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Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry

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Abstract

Our previous study indicated that star fruit (*Averrhoa carambola L.*) is a very good source of natural antioxidants. However, it was still not clear which compounds were responsible for its antioxidant properties. The purpose of this study is to separate and identify compounds that contribute to total antioxidant activity in star fruit using HPLC and mass spectrometry (MS). HPLC coupled with a diode array detector (DAD) was used to characterise antioxidant peak in the juice or residue extract through spiking with free radicals. By analysing the antioxidant capacity and chromatograms of fractions from solid phase extraction, main antioxidants were attributed to phenolic compounds. The peaks were identified as L-ascorbic acid, (−)epicatechin and gallic acid in gallotannin forms. Other antioxidant peaks were further investigated using HPLC-ESI-MS-MS. Identification was confirmed with electronspray ionisation (ESI) MS-MS spectra of pure standards and singly-linked proanthocyanidins from pycnogenol. The major antioxidants were initially attributed to singly-linked proanthocyanidins that existed as dimers, trimers, tetramers and pentamers of catechin or epicatechin.
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Keywords: Food analysis; Star fruit; Polyphenols; Antioxidants; Epicatechin; Proanthocyanidins

1. Introduction

Fruits and vegetables are one of the main sources of antioxidants in our diets. Many clinical researches support the fact that consuming fruits and vegetables is beneficial to age-related diseases, cancers, heart diseases, etc [1–4]. This could be mainly attributed to those antioxidants contained in them.

Our previous study showed that star fruit is a good source of natural antioxidants and can effectively scavenge free radicals [5]. Setiawan and coworkers reported the content of carotenoids in star fruit [6], which exhibit their antioxidant activity mainly by physical quenching effects. Although star fruit is rich in Vitamin C, an effective antioxidant, it contributes only a little to its total antioxidant activity (TAA) [5]. One objective of this study is to investigate whether phenolic compounds are the main antioxidants in star fruits. Furthermore, it is meaningful to study antioxidant composition of star fruit to understand its antioxidant mechanism and amend its nutrition data. To some extent, HPLC could be the most useful tool for identification and quantification of phenolic compounds [7–11]. In the present work, we have used HPLC with solid phase extraction to characterize those major antioxidants in star fruit, and further identified those antioxidant peaks by HPLC-ESI-MS-MS.

2. Experimental

2.1. Reagents

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), L-ascorbic acid, (−)epicatechin, potassium persulfate were from Sigma (MO, USA); (+)catechin hydrate from Chem. Aldrich Co. (WI, USA); gallic acid from Acros Organics (NJ, USA), ethanol, acetic acid, methanol and hydrochloric acid from Merck (Dansturdt, Germany).

2.2. Samples

Several batches of star fruits were purchased from local supermarkets or Pasir Panjang wholesale center. Pycnogenol

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was purchased from Nuvanta, Horphag's French Maritime Pine bark.

2.3. Sample preparation for total antioxidant activity (TAA), HPLC-PDA and HPLC-ESI-MS assays

2.3.1. Liquid–liquid extraction (LLE) of antioxidants

Star fruit was homogenized using a blender, centrifuged and filtered under vacuum. The liquid portion was refrigerated immediately. The solid residue portion was kept at −18 °C and used for extraction. The extraction was carried out at optimised extraction conditions, i.e. 50% aqueous acetone at 90 °C for 45 min. The extract was evaporated to remove solvent under reduced pressure, topped up to a certain volume with water and kept at −18 °C in fridge. The residue extract and juice were filtered with 0.5 μm filter and then used directly for other experiments.

2.3.2. Solid phase extraction of antioxidants

The mixture of juice and residue extract was subjected to solid-phase extraction using a 3 ml end-capped C_{18} column (Isolute) previously activated with methanol and conditioned with pure water. Two milliliters of juice were introduced into column. Three milliliters of water was used to elute most of organic acid and sugar (FRC 1), and then 1.5 ml of methanol was used to elute and the fraction was collected (FRC 2), and then another 1 ml of methanol was used to elute large molecular compounds (FRC 3). The TAA of all fraction collections was measured by $ABTS^{+\bullet}$ scavenging assay.

2.4. TAA assay by ABTS free radical scavenging assay

The TAA assay was carried out on the Ultraspec 3000 UV-Vis spectrophotometer (Pharmacia Biotech Ltd., Cambridge CB4 4FJ, England). The procedure was described by Leong and Shui [5] with the results expressed as mg/100 g L-ascorbic acid equivalent antioxidant capacity (AEAC).

2.5. Identification and quantification of antioxidant peak in star fruit by HPLC-PDA assays

A previously-developed method was applied for the identification and quantification of antioxidants [7]. The HPLC system consisted of a Shimadzu HPLC (Model LC-10ATvp two Pumps and DGU-14A Degasser) equipped with a photo-diode array detector (Model SPD-M10A$_{VP}$) (Shimadzu, Kyoto, Japan). The separation was performed on a Shim-Pack VP-ODS column (250 mm × 4.6 mm i.d.) (Shimadzu, Kyoto, Japan) with a guard column (GVP-ODS, 10 mm × 4.6 mm i.d.).

Twenty microlitres of juice or extracts was injected into the HPLC for L-ascorbic acid (AA) and (−)epicatechin assays. Three millilitres of juice/extract and 4.5 ml of $ABTS^{\bullet+}$ solution are mixed to react for 1 h and then passed through 0.5 μm filter and injected for HPLC assay. Blanks of juice with water and $ABTS^{\bullet+}$ with water were also analysed.

The gallic acid content in gallotannin forms was measured as described in [12]. Briefly, juice and extracts of residue were mixed according to their volumetric ratio. One milliliter of mixed solution, in triplicate, was pipetted into culture test tube connected, and then 0.1 ml of 22 N sulfuric acid was added to it. The contents were frozen and air was removed from these tubes by using a vacuum pump. These tubes were kept at 100 °C for hydrolysing gallotannins to gallic acid. After hydrolysis, the volume was made up to 10 ml with pH value of around 4.0 by adding 0.5 N sodium hydroxide and distilled water. The hydrolysed supernatant was filtered through 0.5 μm filter and injected for HPLC assay of gallic acid. The contents of gallic acid in gallotannin forms were also tested respectively in juice and extracts using the procedure described above.

2.6. ESI-MS and HPLC-DAD-ESI-MS analyses

A Finnigan/MAT LCQ ion trap mass spectrometer (San Jose, CA, USA) equipped with TSP 4000 HPLC system, which includes UV6000LP PDA detector, P4000 quaternary pump and AS3000 autosampler was used. The heated capillary and spray voltage were maintained at 250 °C and 4.5 kV, respectively. Nitrogen is operated at 80 psi for sheath gas flow rate and 20 psi for auxiliary gas flow rate. The full scan mass spectra from *m/z* 50–2000 were acquired both in positive and negative ion mode with a scan speed of 1 s per scan. Tandem mass spectrometry was performed using helium as collision gas, operated at 0.8 mtorr, and the collision energy was set from 10 to 80 and 50% was found to be suitable to obtain extensive fragment ions of proanthocyanidins in pycnogenol and star fruit.

For the HPLC-DAD-ESI-MS assay of juice/extract, the instrument was set to measure following events: (1) UV chromatogram at 280 nm; (2) UV spectra of individual peaks; (3) TICs; (4) zoom scan was applied for measuring isotopic distances at *m/z* 291, 579, 867, 1155 and 1443, respectively; (5) MS-MS was used to break down the most abundant $[M+H]^+$ or $[M-H]^-$. Chromatographic separations were done on a Shim-Pack VP-ODS column (250 mm × 4.6 mm i.d.) (Shimadzu, Kyoto, Japan) with a guard column (GVP-ODS, 10 mm × 4.6 mm i.d.) under the following elution conditions: flow rate: 600 μl min^{-1}; room temperature (27 °C); solvent A, 0.1% formic acid in water; solvent B, methanol, starting from 20 to 50% B in 20 min, from 50 to 90% B in 25 min, and from 90 to 20% B in 5 min for washing and reconditioning of the column.

Manual injection for ESI-MS or EIS-MS-MS was performed with a constant flow rate of 300 μl min^{-1} of 50/50 solvent A to B. The collision induced dissociation (CID) spectra of (−)epicatechin, (+)catechin, and singly-linked proanthocyanidins from pycnogenol were recorded.

3. Results and discussion

3.1. Identification and quantification of antioxidants by HPLC-DAD

As reported previously, star fruit is an excellent free radical scavenger [5]. However, it is still not clear which compounds are responsible for this scavenging ability. To investigate the major antioxidants in star fruit, antioxidant peaks were firstly identified by comparing the chromatogram of juice/extract to that of reaction solution of juice and free radical. Secondly, the percentage contributions of TAA by compounds, which could be identified by HPLC-DAD, were calculated using their reaction stoichiometry with free radicals.

3.1.1. Identification of antioxidant peaks by HPLC with spiking test

As reported previously, star fruit is a good source of Vitamin C, however, Vitamin C only slightly contributes to TAC [5]. This is consistent with present results. In SPE fractions, FRC 2, which mainly contains peaks eluted after 30 min, accounted for around 80% of TAA. FRC 1, which mainly collected polar organic acids prior to 30 min including Vitamin C, accounted for less than 20% of TAA. FRC 3, which may mainly collect those more non-polar components, accounted for less than 5% of TAA. Therefore, peaks eluted after 30 min might be major antioxidants in star fruit. As described previously [9], phenolic compounds were eluted after 30 min, therefore, those peaks with retention time after 30 min in Fig. 1 might be major antioxidants. Moreover, those peaks circled in Fig. 1, which included L-ascorbic acid ($R_t = 14.3$ min), (−)epicatechin ($R_t = 48.3$ min) and several other peaks with a maximum absorbance at around 280 nm, disappeared or were significantly diminished after spiking with $ABTS^{\bullet+}$. Similar antioxidant peaks were observed in juice and residue extract except for content of L-ascorbic acid. As (+)catechin was eluted at 44.3 min in this program [9], this indicated that those compounds, which were eluted between (+)catechin and (−)epicatechin, were major antioxidants in star fruit. In addition, other than $ABTS^{\bullet+}$, $DPPH^{\bullet}$ could also be used as a tool for rapidly characterizing antioxidants in biological samples [13]. However, care should be taken when only $DPPH^{\bullet}$ was used as some antioxidants such as tyrosine (unpublished data using reaction conditions described by Leong and Shui [5]) and

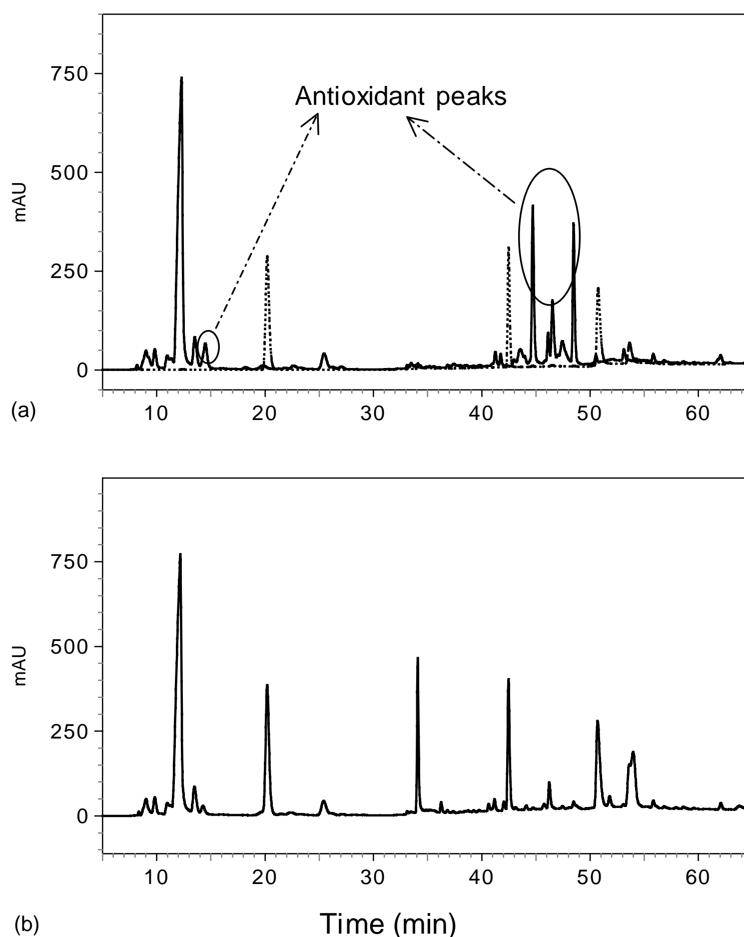

Fig. 1. Chromatograms of free radical spiking test. HPLC conditions as described in [9], wavelength, 215 nm. (a) solid line: chromatogram of juice with water; dashed line: chromatogram of $ABTS^{\bullet+}$ with water; (b) chromatogram of reaction solution of juice and $ABTS^{\bullet+}$.

Table 1
AEAC of star fruit at different extraction conditions

Extraction module	AEAC (mg/100 g)	Vitamin C[a]		Epicatechin[b]		Gallic acid	
		Concentration (ppm)	TAA (%)	Concentration (ppm)	TAA (%)	Concentration (ppm)	TAA (%)
Juice with residue extract at optimised condition	293.8	80.5	2.74	73.1	4.2	19.5	2.1
Juice with residue extract at 30 °C	227.8	80.5	3.53	70.8	5.3	n.d.	n.d.

n.d.: not determined.
[a] Two and 5.8 were used as stoichiometry of the reaction of Vitamin C and (−)epicatechin with $ABTS^{\bullet+}$ respectively.
[b] Calculated using content of hydrolysed gallic acid content and stoichiometry of 6.3 for reaction between gallic acid and $ABTS^{\bullet+}$.

some other phenolic compounds [14] have $ABTS^{\bullet+}$ scavenging activity but are not reactive to $DDPH^{\bullet}$.

3.1.2. Contribution of antioxidant activity by Vitamin C, (−)epicatechin and gallic acid in gallotanin forms

As can be seen from Table 1, optimum extraction gives 29% higher AEAC value than that at room temperature. Of the three compounds quantified, their total contribution is less than 10% of TAA both at room temperature and optimum extraction module. This indicated that other antioxidants unidentified by HPLC-DAD might be major antioxidants.

3.2. Identification of antioxidanst by HPLC and mass spectrometry

As reviewed previously, HPLC coupled with mass spectrometry has been widely used to identify phenolic compounds, and ESI mass spectrometric liquid interface was thought to provide advantages in terms of sensitivity and capability to analyze large, thermally labile and highly polar compounds [15,16]. Initial total ion chromatograms (TICs) of star fruit juice/extract, which were obtained by HPLC-ESI-MS at both positive and negative modes, indicated that those major antioxidant peaks were proanthocyanidins.

To confirm the existence of proanthocyanidins, mass spectrometry of proanthocyanidins in pycnogenol was investigated and compared with those of star fruit juice/extract. Pycnogenol is an extract from French maritime pine bark (PBE). It is a highly standardized mixture of certain polyphenolic compounds. Proanthocyanidins (Fig. 2), which are formed by catechin and epicatechin units with a degree of polymerization of up to heptamer, constitute 75% of its weight [17]. The CID mass spectrum of proanthocyanidins will be discussed below and compared with those of star fruit juice/extract.

3.2.1. ESI-MS-MS of catechin/epicatechin and pycnogenol proanthocyanidins

In ESI-MS, deprotonated molecular ions represented the base peak in the negative ion spectra. In contrast, protonated molecular ions were the base peaks in the positive ion mode. CID spectrum obtained from positive and/or negative parental ions can further give structural information.

Proanthocyanidins in wine have been reported to be better detected in the negative mode than in the positive mode [18]. Recently, RP-HPLC-ESI-MS-MS was successfully applied to analyse commercial vegetable tanning agents, and fragmentation pattern at negative mode was proposed [19]. Fragmentation patterns at both positive and negative modes have also been discussed by Rhor and coworkers, who have reported that fragmentation occurred more extensively in the positive mode because the abundance of molecular ions in the negative mode was more pronounced [20]. In addition, it was found that, in this study, the signal intensity of antioxidant peaks at positive mode was significantly higher than that at negative ion mode probably due to differences on experimental and instrumental conditions such as HPLC mobile phase, mass spectrometric conditions. Therefore, the positive ion mode was chosen to elucidate their mass spectrometric behaviour because it provided more extensive fragment ions and appeared more sensitive for further LC-MS-MS analysis of phenolic compounds in star fruit juice/extract.

As (−)epicatechin and (+)catechin are constitutive units of proanthocyanidins, the fragmentation pattern of (+)catechin or (−)epicatechin may provide useful

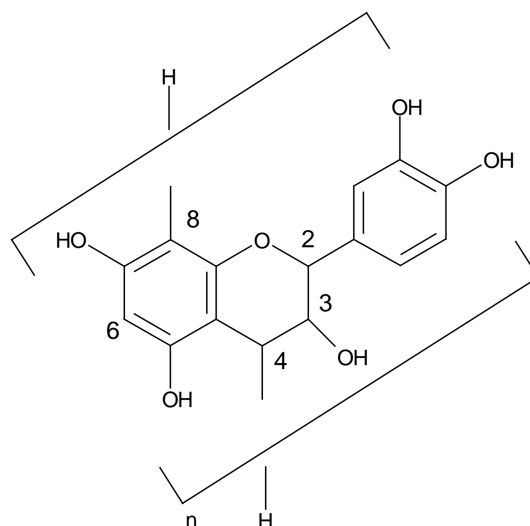

Fig. 2. Chemical structure of main proanthocyanidins in pycnogenol.

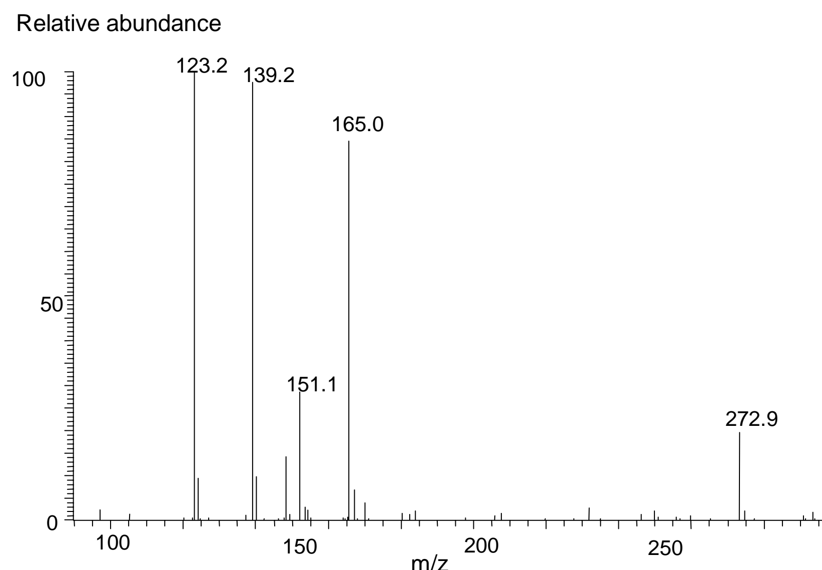

Fig. 3. CID (MS-MS Scan) spectrum at *m/z* 291 of (−)epicatechin, collision energy: 35%.

information for identification of proanthocyanidins. The CID spectrum of parent ion at *m/z* 291 for (−)epicatechin is displayed in Fig. 3 and consisted of daughter ions at *m/z* 139, 123, 165. The isomer (+)catechin gave the same fragment ions, since mass spectrometry cannot distinguish between stereoisomers. The fragment ions peaks obtained here are similar to those obtained by using thermospray-MS-MS [21]. As described by Rhor and coworkers, the fragment ion *m/z* 139 resulted from retro-Diels-Alder fission (RDA-F) of the heterocyclic ring system, and the fragment ion *m/z* 123 resulted from cleavage between C2–C3 and O–C2 of the pyran ring [20]. Another main fragment ion *m/z* 165 might result from cleavage between C4–C5 and O–C2 of the pyran ring.

The CID spectra of proanthocyanidin dimers (*m/z* 579) and trimers (*m/z* 867) in commercial pycnogenol are given in Fig. 4. The main fragment ions of parent ions at *m/z* 579 were *m/z* 427 $[M + H - 152]^+$ from RDA-F of the heterocyclic rings, 409 $[M + H - 170]^+$ from RDA-F of the heterocyclic rings and loss of water, *m/z* 291 $[M + H - 288]^+$ from interflavanic bond cleavage, 453 $[M+H-126]^+$ from cleavages between C4–C5 and O–C2 of one of pyran rings. Similarly, fragment ions of *m/z* 867 included those at *m/z* 579 $[M+H-288]^+$ from interflavanic bond cleavage, *m/z* 715 $[M+H-152]^+$ from RDA-F of the heterocyclic rings, *m/z* 697 $[M + H - 170]^+$ from RDA-F of the heterocyclic rings and loss of water, *m/z* 427$[M + H - 288 - 152]^+$ from interflavanic bond cleavage plus RDA-F of the heterocyclic rings, *m/z* 409 $[M + H - 288 - 170]^+$ from interflavanic bond cleavage and RDA-F of the heterocyclic rings and loss of water, etc. In addition, another main fragment ion *m/z* 577 $[M+H-290]^+$ might arise from interflavanic bond cleavage following the quinone-methide mechanism [22].

3.2.2. Identification of antioxidants by HPLC-ESI-MS

It was found that the contribution of TAA by residue was 82.7%, and juice only accounted for 17.3% while it account for over 85% (w/w) of total weight. Therefore, major antioxidants existed in residue rather than juice. Fig. 5 shows the results of HPLC-DAD-ESI-MS analysis of residue extract. As discussed above, the main antioxidants in juice/residue were found to be those peaks near (+)catechin (R_t = 13.8 min) and (−)epicatechin (R_t = 18.2 min). The negative and positive ions of those major peaks are listed in Table 2. Each major antioxidant peak identified was named compound **1–5** respectively. As the parent ions and fragment ions observed at negative ion mode were not as sensitive as those observed at positive ion mode, they were not used for structural elucidation but only for confirming the existence of compounds interested. One ion peak at *m/z* 291 (R_t = 3.7 min), which was eluted before Vitamin C (R_t = 5.7 min), was not identified as a catechin or epicatechin isomer because no corresponding ion peak at *m/z* 289 was observed and it did not give a CID spectrum similar to that of catechin. In addition, this peak was also not identified as an antioxidant peak because no peaks before Vitamin C was identified as antioxidant peak in the spiking test.

In HPLC-ESI-MS, compound **1** showed a $[M + H]^+$ ion at *m/z* 579, a $[M + Na]^+$ at 601 and a $[M - H]^-$ at 577. Distances between isotopic peaks are 1 amu, thus supporting that the molecular ions were singly charged species. Therefore, the molecular weight of compound **1** is around 578. As shown in Fig. 6a and Table 2, CID spectrum of compound **1** at *m/z* 579 gave fragment ions at *m/z* 427 $[M+H-152]^+$ from RDA-F of the heterocyclic rings, *m/z* 409 $[M+H-170]^+$ from RDA-F of the heterocyclic rings and loss of water, and *m/z* 291 $[M + H - 288]^+$ from interflavanic bond cleavage, etc. This fragmentation pattern is

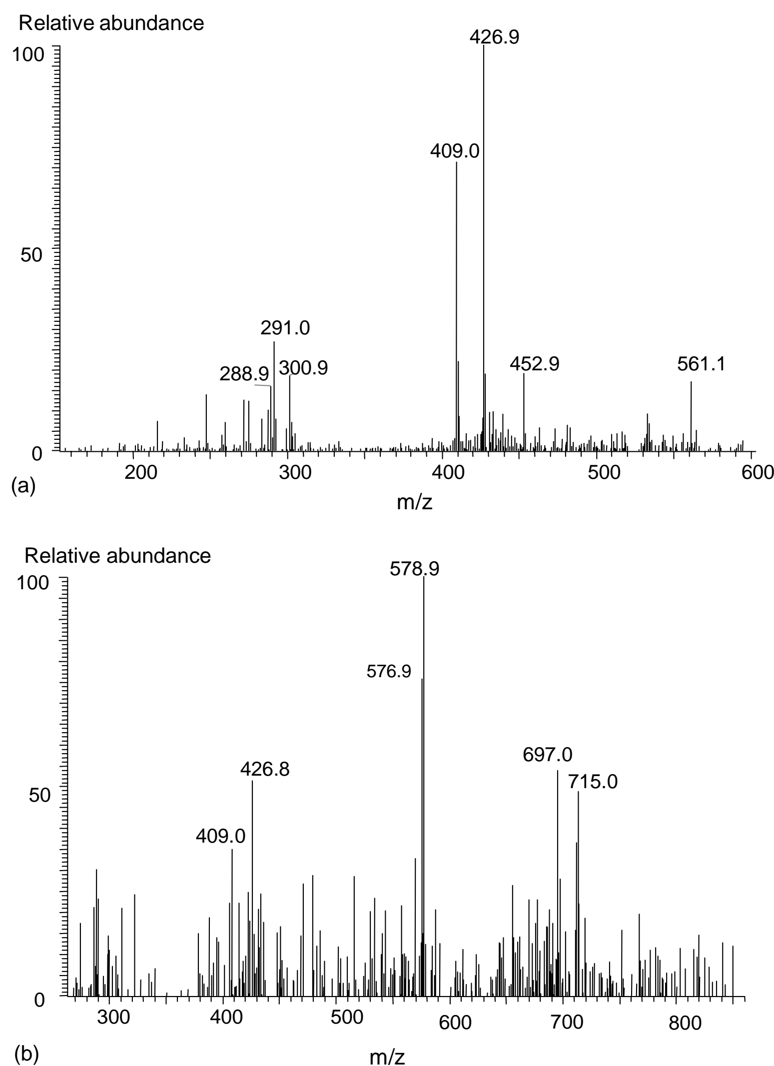

Fig. 4. CID (MS-MS Scan) spectra of pycnogenol proanthocyanidins, collision energy: 50%. (a) corresponding to the parent ion(s) at *m/z* 579; (b) corresponding to the parent ion(s) at *m/z* 867.

Table 2
Positive and negative ions of major antioxidant peaks

R_t (min)	ESI-MS (*m/z*)			Major CID fragments of $[M \pm H]^{+/-}$					Chemical structure
	Mode	$[M \pm H]^{+/-}$	$[M + Na]^{+}$	$[M \pm H - 170]^{+/-}$	$[M \pm H - 152]^{+/-}$	$[M \pm H - 288]^{+/-}$	$[M \pm H - 126]^{+/-}$	Other *m/z* ions	
13.98 (**1**)	+	579	601	409	427	291	453		Dimer
	−	577		407	425	289	451		
15.42 (**2**)	+	867	889	697	715	579	741	Similar to dimer	Trimer
	−	865		695	713	577	739		
15.67 (**3**)	+	1155	1177	985	1003	867		Similar to trimer and dimer	Tetramer
	−	1153			1001				
15.96 (**4**)	+	1443	1465	1273	1291	1155	1317	Similar to tetramer, trimer and dimer	Pentamer
	−	1441							
18.21 (**5**)	+	291			139		165	123, 151, 273	Epicatechin
	−	289						245, 205, 179	

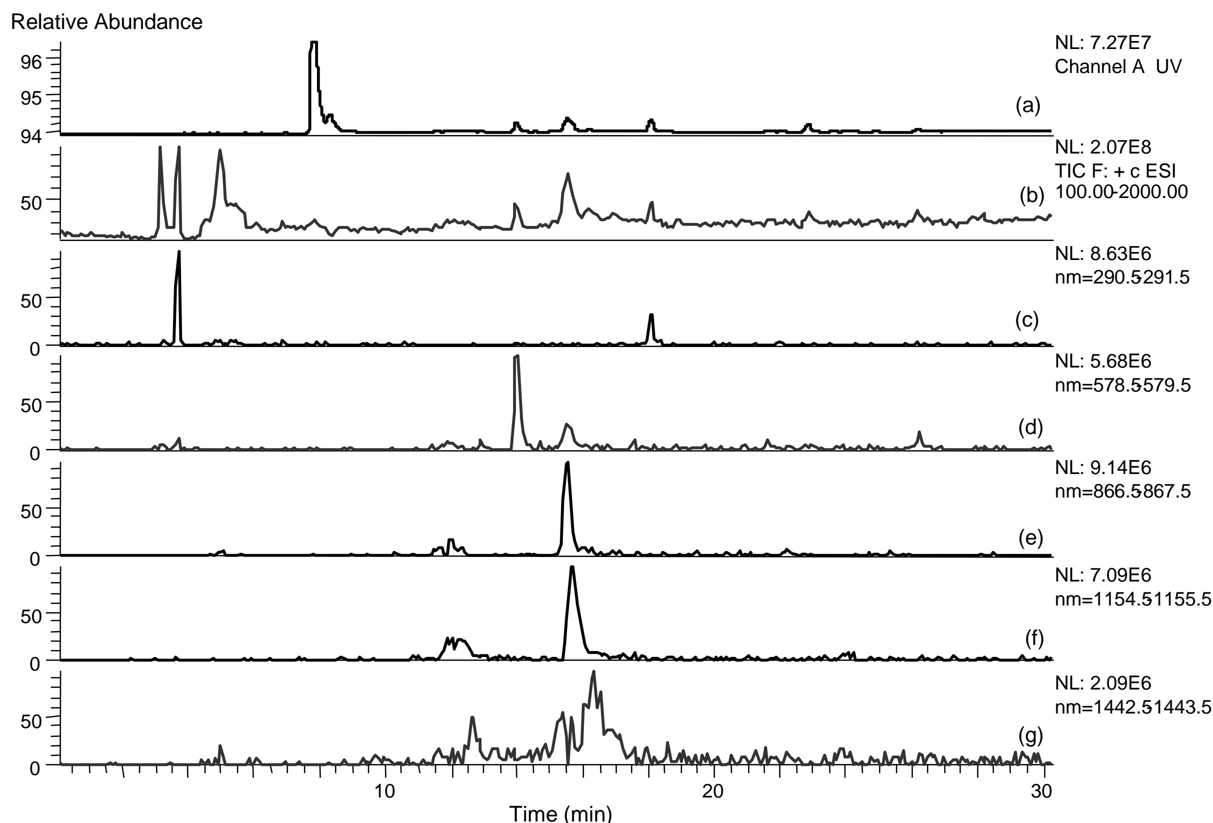

Fig. 5. HPLC-DAD-ESI-MS analysis of residue extract. (a): Chromatogram at 280 nm; (b): TIC at positive mode. Ion traces of (c) to (g) corresponding to monomers (*m/z* 291), dimers (*m/z* 579), trimers (*m/z* 867), tetramers (*m/z* 1155) and pentamers (*m/z* 1443), respectively.

the same as proanthocyanidin dimer from pycnogenol. In addition, the UV spectrum of compound **1** is similar to that of (+)catechin/(−)epicatechin. Therefore, compound **1** was identified to be a singly-linked proanthocyanidin dimer. As shown in Fig. 5, besides compound **1**, other minor isomers with retention times of 12.00, 12.61, 15.5 and 26.2 min, respectively were detected at *m/z* 577 and showed fragmentation patterns similar to that of compound **1**.

Similarly, the molecular weight of compound **2** was found to be around 866. It shows an UV spectrum similar to that of (+)catechin/(−)epicatechin and a molecular weight of $[290 \times 3 - (n - 1) \times 2]$. As shown in Fig. 6b and Table 2, CID spectrum of compound **2** at *m/z* 867 gave fragment ions at *m/z* 715 $[M + \mathrm{H} - 152]^+$, 697 $[M + \mathrm{H} - 170]^+$, 579 $[M + \mathrm{H} - 288]^+$, 577 $[M + \mathrm{H} - 290]^+$, etc. The fragment ions were similar to those of proanthocyanidin trimer, and the fragmentation pattern should also be similar to that of proanthocyanidin trimer from pycnogenol. Based on above results, compound **2** was identified as a proanthocyanidin trimer. The isomers of compounds **2** in both juice and extract might exist at low concentrations as intensities of their ion peaks were much lower than compound **2** (Fig. 5).

Compounds **3** has the molecular weight of around 1154 $[290 \times 4 - (4 - 1) \times 2]$ and UV spectra similar to those of (+)catechin/(−)epicatechin. As shown in Fig. 6c and Table 2, CID spectrum of compound **3** at *m/z* 1155 gave fragment ions at *m/z* 1003 $[M + \mathrm{H} - 152]^+$, *m/z* 985 $[M + \mathrm{H} - 170]$, *m/z* 867 $[M + \mathrm{H} - 288]^+$, 579 $[M + \mathrm{H} - 288 \times 2]$, *m/z* 865 $[M + \mathrm{H} - 290]^+$ and 577 $[M + \mathrm{H} - 288 - 290]^+$. The fragment ions might arise from the fragmentation pattern similar to those of dimmers or timers. Therefore, compound **3** was identified as a singly-linked proanthocyanidin tetramer. A few isomers of compound **3** with retention times of 11.87, 12.26 and 23.8 min were also observed with much lower intensities in all residue extracts and most batches of juice, while two dimers with retention times of 11.87 and 12.26 min were detected to have around 50% intensity of compound **3** in two batches of juice. However, the ion intensities of the two isomers in whole star fruit were still much lower than that compound **3**. The results agreed with above result, which shows that the contribution of TAA by residue was 82.7% and major antioxidants exited in residue rather than juice. Therefore, compound **3** was identified as the major tetramer in star fruit.

For compound **4**, its molecular weight was around 1442 $[290 \times 5 - (5 - 1) \times 2]$ and UV spectra was similar to those of (+)catechin/(−)epicatechin. As shown in Fig. 6d and Table 2, CID spectrum of compound **4** at *m/z* 1443 gave fragment ions at *m/z* 1291 $[M + \mathrm{H} - 152]^+$, *m/z* 1273 $[M + \mathrm{H} - 170]^+$, 1155 $[M + \mathrm{H} - 288]^+$, 867 $[M + \mathrm{H} - 288 \times 2]^+$, 579 $[M + \mathrm{H} - 288 \times 3]$, 1153 $[M + \mathrm{H} - 290]^+$, 865

$[M+H-288-290]^+$ and 577 $[M+H-288\times2-290]^+$. The fragment ions might arise from the fragmentation pattern similar to those of of tetramers or trimers in pycnogenol or star fruit. Therefore, compound **4** was identified as a singly-linked proanthocyanidin pentamer. While compound **4** was the major pentamer in star fruit, a few of isomers with retention times of 12.6 and 15.2 min were also observed.

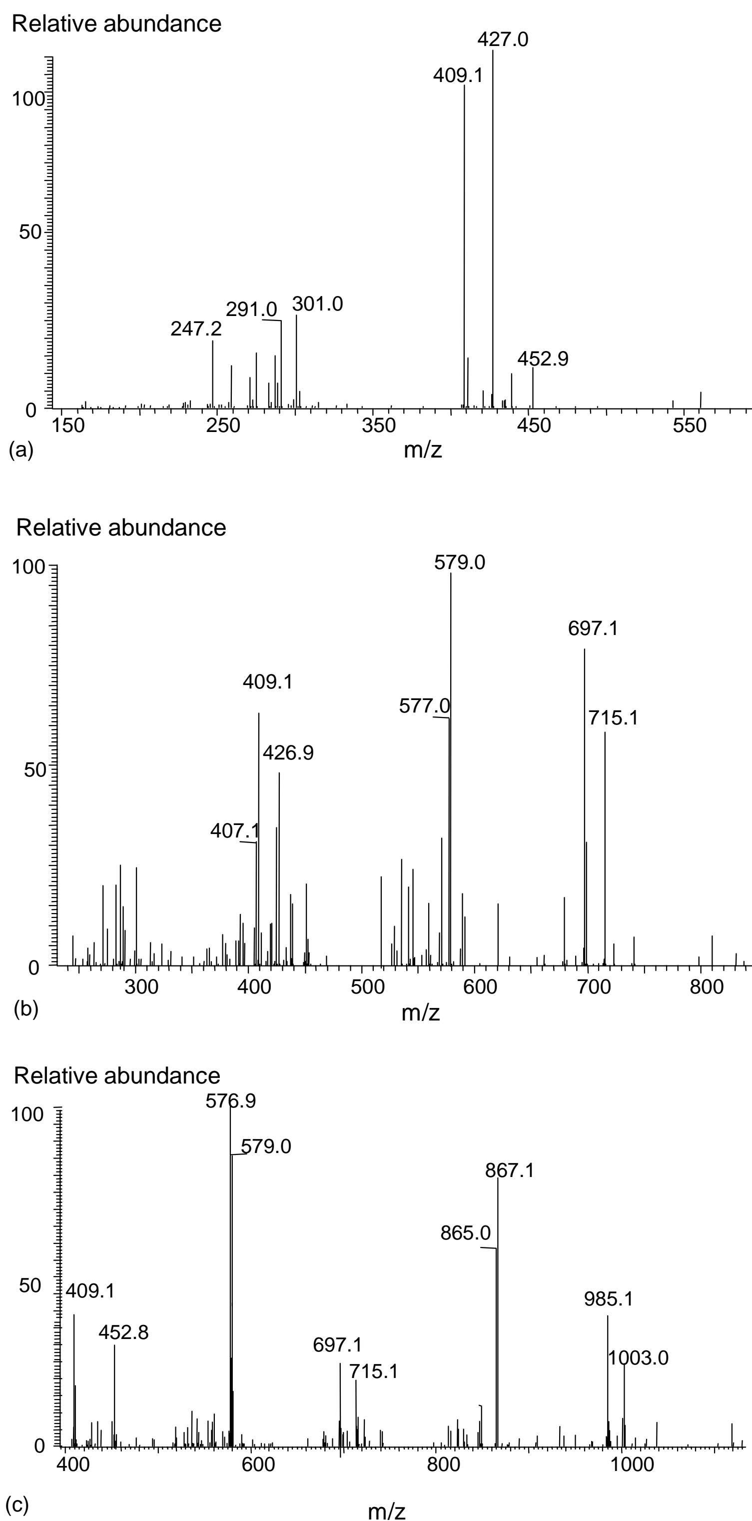

Fig. 6. CID (MS/MS Scan) spectra of antioxidant peaks in residue extract, collision energy: 50%. (a) CID spectrum corresponding to the parent ion(s) at m/z 579; (b) CID spectrum corresponding to the parent ion(s) at m/z 867; (c) CID spectrum corresponding to the parent ion(s) at m/z 1155; (d) CID spectrum corresponding to the parent ion(s) at m/z 1443.

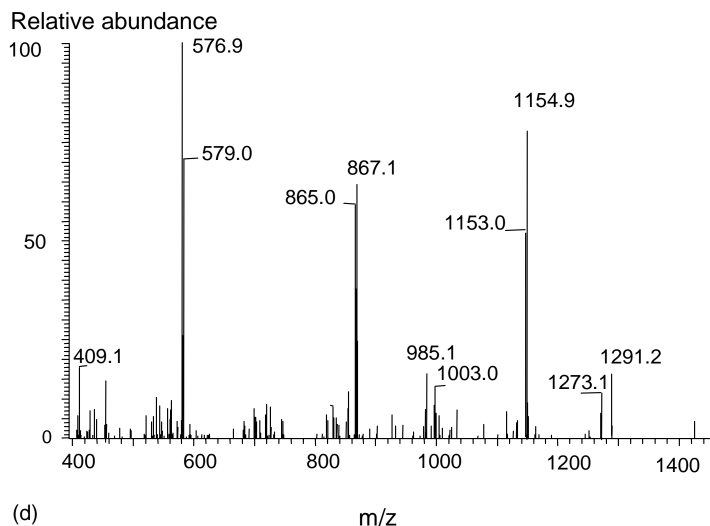

Fig. 6. (*Continued*).

Compound **5** was identified as (−)epicatechin by comparison of the UV and CID spectra with those of the authentic standard. In addition, the precipitation of bovine protein after addition of star fruit extract/juice indicated the existence of tannins. The results from extract solvent experiment showed that acetone/water gave more effective extraction than alcoholic solvents (unpublished data). This could be due to the fact that acetone inhibits tannin-protein interaction and thus increases extraction efficiency of tannins. Therefore, the major antioxidants were preliminarily attributed to condensed tannins i.e. proanthocyanidins.

4. Conclusion

Based on TAA assays of SPE fractions and HPLC assays of SPE fractions, juice/extract and reaction solution with free radicals, the major antioxidants in star fruit were investigated and further identified by tandem mass spectrometry. Phenolic compounds were found to be the major antioxidants in star fruit. (−)Epicatechin and proanthocyanidins, which existed as dimers through pentamers, were reported in star fruit for the first time and preliminarily considered as major phenolic compounds in star fruit.

The antioxidant properties of star fruit in different systems had been studied and will be reported later. As proanthocyanidins have attracted increasing attention due to their potential health benefits, the next step of our research is to quantify them for the initial understanding of the possible dietary intake of these compounds.

References

[1] B.N. Ames, M.K. Shigenaga, T.M. Hagwn, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 7915.
[2] B. Halliwell, Ann. Rev. Nutr. 16 (1996) 33.
[3] K.B. Michels, E. Giovannucci, K.J. Joshipura, B.A. Rosner, M.J. Stampfer, C.S. Fuchs, G.A. Colditz, F.E. Speizer, W.C. Willett, J. Natl. Cancer I. 92 (2000) 1740.
[4] M.S. Van Duyn, E. Pivonka, J. Am. Diet Assoc. 100 (2000) 1511.
[5] L.P. Leong, G. Shui, Food Chem. 76 (2002) 69.
[6] B. Setiawan, A. Sulaeman, D.W. Giraud, J.A. Driskell, J. Food Comp. Anal. 14 (2001) 169.
[7] G. Shui, L.P. Leong, J. Chromatogr. A 977 (2002) 89.
[8] J.J. Dalluge, B.C. Nelson, J. Chromatogr. A 881 (2000) 411.
[9] A. Escarpa, M.C. Gonzalez, J. Chromatogr. A 830 (1999) 301.
[10] P. Sarni-Manchado, E. Le Roux, C. Le Guerneve, Y. Lozano, V. Cheynier, J. Agricult. Food Chem. 48 (2000) 5995.
[11] H. Chen, Y.G. Zuo, Y.W. Deng, J. Chromatogr. A 913 (2001) 387.
[12] FAO/IAEA working manual: quantification of tannins in tree foliage, 2000, IAEA, Viena, Italy.
[13] M. Carini, R.M. Facino, G. Aldini, M. Calloni, L. Colombo, Rapid Commun. Mass Spectrom. 12 (1998) 1813.
[14] M. Wang, J. Li, M. Rangarajan, Y. Shao, E.J. LaVoie, T.C. Huang, C.T. Ho, J. Agricult. Food Chem. 46 (1998) 4869.
[15] M. Careri, A. Mangia, M. Musci, J. Chromatogr. A 794 (1998) 263.
[16] M. Careri, F. Bianchi, C. Corradini, J. Chromatogr. A 970 (2002) 3.
[17] L. Packer, G. Rimbach, F. Virgili, Free Radical Biol. Med. 27 (1999) 704.
[18] H. Fulcrand, S. Remy, J.-M. Souquet, V. Cheynier, M. Moutounet, J. Agricult. Food Chem. 47 (1999) 1023.
[19] B. Zywicki, T. Reemtsma, M. Jekel, J. Chromatogr. A 970 (2002) 191.
[20] G.E. Rohr, G. Riggio, B. Meier, O. Sticher, Phytochem. Analysis 11 (2000) 113.
[21] Y.Y. Lin, K.J. Ng, S. Yang, J. Chromatogr. 629 (1993) 389.
[22] J.J. Karchesy, L.Y. Foo, E. Barofsk, B. Arbogast, D.F. Barofsky, J. Wood Chem. Technol. 9 (1989) 313.