P-Coumaroylquinic acid peak.

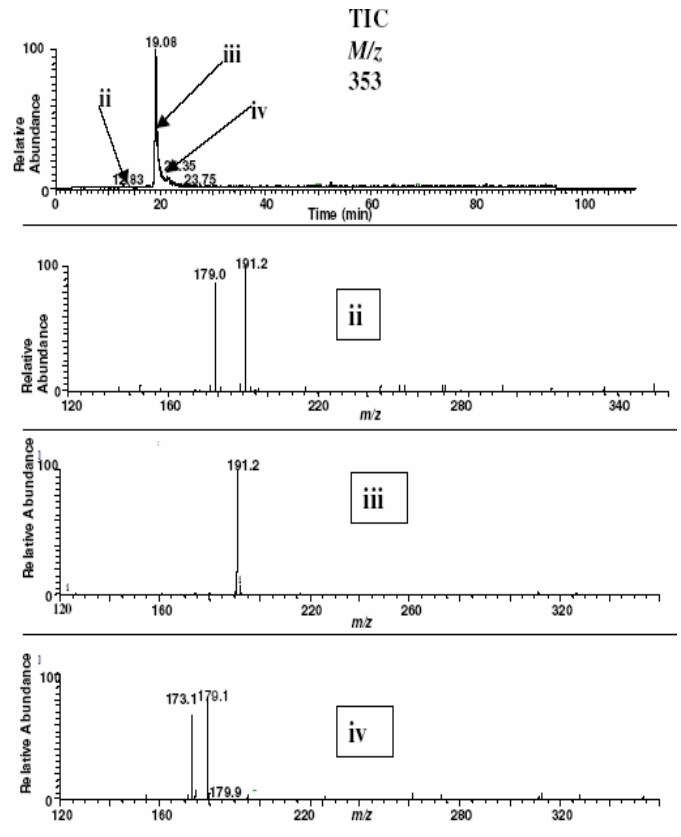

Figure 2. MS^2 Spectra for isomeric caffeoylquinic acid. ii, iii, iv indicates caffeoylquinic isomers eluted at 12.8,19, 22.3 min respectively.

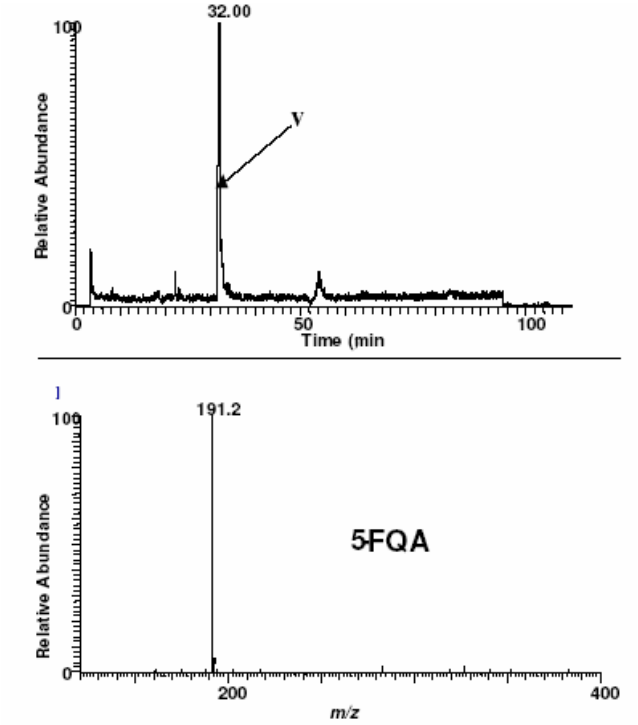

Figure 3. MS^2 Spectra of feruloylquinic acid. V is the feruloylquinic parent ion eluted at 32 min.

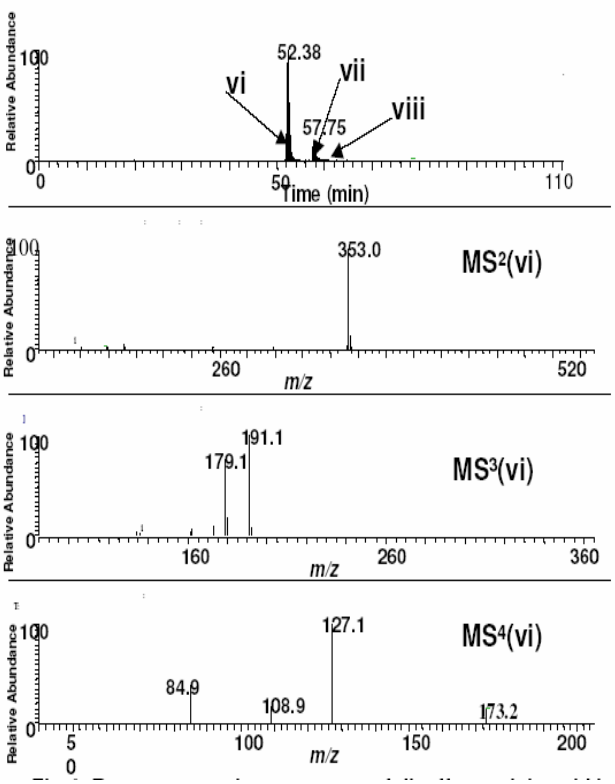

Figure 4. Represents a chromatogram of dicaffeoyquinic acid isomers: vi, vii, viii. MS^2, MS^3 MS^4 Spectra for 3,5 dicaffeoyquinic acid also indicated in the mass spectra.

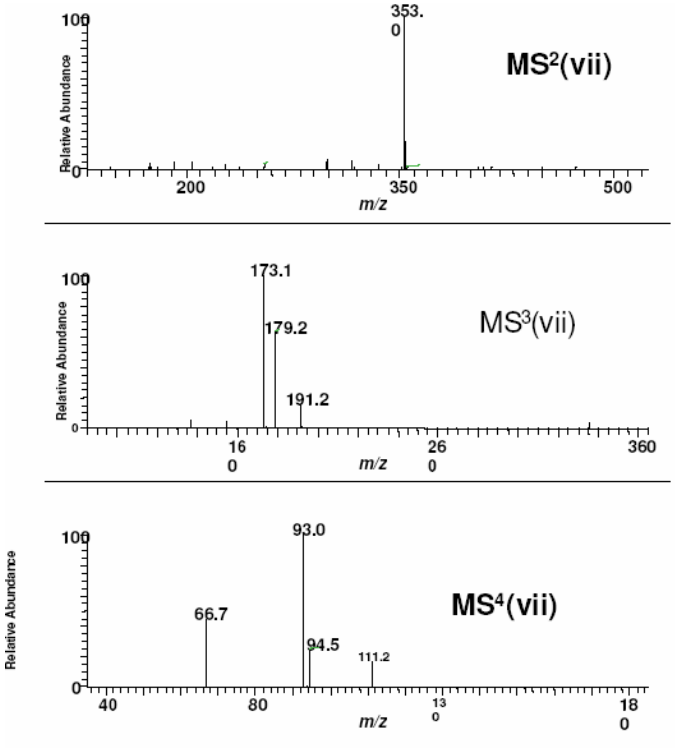

Figure 5. MS^2, MS^3, MS^4 for 4, 5 dicaffeoylquinic acid.

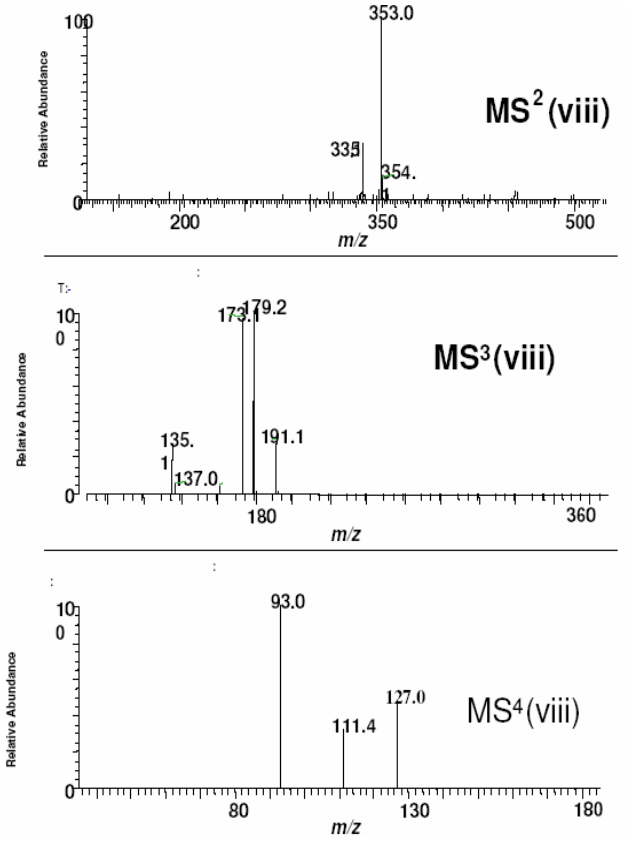

Figure 6. MS^2, MS^3, MS^4 for 3,4 dicaffeoylquinic acid.

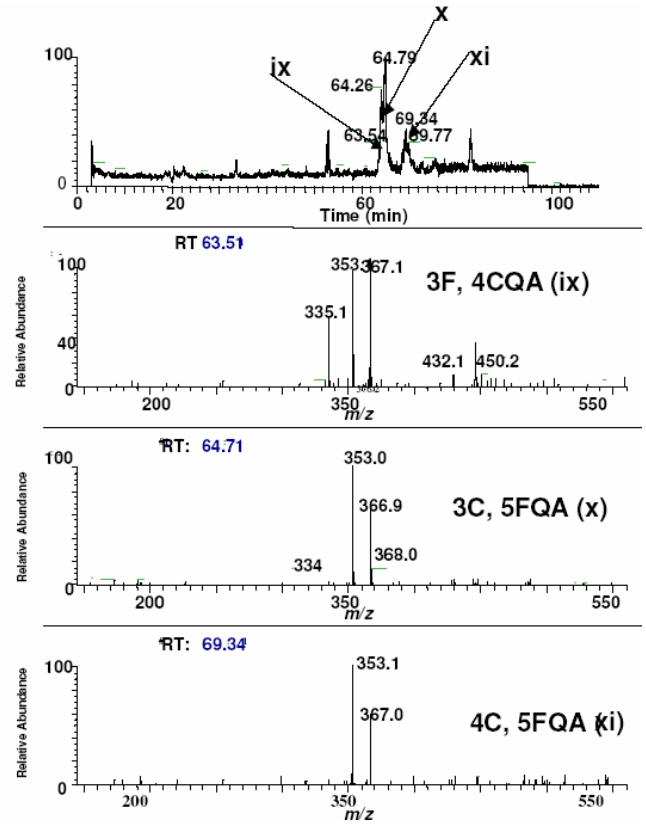

Figure 7. Represents a chromatogram of caffeoylferuloylquinic acid isomers ix, x, xi. MS^2 Spectra for caffeoylferuloylquinic acid isomers *m/z* 529.

diCQA, 3, 5-diCQA and 4, 5-diCQA eluted at *ca* 52, 58 and 60 min respectively (Figure 4). Again this identify-cation was possible by use of hierarchical key (Table 2a and b) and comparison with similar compounds in other substances such as artichoke and coffee (Sanchez-Rabanda et al., 2003; Clifford et al., 2003) respectively. The fragmentation for 3, 4 and 4, 5-diCQA was similar to that observed in coffee bean extract (Clifford et al., 2003).

The two 3, 4 and 4, 5-diCQA gave the expected molecular ion $[diCQA—H^+]^-$ at *m/z* 515 (Figure 4) and MS^2 base peak 353 (Figure 5). Consistent with hierarchi-cal key, 3, 4 and 4, 5 diCQA gave expected MS^3 and MS^4 base peaks at *m/z* 173.5 and *m/z* 93.5 respectively. The distinction between 3, 4 and 4, 5 was based on the intensity of MS^2 secondary ion at *m/z* 335. 3,4-diCQA has more intense MS^2 335 (>20%) compared with 4, 5-diCQA isomer with less than 5%. 3,5-DiCQA had same molecular mass as 3, 4 and 4, 5 diCQA (Figure 5) but its MS^3 base peak was different from those of 3, 4 and 4, 5-diCQA. The MS^3 base peak for 3, 5-diCQA was at *m/z* 191 while for 3, 4 and 4, 5-diCQA was at *m/z* 173. The former can be distinguished by the intensity of the MS^2 secondary ion fragment at *m/z* 335 and MS^3 fragment ion at *m/z* 179. Freeze dried Nsp variety extract contained 3, 4 and 4, 5-diCQA and oven dried Nsp sample contained

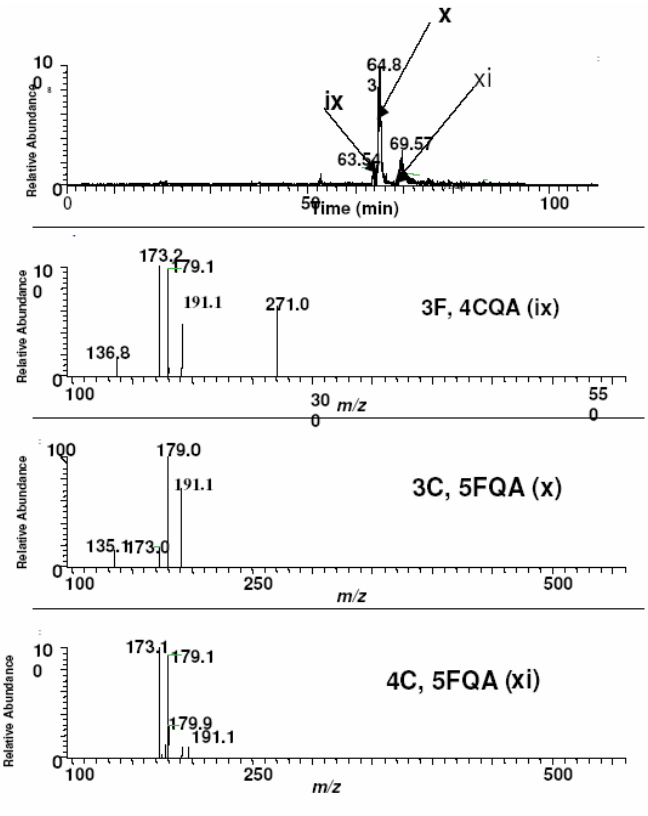

Figure 8. MS^3 Spectra for caffeoylferuloylquinic acid (m/z 529).

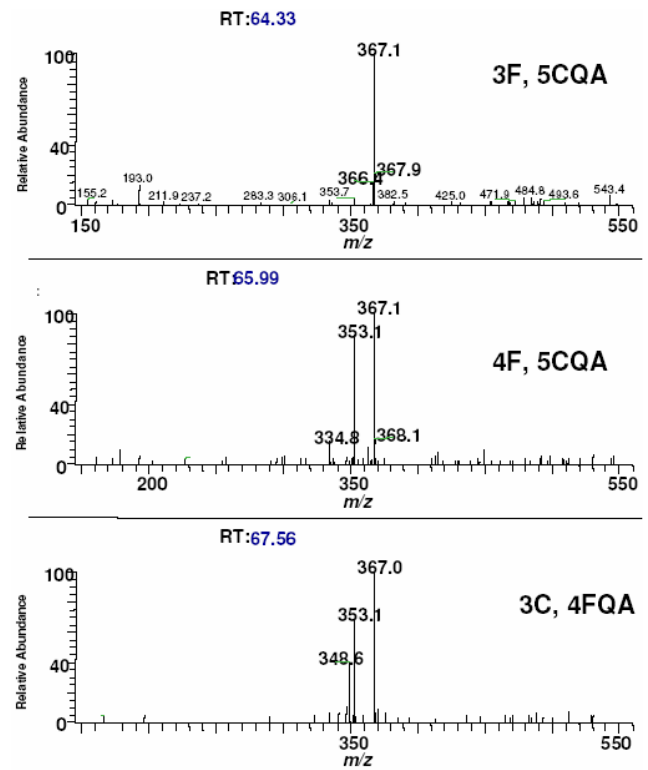

Figure 9. MS2, Spectra for 3F,5CQA; 4F,5CQA; 3C,4FQA

only one isomer 3, 5-diCQA detected at retention time 52 min. The same isomers were detected in the sun-dried Nsp extract. In general the diCQA were eluted between 52 and 59 min comparable with the diCQA from coffee extract.

Caffeoylferuloylquinic acids (CFQA)

More isomers of CFQA were observed in Tz flesh and the peel samples than in the rest of the varieties. The isomers identified in Tz flesh and Tz peel included; 3F 4CQA, 3F 5CQA, 3C 5FQA, 4F 5CQA, 3C 4FQA and 4C 5FQA eluted at *ca* 63.5, 64.3, 64.7, 66.0, 67.6 and 69.3 min respectively. These phenolic compounds, like the rest, were identified based on their parent ion at *m/z* 529 and MS^2 and MS^3 fragments as described in hierarchical key (Table 2a and b) and comparison with the isomers obtained from coffee. All these isomers had a parent ion at *m/z* 529 so the distinction of isomers was based on the MS^3 and MS^2 secondary ions at *m/z* 173 and 353 or 367 respectively (Figure 7 to 10). 3-Caffeoyl-5-feruloylquinic acid was also observed at *ca* 66 min in Nsp freeze-dried extract and in Spk sun-dried extract. This is the first time that CFQA isomers have been detected or identified in sweet potato samples. These compounds were detected in coffee by Clifford et al (2003). It is interesting to note that although sweet potato is not botanically related to coffee it contains the CFQA that so far have not been reported elsewhere.

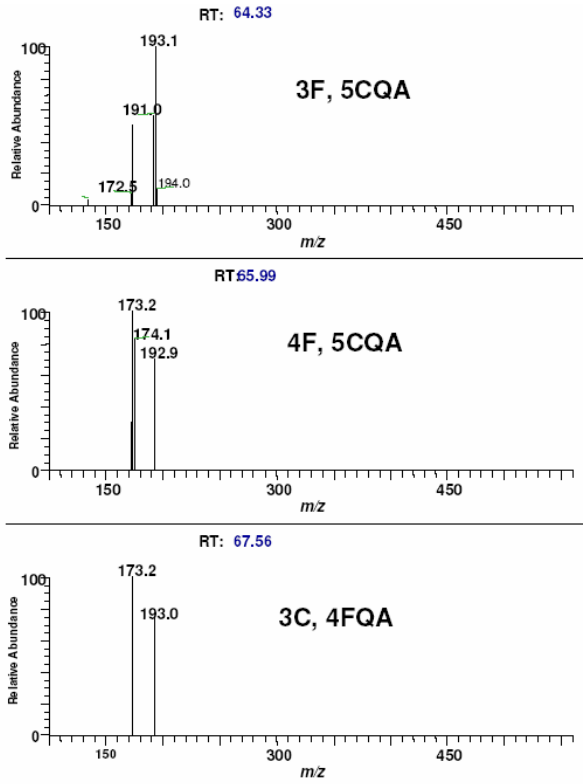

Figure 10. MS^3 Spectra for 3F, 5CQA; 4F,5CQA; 3C,4FQA.

There were some differences observed among the cultivars. CFQA was not observed in sun dried and oven dried Nsp samples. In the Nsp variety, they were probably converted into other products or were simply too low to be detected or were probably destroyed during heating. In general, processing (sun and oven drying) affected the quantity of some of the phenols in sweet potato. Further work would be required to investigate the quantity of each of these isomers of the chlorogenic acids found in sweet potato in fresh samples and processed (sun-dried, freeze-dried and oven dried) samples to assess the impact of processing on phenol content.

Conclusion

The HPLC- MS method has been found to be an excellent tool for the screening of phenolic compounds in plants. By means of this methodology, several phenolic compounds were detected in sweet potato roots and these included; caffeoylquinic acid, dicaffeoylquinic acid, *p*-coumaroylquinic, feruloylquinic acid and six isomers of caffeoylferuloylquinic acid. The existence of these compounds in sweet potato makes it an interesting source of natural antioxidants.

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