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Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals

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Abstract

Our previous study has indicated that star fruit (*Averrhoa carambola* L.) is a good source of natural antioxidants and that polyphenolics are its major antioxidants. In this study, the residue of star fruit, which is normally discarded during juice drink processing, was found to contain much higher antioxidant activity than the extracted juice using several methods for assessing antioxidant activity. Under optimized extraction conditions, the residue accounted for around 70% of total antioxidant activity (TAA) and total polyphenolic contents, however only contributed 15% of the weight of whole fruit. Freeze-dried residue powder, which accounted for around 5% of total weight, had total polyphenolic content of 33.2 ± 3.6 mg gallic acid equivalent (GAE)/g sample and total antioxidant activity of 3490 ± 310 and 3412 ± 290 mg L-ascorbic acid equivalent antioxidant capacity (AEAC) or 5270 ± 468 and 5152 ± 706 mg trolox equivalent antioxidant capacity (TEAC) per 100 g sample obtained by 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) free radical ($ABTS^{+\cdot}$) and 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) scavenging assays, respectively. It was also found to have 510.3 ± 68.1 mol ferric reducing/antioxidant power (FRAP) per gram sample. The residue extract also shows strong antioxidant activity in delaying oxidative rancidity of soya bean oil at 110 °C. Antioxidant activity and polyphenolic profile of residue extracts were compared with extracts of standardized pyconogenol. High performance liquid chromatography coupled with mass spectrometry (HPLC/MS) shows that major proanthocyanidins in star fruit were different from their isomers in pyconogenol. The high content of phenolics and strong antioxidant activity of residue extracts indicate that residue powder may impart health benefits when used in functional food products and that residue extracts should also be regarded as potential nutraceutical resources in future.

Keywords: Star fruit; Antioxidants; Anti-rancidity; Phenolics; Residue; Functional food; Nutraceuticals

1. Introduction

Fruits and vegetables are good dietary sources of natural antioxidants for dietary prevention of degenerative diseases. The main contribution to the antioxidant capacity of a fruit or vegetable is likely to come from a variety of phytochemicals other than vitamin C (Leong & Shui, 2002). Fruits and vegetables contain many antioxidants such as phenolics, thiols, carotenoids and tocopherols, which may protect us against chronic diseases. Antioxidants could reduce oxidative damages to biomolecules by modulating reactive free radicals. Therefore, increased consumption of fruits and vegetables has been recommended. An increasingly growing market for nutraceuticals and functional foods has triggered the study on natural sources of antioxidants and their potential for nutraceutical and functional foods (Cevallos-Casals & Cisneros-Zevallos, 2003; Lachance, 2002; Lachance, Nakat, & Jeong, 2001).

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To avoid loss of nutrition value, it is usually encouraged to consume fruits and vegetables fresh. However, for the purpose of transportation, extension of shelf life, etc., they are also processed into juices and juice drinks. During the processing of juices or juice drinks, agricultural and industrial wastes are likely to be discarded or only used as low-value by-products. As reviewed previously, numerous antioxidants could be extracted from those residual sources (Moure et al., 2001). Recently, apple peel was reported as a value-added food ingredient for food products to promote good health due to its phytochemical contents (Wolfe & Liu, 2003; Wolfe, Wu, & Liu, 2003). Residues of the oil-extract of eight different oilseeds were reported to contain phenolic compounds and showed remarkable antioxidant activities, which can be used as natural antioxidants for the protection of fats and oils (Matthaus, 2002).

Star fruit is grown in the tropic and sub-tropic regions of the world. It is quite a popular fruit and largely planted in Southeast Asia and many other countries. It is usually consumed fresh or made into fruit juice or juice drinks. We have reported that star fruit is a good source of natural antioxidants, and the antioxidants in star fruit were found to be proanthocyanidins, (−)-epicatechin and vitamin C (Leong & Shui, 2002; Shui & Leong, 2004). When star fruit is used to produce fruit drinks, normally only the juice will be used and the residue is often discarded as waste or used to produce low-value by-products. The purpose of this study is to further investigate antioxidant capacities and total phenolic contribution of the residue. In addition, we will also investigate the possibilities of utilizing the residue as a valuable food ingredient or a resource for nutraceutical products in the future.

2. Materials and methods

2.1. Chemicals and reagents

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picryl-hydrazyl, (−)-epicatechin, and potassium persulfate were from Sigma (MO, USA); anhydrous sodium carbonate and, (+)-catechin hydrate from Chem. Aldrich Co. (WI, USA); trolox, gallic acid and tripydyltriazine (TPTZ) from Acros Organics (NJ, USA), Folin–Ciocalteu reagent, ferric chloride, ferrous sulfate from Merck (Darmstadt, Germany).

2.2. Sample preparation and extraction

Several batches of star fruits were purchased from a local supermarket or wholesale center. One brand of commercial Pycnogenol, which is made from Nuvanta, Horphag's French Maritime Pine bark, was purchased from a branch of the Guardian pharmaceutical shop in Singapore. Fresh star fruit obtained from the market was homogenized using a blender, centrifuged and filtered under vacuum. The liquid portion (juice) was used directly for antioxidant capacity and total phenolic assays. The solid portion (residue) was dried into powder by lyophilization, which was found to be around 5% of the total weight. The extraction of phenolic from the dried powder was carried out at different ratios of solvents (acetone or ethanol):water (0%, 30%, 50%, 70% and 100%, respectively), to obtain suitable extraction solvent and ratio of solvent to water. Paired-sample *t*-test was carried out to identify the significant differences between solvents. Under the selected extraction solvent, different extraction temperatures (30, 50, 70 and 90 °C, respectively) were compared. Under the selected solvent and temperature, different extraction times (15, 30, 45, 60 min, respectively), were applied for obtaining a suitable extraction time. The extraction was carried out in sealed glass bottles, which were placed into a water bath at a preset temperature. The extraction efficiencies among different extraction modules were compared by measuring total antioxidant activity (TAA) of individual extracts. The TAA of the star fruit was determined using the $ABTS^{+\cdot}$ decolorization assay. Residue was extracted three times to investigate the necessity of multiple extractions. The first extract of residue (w/v, 1:60) was obtained under optimum extraction conditions. The second extract and the third extract were obtained under similar extraction conditions except for a reduced retention extraction time of 15 min. The three extracts were combined and evaporated at 40 °C under vacuum to remove solvents and then redissolved in water. The antioxidant activity of the three separate extracts were also tested and used to compare the extraction efficiency. The extract of residue was filtered with 0.5 μm membrane filter and then used directly for HPLC and HPLC/MS assay. The antioxidant capacity for juice and extracts was also tested.

Commercial pycnogenol pill was ground into powder and dissolved into 50% aqueous ethanol in a dark bottle for 2 h. The extract was centrifuged and filtered with filter paper (Whatman 1). The filtrate was dried by vacuum evaporation and re-dissolved in 20% aqueous methanol. The obtained solution was filtered with a 0.5 μm membrane filter and then used for HPLC/MS assay. The antioxidant activity of the solution was also tested.

2.3. Total antioxidant activity (TAA) assay by $ABTS^{\cdot+}$ scavenging assay

The TAA assay was carried out on the Ultraspec 3000 UV/Visible Spectrophotometer (Pharmacia Biotech Ltd., Cambridge CB4 4FJ, England). The procedure was described by (Leong & Shui, 2002). Briefly,

$ABTS^{\cdot+}$ was generated by reacting ABTS (7.4 mM) with potassium persulphate (2.6 mM). The solution was diluted to obtain an absorbance of 1.5–2.0 U at 414 nm with pH 4.5 HCl solution before use. An aliquot of 20–80 μl of extract or L-ascorbic acid solution was added to 3 ml of this solution. The changes in absorbance at 414 nm were recorded after 1 h. Antioxidant capacity of the extract was obtained by comparing the change of absorbance at 414 nm in a test reaction mixture containing extracts with that containing L-ascorbic acid and trolox, and expressed as mg L-ascorbic acid equivalent antioxidant capacity (AEAC) and trolox equivalent antioxidant capacity (TEAC) per 100 g sample.

2.4. TAA assay by DPPH· scavenging assay

DPPH· scavenging ability of fruits was measured using the method described by Brand-Williams, Cuvelier, and Berset (1995). A solution of 0.1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol was prepared. An aliquot of 10–40 μl of L-ascorbic acid or fruit/vegetable extract was added to 3 ml of this solution. The absorbance at 517 nm after mixing was measured every 5 min until the reaction reached a plateau. The loss of absorbance was calculated and expressed as mg of L-ascorbic acid equivalent antioxidant capacity (AEAC) and trolox equivalent antioxidant capacity (TEAC).

2.5. TAA assay by ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of plant extracts was determined using a modification of the ferric reducing/antioxidant power (FRAP) assay of Benzie and Strain (1999). The FRAP reagent contained 2.5 ml of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 ml of 0.3 M acetate buffer at pH 3.6. Briefly, 3.0 ml of FRAP reagent, prepared freshly and warmed at 37 °C, was mixed with 40 μl of plant extract and reaction mixtures incubated at 37 °C. Absorbance at 593 nm was determined with reference to a reagent blank containing distilled water which was also incubated at 37 °C for up to 1 h instead of 4 min, which is the original time applied in FRAP assay. Aqueous solutions of known Fe (II) concentrations in the range of 100–2000 μM ($FeSO_4 \cdot 7H_2O$) were used for calibration.

2.6. Total phenolic content by Folin–Ciocalteau assay

Total phenolics were determined using Folin–Ciocalteau reagents (Singleton & Rossi, 1965). Gallic acid standard solution (2.0 mg/ml) was prepared by accurately weighing 0.01 g and dissolving 50 ml of distilled water. The solution was then diluted to give with concentrations working standard solutions of 1.5, 1.0, 0.5, 0.2, and 0.1 mg/ml. Forty microlitres of juice/residue extract or gallic acid standard was mixed with 1.8 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 1.2 ml of sodium bicarbonate (7.5%) was added to the mixture. After standing 60 min at room temperature, absorbance was measured at 765 nm. Results are expressed as mg/g gallic acid equivalents (GAE).

2.7. Anti-rancidity properties of residue extract on soya bean oil

Twenty milliliters of extract solution from 0.2 g of residue was evaporated to around 0.5 ml and then thoroughly mixed with 100 g of soya bean oil. The oil was pipetted into 15 test tubes with each test tube containing 5 g and then subjected to peroxidation at 110 °C. At a specific time, the peroxide values (PV) of the test tubes were determined using the AOCS official method (AOCS, Cd 8-53, 1990). The PV value of blank was also obtained using pure soya bean oil. In addition, soya bean oil containing 140 ppm BHT was also used as comparison.

2.8. High performance liquid chromatography coupled with electronspray ionization and mass spectrometry (HPLC/ESI/MS) analyses

For HPLC/ESI/MS analysis, the apparatus was a Finnigan/MAT LCQ ion trap mass spectrometer (San Jose, CA, USA) equipped with TSP spectra system, which includes a UV6000LP PDA detector, P4000 quaternary pump and AS3000 autosampler. The heated capillary and voltage were maintained at 200 °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 modes with a scan speed of 1 s/scan. Chromatographic separations were done on a Shim-Pack VP-ODS column (250 × 4.6 mm i.d.) (Shimadzu, Kyoto, Japan) with a guard column (GVP-ODS, 10 × 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.

3. Results and discussion

3.1. Extraction of antioxidants from star fruit

The effects of solvents and ratios of solvent to water on extract antioxidant capacity, which is proportional

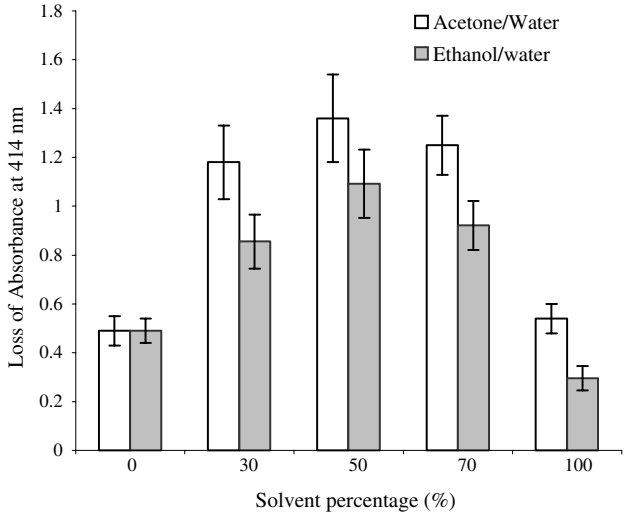

Fig. 1. Effects of solvents and solvent ratios on extraction efficiency. Percentage given is the percentage of acetone or ethanol. Extraction temperature: 90 °C; extraction time: 45 min.

to loss of absorbance at 414 nm upon addition of extract, are reported in Fig. 1. The acetone/water extract system obtained higher extraction efficiency than the ethanol/water system. A paired-sample t test indicated that there are significant differences between acetone and ethanol at the solvent ratio of 30% ($p < 0.05$), 50% ($p < 0.10$), 70% ($p < 0.05$) and 100% ($p < 0.05$). The 50/50 mixture of acetone/water gave highest extraction efficiency of antioxidant activity. Extraction efficiency on homogenized star fruit at room temperature gave similar trends on another four batches of star fruits (data not shown). Therefore, 50% aqueous acetone was chosen as the extraction solvent.

The effects of temperature on extraction efficiency of are shown in Fig. 2. Of the five batches of star fruits used for optimum extraction temperature test, the

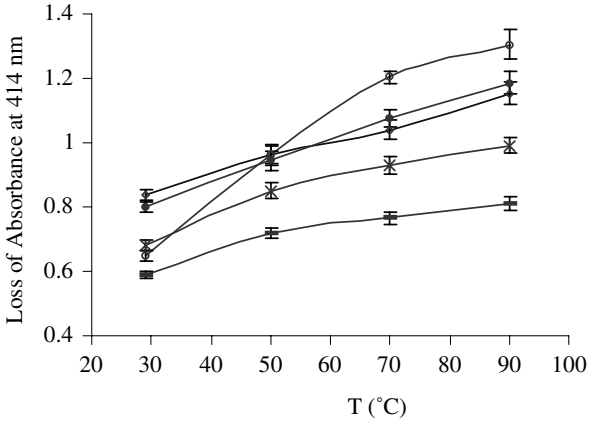

Fig. 2. Effects of temperature on extraction efficiency. Each line represents one batch of star fruit with triplicates. Extraction time: 45 min; solvent, 50% aqueous acetone solution.

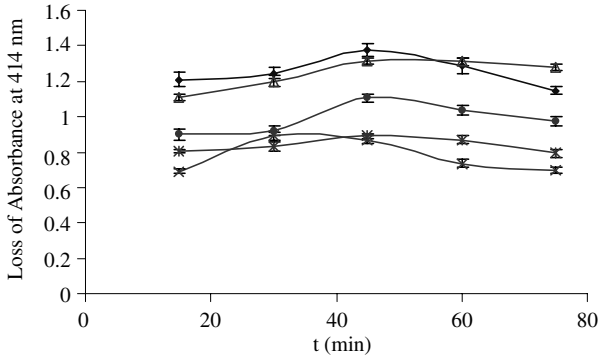

Fig. 3. Effects of time on extraction efficiency. Each line represents one batch of star fruit with triplicates. Extraction temperature, 90 °C; solvent, 50% aqueous acetone solution.

extraction efficiency increased with increased temperature. A major increase was observed especially from 30 to 75 °C. Considering stability of antioxidants at high temperature, 90 °C was chosen as a suitable extraction temperature.

Fig. 3 gives the consequences of different extraction times on the efficiency of extraction. Extraction efficiency increased with time and reached a highest value from 30 to 60 min. Of the five batches of star fruits tested, four gave highest extraction efficiency at 45 min, only one batch gave highest value at 30 min. Therefore, 45 min was chosen as the optimum extraction time.

As a summary, the optimum conditions used for extraction are 50% acetone as extraction solvent at 90 °C for 45 min.

3.2. Distribution of antioxidants in star fruit

Our previous study showed that proanthocyanidins, which existed as singly-linked dimers through pentamers, were most likely to be the major antioxidants in star fruit (Shui & Leong, 2004). As shown in Table 1, the first extract accounted for 73% of total antioxidant activity in star fruit, and the second and third extract accounted for 7% and 2%, respectively. Juice only accounted for 17% of TAA while it may account for about 85% of the total weight. Therefore, the majority of antioxidants existed in residues other than the juice.

3.3. Correlations between TAA and total phenolic content

In this study, samples were taken from juice, single residue extracts at 30, 50, 70 and 90 °C, single residue extracts at different acetone percentage at 90 °C, and multiple extracts at 90 °C, respectively. The total antioxidant activity of juice and residue extracts correlated well with their total phenolic content (Fig. 4).

Table 1
Antioxidant contribution in processed star fruit solution

	Juice	Residue			TAA
		Extract (1st)	Extract (2nd)	Extract (3rd)	
ΔA[a]	0.855 ± 0.045	1.380 ± 0.051	0.140 ± 0.009	0.078 ± 0.002	
Volume (ml)	153	400	400	240	
$\Delta A \times$ Vol	130.8	552	56	18.7	757.5[b]
Percentage (%)	17.27	72.87	7.39	2.47	100
		82.73			

$N \pm$ SD, $N = 3$.
[a] Absorbance change upon addition of 10 μl of solution.
[b] TAA is the sum of $\Delta A \times$ Vol of juice, the first extract, the second extract and the third extract.

The result is consistent with our previous result, which indicated that polyphenolic compounds are the major antioxidants in star fruit (Shui & Leong, 2004). It was found that the residue accounted for over 70% of the total polyphenolic content in whole fruits.

3.4. Inhibition of lipid peroxidation

Fats and lipids are subject to oxidative damage, which is associated with the formation of peroxides and hydroperoxides which may be detrimental to health. Unsaturated fats are particularly susceptible to this type of damage. Antioxidants prevent the formation of these products and their further breakdown. The breakdown products give foods off-flavors and off-odors, and eventually lead to rancidity. Antioxidants are therefore able to slow the process of rancidity and greatly increase the shelf-life of foods.

Butylated hydroxytoluene (BHT) is a synthetic antioxidant widely used in the food industry to slow the development of off-flavors, odors and colour changes caused by auto-oxidation, mostly in foods with high fats and oils. For the purpose of safety, the US Food and Drug Administration (FDA) limit the use of BHT to 0.02% or 200 ppm of the oil or fat content of a food product.

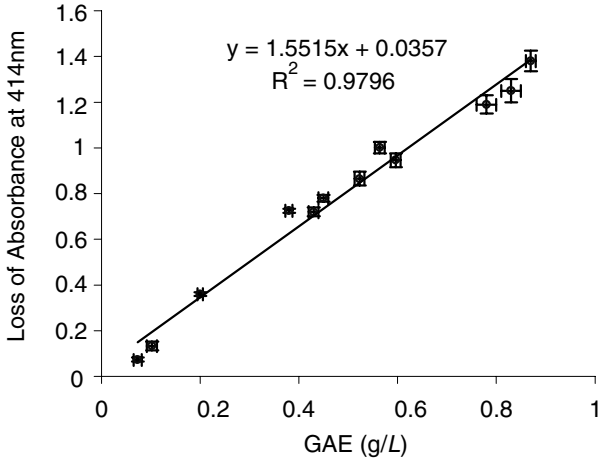

Fig. 4. Correlation of antioxidant capacity assessed by loss of absorbance at 414 nm in the ABTS radcal scavenging assay plotted against the total phenolic content expressed as GAE.

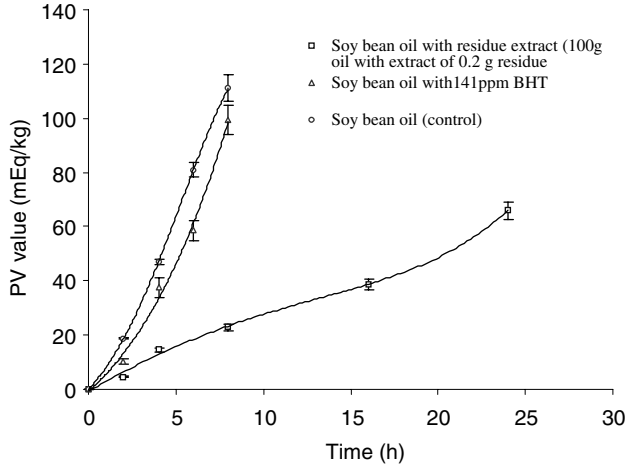

Fig. 5. Effects of BHT and residue extract on peroxidation of soya bean oil.

It was found in this study that antioxidants obtained from the residue extract significantly slowed the process of rancidity of oil to a large extend compared to BHT (Fig. 5). This result shows that antioxidants obtained from star fruit have a potential to be used for the prevention of oil rancidity.

3.5. Free radical scavenging abilities and FRAP of residual extract

As can be seen from Table 2, the AEAC value of residue is extremely high (more than 3000 mg AEAC/100 g of freeze-dried residue powder). The total phenolic content and FRAP value were also very high. These indicated that residues from star fruit were excellent sources of phenolic antioxidants for antioxidant nutraceuticals or functional food additives.

Table 2
Total phenolic content and antioxidant capacity of residue

	Total phenolics GAE mg/g	$AEAC_{ABTS}$ mg/100 g	$TEAC_{ABTS}$ mg/100 g	$AEAC_{DPPH}$ mg/100 g	$TEAC_{DPPH}$ mg/100 g	FRAP μmol/g
Residue (dry basis)	33.2 ± 3.6	3490 ± 310	5270 ± 468	3412 ± 290	5152 ± 706	510.3 ± 68.1

Based on the freeze-dried residue powder. $N \pm SD$, $N = 3$.

3.6. Comparison of phenolic profile and antioxidant capacity of residue and pycnogenol pills

In this study, it was found that $AEAC_{ABTS}$ of residue from ten star fruits was close to that of a bottle of pycnogenol containing 40 pills while the cost of the latter was eight times more than the former. As residue in juice drink processing might be discarded, its commercial potential was significantly underestimated.

As reported previously (Shui & Leong, 2004), HPLC/ESI/MS/MS spectra of major antioxidants in star fruit were similar to ESI/MS/MS spectra of pycnogenol proanthocyanidins and thus proanthocyanidins, which existed as dimers through pentamers in star fruit, were characterised in star fruit. However, as mass spectrometry might not distinguish isomers, the proanthocyanidin profile of star fruit might be different from that of pycnogenol. The major antioxidants also eluted between (+)catechin ($R_t \approx 14$ min) and (−)epictechin ($R_t \approx 18$ min) (Shui & Leong, 2004). Fig. 6 shows averaged mass spectra profiles (13–19 min) from the sample used in this study. Here $[M + H]^+$ of hexamer ion at m/z 1731 was also observed. This is probably due to seasonal variations on the quality of star fruit or different cultivars of star fruit used in experiments.

Pycnogenol is an extract from French maritime pine bark (PBE). It is a highly standardized mixture of certain polyphenolic compounds, which comprises 80–85 wt% of proanthocyanidins. Fig. 7(a) and (b) show chromatographic elution profiles of proanthocyanidins in pyconogenol and star fruit residue extract, respectively. Table 3 summarises the chromatographic profiles of proanthocyanidins in star fruit and pycnogenol. It is found that proanthocyanidin profile in star fruit and that in pycnogenol are different. For example, (−)epicatechin ($R_t = 18.28$ min) was the major monomer in star fruit, and (+)catechin ($R_t = 14.05$) was the major monomer in pycnogenol. In addition, major proanthocyanidin isomers in pycnogenol were found to elute faster than proanthocyanidin isomers in star fruit. For example, the elution times for other major proanthocyanidin dimer through hexamer in star fruit were 14.10, 15.66, 15.58, 16.01, 16.18 min, respectively, compared to corresponding elution times in pyconogenol that are 10.73, 10.40, 8.26, 10.20, 11.81 min, respectively (Table 3). This may imply that major constituents of proanthocyanidins in star fruit are (−)epicatechin while (+)catechin are major constituents of proanthocyanidins in pycnogenol.

High content of polyphenolics, mainly proanthocyanidins, contributes to the high antioxidant capacity of the residue extract of star fruit. This suggests its great commercial potential as a nutraceutical resource or functional food ingredient. Further research on structural

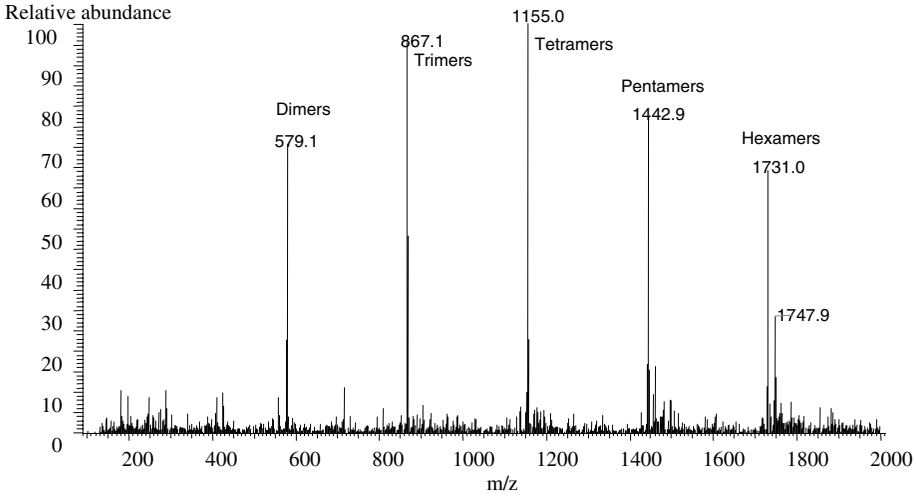

Fig. 6. Averaged (13–19 min) mass spectra profile of proanthocyanidins in star fruit.

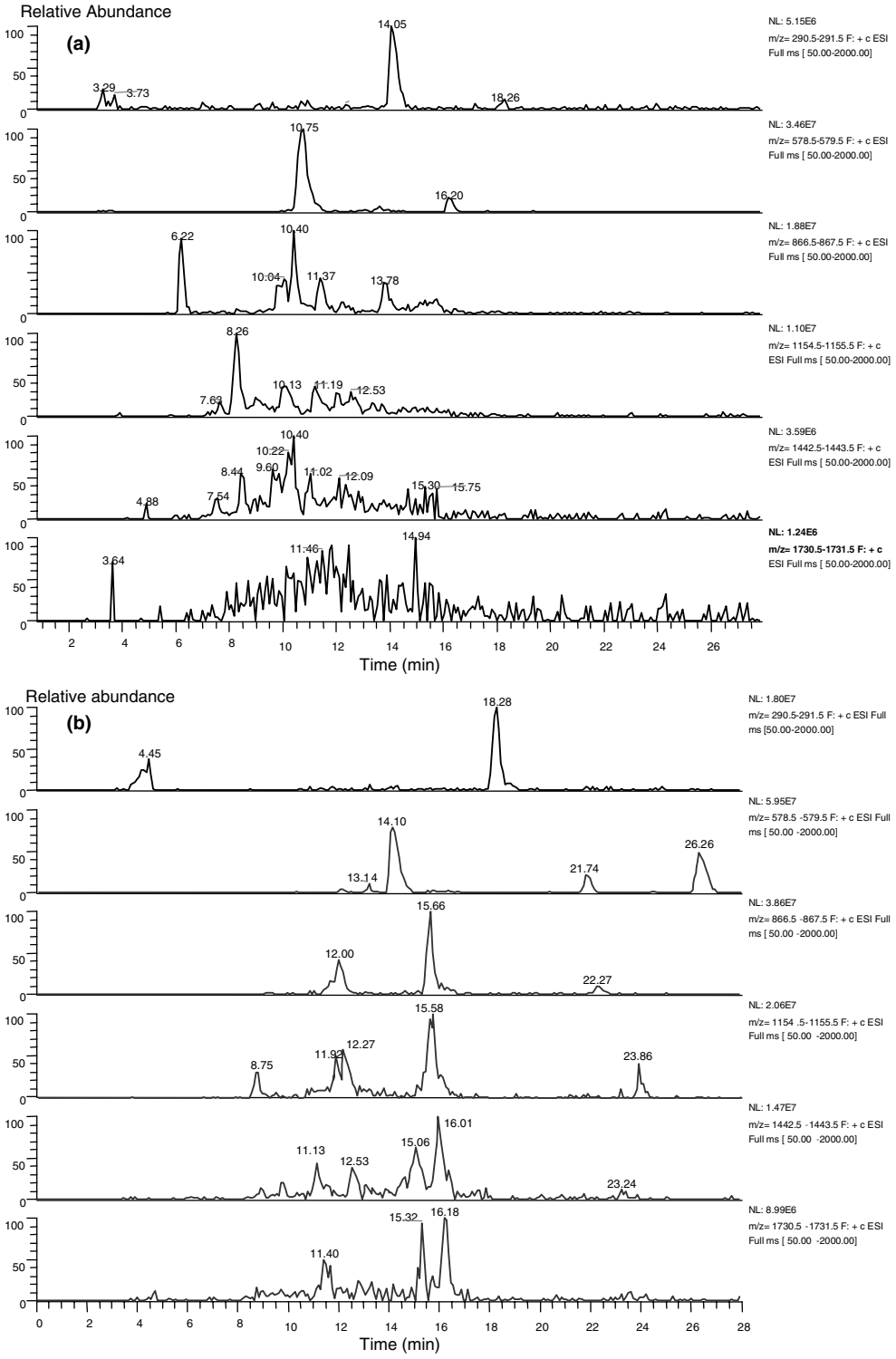

Fig. 7. Ion traces of proanthocyanidins in pycnogenol and star fruit. (a) Ion traces of monomers through hexamers in pycnogenol. (b) Ion traces of monomers through hexamers in star fruit.

identification and quantification of proanthocyanidins in star fruit is necessary to understand its proanthocyanidin profiles and possible dietary intake of these compounds. While pycnogenol have been reported and claimed for its health effects (Devarajet al., 2002; Maritim, Dene, Sanders, & Watkins, 2003; Packer, Rimbach, & Virgili, 1999; Rohdewald, 2002; Youm & Kim, 2003), the potential health effects of star fruit need also be studied.

Table 3
Comparisons of elution times of proanthocyanidins in pycnogenol and star fruit

Compounds	Retention times (min) and relative abundance (in brackets)			
Monomers				
Pycnogenol	14.05(100)			
Star fruit	18.28(100)			
Dimers				
Pycnogenol	10.73(100)	16.20(17)		
Star fruit	14.10(100)	21.74(26)	26.26(50)	
Trimers				
Pycnogenol	10.40(100)	11.37(40)		
Star fruit	15.66(100)	12.00(44)	22.31(12)	
Tetramers				
Pycnogenol	8.26(100)	10.13(40)		
Star fruit	15.58(100)	11.92(50)	12.27(55)	23.86(35)
Pentamers				
Pycnogenol	10.20(100)			
Star fruit	16.01(100)	15.06(50)	11.13(35)	12.53(15)
Hexamers				
Pycnogenol	11.81(100)			
Star fruit	16.18(100)	15.28(90)		

References

AOCS. 1990. *Official method* Cd 8-53, American oil chemists society, Champaign, II.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologi, 26*, 25–30.

Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology, 299*, 15–27.

Cevallos-Casals, B. A., & Cisneros-Zevallos, L. (2003). Stoichiometric and Kinetic Studies of Antioxidants from Andean Purple Corn and Red-Fleshed Sweetpotato. *Journal of Agricultural and Food Chemistry, 51*, 3313–3319.

Devaraj, S., Vega-Lopez, S., Kaul, N., Schonlau, F., Rohdewald, P., & Jialal, I. (2002). Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. *Lipids, 37*, 931–934.

Lachance, P. A. (2002). Nutraceuticals, for real. *Food Technology, 56*, 20–20.

Lachance, P. A., Nakat, Z., & Jeong, W. S. (2001). Antioxidants: an integrative approach. *Nutrition, 17*, 835–838.

Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry, 76*, 69–75.

Maritim, A., Dene, B. A., Sanders, R. A., & Watkins, J. B. (2003). Effects of pycnogenol treatment on oxidative stress in streptozotocin-induced diabetic rats. *Journal of Biochemical and Molecular Toxicology, 17*, 193–199.

Matthaus, B. (2002). Antioxidant activity of extracts obtained from residues of different oilseeds. *Journal of Agricultural and Food Chemistry, 50*, 3444–3452.

Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Dominguez, H., et al. (2001). Natural antioxidants from residual sources. *Food Chemistry, 72*, 145–171.

Packer, L., Rimbach, G., & Virgili, F. (1999). Antioxidant activity and biologic properties of a procyanidin- rich extract from pine (*Pinus maritima*) bark, pycnogenol. *Free Radical Biology and Medicine, 27*, 704–724.

Rohdewald, P. (2002). A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. *International Journal of Clinical Pharmacology and Therapy., 40*, 158–168.

Shui, G. H., & Leong, L. P. (2004). Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *Journal of Chromatography A, 1022*, 67–75.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Youm, J. A., & Kim, Y. G. (2003). Examination of the antioxidant potential of pycnogenol under conditions of oxidative stress in Escherichia coli mutants deficient in HP1 and superoxide dismutase activities. *Journal of Microbiology, 41*, 28–33.

Wolfe, K. L., & Liu, R. H. (2003). Apple peels as a value-added food ingredient. *Journal of Agricutural and Food Chemistry, 51*, 1676–1683.

Wolfe, K. L., Wu, X. Z., & Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of Agricutural and Food Chemistry, 51*, 609–614.