Antioxidant capacity and hydrophilic phytochemicals in commercially grown native Australian fruits

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A R T I C L E   I N F O

A B S T R A C T

Hydrophilic phytochemicals and antioxidant capacities of eight commercially grown native Australian fruits were determined. Kakadu plum (Terminalia ferdinandiana) contained a 6-fold higher level of total phenolic compounds and quandong (Santalum acuminatum) a 1.9-fold higher level of total phenolic compounds (TP, Folin–Ciocalteu assay) than blueberry (Vaccinum sp., cv. Biloxi). Both fruits displayed superior oxygen radical-scavenging capacity (ORAC-H assay) that was, respectively 4.1-fold and 6.5-fold of that of blueberry. The total reducing capacity (TRC; ferric reducing antioxidant power (FRAP) assay) of Kakadu plum and quandong exceeded the TRC of blueberry, respectively, 13.1- and 2.3-times. The primary sources of antioxidant capacities in the evaluated fruits were phenolic acids (benzoic and cinnamic) and flavonoids (flavonols, flavanones and anthocyanins) tentatively detected by liquid chromatography–mass spectrometry (LC–PDA–MS/MS). A high level of vitamin C was recorded for Kakadu plum and Australian citrus fruits. The major organic acids detected were citric and malic acid.

1. Introduction

Epidemiological studies have consistently shown that a high uptake of fruits/vegetables is associated with reduced risk of developing chronic diseases. Among the phytonutrients responsible for this health benefit are phenolic compounds. Their preventative effects against the development of degenerative diseases, such as cancer (Hertog et al., 1996), cardiovascular diseases (Vita, 2005), neural degeneration (Youdim, Spencer, Schroeter, & Rice-Evans, 2002), diabetes and obesity (Tsuda, Horio, Uchida, Aoki, & Osawa, 2003) have been reported. Phenolic compounds are generally strong antioxidants and a primary explanation of their action is protection of cell constituents against oxidative damage through the scavenging of free radicals, thereby averting their deleterious effects on nucleic acids, proteins and lipids in cells (Rice-Evans, 2001). Phenolics interact directly with receptors or enzymes involved in signal transduction (Moskaug, Carlsen, Myhrstad, & Blomhoff, 2005) which clearly indicates that they play a specific role in our physiology. Understanding the role of dietary phenolics will certainly open new prospects for their utilisation in health-promoting foods. Identifying novel sources of phenolic compounds and further understanding of their properties will be of interest to the food industry.

Utilisation of local edible plants for food is important from an economical point of view. Exploring these plants as sources of physiologically active compounds offers enormous opportunities for the development of novel foods. Over recent decades, a number of endemic edible Australian plants have entered commercial production (Ahmed & Johnson, 2000). From the large variety of plants in the wild, growers have selected the most productive forms, suitable for large plantations and commercialisation. This study evaluated native Australian fruits of primary importance to the Australian Native Food Industry.

Kakadu plum (Terminalia ferdinandiana Exell, Combretaceae) grows in the Northern Territory and Western Australia (Wrigley, 1988). The fruit is yellow–green, almond-sized and contains one large seed. It is fibrous and is used predominantly in the form of a powder.

Davidson's plum (Davidsonia pruriens F. Muell., Cunoniaceae) originates from north Queensland. The fruit resembles a large (3–5 cm in diameter) plum, intensively pigmented, sour and tangy. Due to its large yields (Ahmed & Johnson, 2000) and wide application, in both sweet and savoury products, it is rated among the best of native Australian fruits.

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Quandong (*Santalum acuminatum*, A.D.C., *Santalaceae*) was an important staple for the Australian Aboriginal population (Brand-Miller & Holt, 1998). It is widely distributed throughout most southern regions of mainland Australia, including the arid centre of the country, and in Western Australia. The tree is hemi-parasitic and can attach to the roots of a wide range of species (Grant & Buttrose, 1978) which creates an opportunity to extend the areas of quandong growth through utilisation of roots of the plants that are drought-resistant. The fruit is bright red with a firm fleshy layer surrounding an edible stone. The flesh is a good source of carbohydrate (17%) and fibre (4%) (Brand-Miller & Holt, 1998) and is used for fruit-type flavour in sweet and savoury products.

Riberry (*Syzygium luehmannii* (F. Muell.) L.A.S. Johnson, *Myrtaceae*) occurs in sub-tropical rainforests on the east coast of Australia. The fruit, a pink to red berry with a clove/cinnamon flavour, contains a single seed or is seedless. The berries are used in a similar way to blueberries. The whole fruit can be blended for use in ice-cream and is becoming popular with restaurants and food processors. The current production value (3–5 tonnes from cultivation and smaller amount from wild harvest) is lower than the demands but newly established orchards are expected to reduce this shortage (*Russel Glover*, *Woolgoolga Rainforest Products*; *personal communication*).

Lemon aspen (*Acronychia acidula* F. Muell, *Rutaceae*) is a small to medium-sized native tree of highland rainforests from central to north Queensland. The fruit is small, yellow, and approximately 20–25 mm in diameter, with small dark seeds encased in husks. It has a spicy citrus aroma and a strong, acidic lemon flavour (Low, 1991) and is used in sweet and savoury products. Current retail product categories include simmer sauces, chutney, relishes and aspen-flavoured mineral water.

Australian desert lime (*Citrus glauca* (Lindl.) Burkill; *Rutaceae*) grows in Queensland and New South Wales, with some isolated occurrences in central South Australia. The tree is 2–4 m high, bearing small (4 cm diameter) green to yellow-coloured fruit (Low, 1991), which is used for distinctive, recognisably citrus flavour in sweet and savoury products. The production volume reaches 10–15 tonnes per annum. Desert lime has been identified as one of the most resilient *Citrus* species, being comparatively heat-, drought-, and cold-tolerant.

Finger lime (*Citrus australasica* F. Muell; *Rutaceae*) originates from the rainforests in southern Qld and northern NSW. The oblong fruit is about 6–7 cm long and about 1 cm in diameter. Forms that bear fruits of different colours (green, yellow, pink, red and purple) have been selected from the wild and are grown commercially. The cylindrical fruit has globular vesicles resembling caviar. These are becoming popular as a gourmet food. The fruit is made into drink, marmalade, pickle and is even dried and used as a flavouring spice.

Previous evaluation of the above-listed fruits, for the presence of water, protein, fat, carbohydrates, selected vitamins and minerals, has indicated a similar composition to common western foods in the same category (Ahmed & Johnson, 2000; Miller, James, & Maggiore, 1993). Our research has focused on their evaluation as a source of health-promoting phytochemicals, including major hydrophilic and lipophilic compounds, as well as their antioxidant capacities, and comparison of their composition with other commonly consumed fruits. In the present paper, we have characterised the commercially produced Australian fruits with regard to their antioxidant capacity, the level of total phenolics and identity of the major hydrophilic phytochemicals: phenolic compounds identified by liquid chromatography–mass spectrometry (LC/MS) and organic acids identified by high performance liquid chromatography with a diode array detector (HPLC–DAD). It should be noted that the results obtained in this study originate from a single lot of samples produced during one vegetative season, using plant sources selected by the Australian Native Food Industry Ltd. Variations in the levels of phenolic compounds and antioxidant capacities, arising from genetic diversity and environmental factors, were not evaluated in this study.

2. Materials and methods

2.1. Plant material

Samples were selected and provided by the Australian Native Food Industry Ltd. (ANFIL). Australian desert lime was obtained from the Australian Desert Limes company (Queensland, Australia). Kakadu plum, green finger limes, pink finger limes and lemon aspen were obtained from the Australian Produce Company Pty. Ltd. (Queensland, Australia). Davidson's plum was obtained from the Australian Rainforest Products (New South Wales, Australia). A dry sample of quandong was supplied by the Australian Native Food "Outback Pride" and a frozen sample was purchased from the Tanamera Bush Foods, (South Australia, Australia). The riberry sample was supplied by the Woolgoolga Rainforest Products, (NSW, Australia). Blueberries (*Vaccinum* spp., cv. Biloxi) from the Blueberry Farm of Australia, Corrindi (NSW, Australia), were used as a reference and were from a very late harvest after most harvesting for fresh market sales was completed. The frozen samples were freeze-dried on arrival. In the case of plums, the fruits were defrosted to allow stone removal, immediately frozen using liquid nitrogen and freeze-dried. The freeze-dried samples were finely ground and placed in air-tight containers. Subsequently they were stored at $-20\,°C$ until analysed.

2.2. Reagents and standards

Unless otherwise stated, all chemicals were purchased from Sigma–Aldrich (Sydney, Australia) and were of analytical or HPLC grade. Cyanidin 3-glucoside, cyanidin 3-sambubioside, cyanidin 3-rutinoside, and cyanidin 3,5-diglucoside, were purchased from Polyphenols Laboratories AS (Hanaveien, Norway). Deionised water was used throughout.

2.3. Extraction of hydrophilic compounds

An aliquot of the ground sample (250 mg) was extracted with 5 ml of 80% aqueous methanol/1.0% HCl (v/v) under a nitrogen atmosphere to prevent oxidation. The samples were sonicated for 10 min, centrifuged [10 min, 5000 rpm; centrifuge Jouan C3i (Jouan S.A., France) rotor AC 100.10], and the supernatants collected. The pellets were re-extracted two more times. Aliquots of the combined supernatants (15 ml) were filtered with a 13 mm × 0.45 µm polytetrafluoroethylene (PTFE) membrane, flushed with nitrogen and stored at $0.5\,°C$ until analysed. The extraction was carried out in triplicate for each sample. The analysis was conducted within 3 days.

2.4. Total phenolic content (Folin–Ciocalteu assay)

The total phenolic content (TP) was determined using the Folin–Ciocalteu (F–C) assay (Singleton & Rossi, 1965). Diluted extracts were directly assayed at 600 nm, with gallic acid as a standard. Measurements were done in microplates, using a microplate reader model Multiscan RC, version 4 (Labsystems, Finland) operated by the DeltaSoft3 programme (Elisa Analysis for the Macintosh with interference for the Multiscan Microplate Readers, BioMetallics, Inc., 1995). The analysis was conducted in triplicate. Ascorbic acid readily reacts with the F–C reagent and enhances the F–C value. To avoid this effect, the level of ascorbic acid was measured be-

fore the medium was alkalised with Na_2CO_3 (the assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form blue complexes) and then subtracted from the F–C value (Singleton & Rossi, 1965). Results were expressed as micromoles of total phenolics (gallic acid equivalents, GAE) per gramme fresh weight (µmol GAE/gFW) or per gramme dry weight (µmol GAE/gDW). In the case of plums, the stone was removed and fresh weight was corrected to reflect the amount of compounds present in the edible part.

2.5. FRAP (ferric reducing antioxidant power) assay

The assay was conducted according to Benzie and Strain (1996) with minor modifications. Thirty microliter of water and 10 µl of fruit extracts (diluted, as needed to obtain a clear reading) were mixed with 200 µl of FRAP reagent, consisting of ferric chloride and 2,4,6-tripyridyl-s-triazine (TPTZ). The absorbance was measured after 4 min at 600 nm. The reducing capacity was calculated, using the absorbance difference between sample and blank and a further parallel Fe(II) standard solution. Results were expressed as micromoles of Fe^{2+} per gramme fresh weight (µmol Fe^{2+}/gFW). Measurements (in triplicate) were done in microplates, as described for total phenolics.

2.6. Oxygen radical absorbance capacity for hydrophilic compounds (ORAC–H) assay

The ORAC–H assay for oxygen radical-scavenging capacity was conducted according to Prior, Wu, and Schaich (2005) and Ou, Hampsch-Woodill, and Prior (2001). The samples (in triplicate) were mixed with a fluorescein (15 nM) solution and a solution of 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH, 360 mM), both in phosphate-buffered saline (PBS, 75 mM, pH 7.0). Both AAPH and PBS buffer were warmed to 37 °C prior to use. The fluorescence was recorded until it reached zero (excitation wavelength 495 nm, emission wavelength 515 nm) in a Varian Cary Eclipse Fluorescence Spectrophotometer (Varian Australia Pty. Ltd.) equipped with an automatic thermostatic autocell holder at 37 °C. A calibration curve was constructed, daily, by plotting the calculated differences of area under the fluorescein decay curve between the blank and the sample for a series of standards of trolox solutions in the range of 6.25–75 µg/l. The results were expressed as µmol trolox equivalents per 100 g fresh weight (µmol T E/gFW).

2.7. Analysis of phenolic compounds by high performance liquid chromatography–diode array detector (HPLC–DAD) and liquid chromatography–photo–diode array–mass spectrometry (LC–PDA–MS/MS)

2.7.1. HPLC–DAD analysis

Quantification of phenolic compounds in extracts was carried out using a high performance liquid chromatography system that consisted of two LC-10AD pumps, SPD-M10A diode array detector (DAD), CTO-10AS column oven, DGU-12A degasser, SIL-10AD auto-injector and SCL-10A system controller (Shimadzu Co., Kyoto, Japan), equipped with a 250 × 4.6 mm i.d., 5 µm particle size Luna C18(2) column (Phenomenex, NSW, Australia). The following solvents in water with a flow rate of 1.0 ml/min were used: A, 0.5% trifluoroacetic acid (TFA) in water and B, 95% acetonitrile and 0.5% TFA in water. The elution profile was a linear gradient elution for B of 10% over 10 min, followed by an increase to 50% over 45 min, and then to 80% over 15 min. The column was washed with 100% solvent B for 10 min. Analytical HPLC was run at 25 °C and monitored at 280 (hydroxybenzoic acids and flavanols), 326 (hydroxycinnamic acids, stilbenes), 370 (flavonols) and 520 nm (anthocyanins). Hydroxybenzoic acids and flavanols were quantified as gallic acid equivalents (µmol GA E/gFW), cinnamic acids were quantified as chlorogenic acid equivalents (µmol CHA E/gFW), flavonols and stilbenes were quantified as rutin equivalents (µmol R E/gFW) and anthocyanin compounds were quantified as cyanidin 3-glucoside equivalents (µmol C3G E/gFW).

2.7.2. LC–PDA–MS/MS analysis

LC–PDA–MS/MS analysis was carried out on a Quantum triple stage quadrupole (TSQ) mass spectrometer (ThermoFinnigan, NSW, Australia) equipped with a quaternary solvent delivery system, a column oven, a photo-diode array (PDA) detector and an autosampler. An aliquot (20 µl) from each extract was chromatographed on a Luna C_{18}(2) analytical column (150 mm × 2.1 mm, 5 µm particle size), (Phenomenex, NSW, Australia), which was heated to 30 °C in an oven. The mobile phase consisted of 0.5% formic acid in water (A) and 0.5% formic acid in acetonitrile (B) at the rate of 220 µl/min. A linear gradient was used (0% B to 100% B over 40 min). Ions were generated using an electrospray source in the positive or negative mode, under conditions set following optimisation, using solutions of cyanidin-3-glucoside, chlorogenic acid and rutin. The PDA was monitoring signals at 520, 370, 320 and 280 nm. MS experiments, in the full scan (precursor and product-specific) and the selected reaction monitoring (SRM) modes, were carried out.

2.8. Extraction and analysis of vitamin C and organic acids

Organic acids were extracted from powdered samples and stabilised using 4.5% meta-phosphoric acid according to Vazquez-Oderiz, Vazquez-Blanco, Lopez-Hernandez, Simal-Lozano, and Romero-Rodriguez (1994). A freeze-dried powder (50 mg) of each sample was mixed with 1500 µl of 4.5% meta-phosphoric acid, vortexed and sonicated for 5 min to enhance the extraction process. Subsequently, the samples were centrifuged (5 min, 12000 rpm), the supernatants were collected and the extraction was repeated twice. The supernatants were pooled (4.5 ml). The extracts were prepared and analysed in triplicate. Representative samples (10 µl) were injected into HPLC (equipment details as above). The compounds were separated, under isocratic conditions using water acidified with sulphuric acid to pH 2.2, following the method of Vazquez-Oderiz et al. (1994). Detection was carried out at 215 and 245 nm at a flow rate of 1.0 ml/min. Vitamin C was identified by comparing the retention time (at 215 nm) and characteristic UV–VIS spectra with those of synthetic L-ascorbic acid (Sigma, Sydney, Australia). The results were quantified using an L-ascorbic acid calibration curve and calculated as milligrammes of vitamin C per gramme fresh weight (mg/gFW). Similarly, standards of citric acid, L-malic acid and oxalic acid (Sigma, Sydney, Australia) were monitored at 245 nm and their chromatograms were used to construct the relevant calibration curves and to quantify the levels of organic acids.

3. Results and discussion

3.1. Folin–Ciocalteu assay and HPLC quantification of phenolic compounds

Among the commercially produced native Australian fruits evaluated in this study, Kakadu plum and quandong differed significantly from all other sources and the blueberry reference in respect to the level of TP. The level for Kakadu plum reached 160 ± 1.2 µmol GAE/gFW (or 114 ± 8.8 µmol GAE/gFW when the weight of a stone was included; Table 1). The second highest level of 50.4 ± 0.4 µmol GAE/gFW was displayed by quandong. The F–C assay, based on the reaction of phenolic compounds with a colori-

Table 1
Total phenolic content and antioxidant capacity of selected commercially grown native Australian fruits. The data are means ± SD of 3 determinations.

Fruit	DW/FW ratio	Total phenolics (FC) (µmol GA E/gFW)	HPLC quantification[*] of phenolic compounds				FRAP (µmol Fe+2/gFW)	ORAC-H (µmol TEq/gFW)
			280 nm (µmol GA E/gFW)	326 nm (µmol CHA E/gFW)	370 nm (µmol R E/gFW)	520 nm (µmol C3G E/gFW)		
Australian desert lime[**]	0.1957	10.8 ± 0.4	10.8 ± 1.6	1.32 ± 0.1	–	–	34.8 ± 2.3	44.9 ± 5.1
Kakadu plum[**]	0.1219	114 ± 8.8	69.8 ± 2.10	–	–	–	691 ± 48.4[***]	315 ± 33.7[***]
	0.1712[***]	160 ± 1.2[***]	98.0 ± 3.00[***]					
Lemon aspen	0.1549	9.5 ± 0.3	17.6 ± 0.70	–	–	–	14.0 ± 2.4	132 ± 11.4
Davidson's plum	0.0714	14.1 ± 1.0	13.9 ± 0.20	–	T	0.76 ± 0.01	53.9 ± 4.0[***]	83.1 ± 10.9[***]
	0.0804[***]	15.9 ± 1.3[***]	15.4 ± 0.30[***]			0.85 ± 0.02[***]		
Finger lime (green)[**]	0.2090	6.8 ± 0.4	8.7 ± 0.6	1.4 ± 0.03	–	–	12.6 ± 0.5	45.9 ± 6.6
Finger lime (pink)[**]	0.1880	9.2 ± 0.5	10.6 ± 2.7	0.45 ± 0.01	–	0.06 ± 0.01	23.2 ± 0.8	65.1 ± 12.8
Riberry	0.0881	7.5 ± 0.7	11.9 ± 0.41	–	0.17 ± 0.01	0.69 ± 0.05	33.2 ± 1.9	49.9 ± 6.4
Quandong	0.2331	50.4 ± 0.4	–	30.1 ± 0.91	1.15 ± 0.01	0.37 ± 0.01	123 ± 0.6	501 ± 64.1
Blueberry	0.1332	26.5 ± 0.6	–	3.9 ± 0.05	–	11.5 ± 0.30	52.4 ± 2.78	76.9 ± 13.3

[*] HPLC quantification of phenolic compounds was calculated as: µmol gallic acid E/gFW for compounds detected at 280 nm, µmol chlorogenic acid E/gFW for compounds detected at 326 nm, µmol rutin E/gFW for compounds detected at 370 nm and µmol cyanidin 3-glucoside E/gFW for anthocyanins (520 nm).

[**] Results of total phenolic compounds (F–C) corrected for vitamin C.

[***] Stone weight excluded; FRAP: ferric reducing antioxidant power; ORAC-H: oxygen radical absorbance capacity – hydrophilic compounds; µmol TEq/gFW: micromole trolox equivalent/g fresh weight.

metric reagent, represents a fast screening method for measurement of antioxidant capacity of food products and dietary supplements (Prior et al., 2005) and is frequently used, which allows comparison of research data across a number of studies. We have compared the TP values of native Australian fruits with that of a blueberry (*Vaccinum* sp.), which is among fruits with the highest antioxidant capacity due to its high TP content (Wang, Cao, & Prior, 1996). The TP level of Kakadu plum exceeded (by 6-fold), and that of quandong exceeded (by 1.9-fold) the TP level of the blueberry control (Table 1). These two Australian native fruits had higher levels of phenolic compounds per gramme of fresh weight than had most common fruits, such as cranberries (42.2 µmol GAE/gFW), blackberries (38.8 µmol GAE/gFW), apple (12.3–20.4 µmol GAE/gFW) and orange (19.8 µmol GAE/gFW) (USDA database on Oxygen Radical Absorbance Capacity (ORAC) of selected foods, 2007; data were recalculated from mg GAE/gFW to µmol/gFW). The TP levels of all other commercially grown Australian fruits evaluated in this study were lower than the TP of blueberry.

At present quandong is sold in Australia predominantly as a dry fruit. A commercial sample of dry fruit was compared with a sample of frozen quandong. The TP of dry quandong was 193 ± 17.0 µmol GAE/gDW, and was 10.4% lower than the TP of the frozen quandong (216 ± 15.5 µmol GAE/gDW).

Australian desert lime contained 10.8 ± 0.4 µmol GAE/gFW of TP and finger limes contained from 6.8 ± 0.4 to 9.2 ± 0.5 µmol GAE/gFW (Table 1). Finger limes evaluated in this study exhibited similar levels of TP to red (8.6 µmol GAE/gFW) and yellow (10.9 µmol GAE/gFW) forms evaluated earlier (Netzel, Netzel, Tian, Schwartz, & Konczak, 2007). However commercially cultivated riberry, analysed in the present study, had lower TP than had riberry fruits collected from the wild (13.1 µmol GAE/gFW) (Netzel et al., 2007).

The F–C assay is commonly used to estimate the level of phenolic compounds in food products. However, the assay is not specific to phenolic compounds and other redox-active molecules interfere in the measurement in an inhibitory, additive or enhancing manner (Prior et al., 2005; Singleton & Rossi, 1965). Among these molecules are reducing sugars and vitamin C, pectins and organic acids, present in fruits. Moreover, some phenolic compounds (e.g. salicylic acid, present in citrus) are less responsive in this assay than are others (Singleton & Rossi, 1965). Therefore the levels of phenolic compounds in the fruits were evaluated by reversed-phase high performance liquid chromatography.

Overall, the level of phenolic compounds in fruits, as evaluated by HPLC, varied from the data obtained in the F–C assay. For the

blueberry reference, the total level of phenolic compounds, as evaluated by HPLC, was lower than the F–C values (Table 1). Zheng and Wang (2003) have analysed blueberry (cv. Sierra) and found that the TP level, as evaluated in the F–C assay, was 4.12 mg/gFW. Subsequently, they have quantified the level of individual compounds in the blueberry extract by HPLC and found that the sample contained 0.65 mg/gFW of chlorogenic acid and 1.56 mg/gFW of anthocyanins. These values were approximately 50% lower than values obtained in the F–C assay, which is in agreement with our results.

Considerable variation was found in phenolic compounds in various fruits. Kakadu plum extract exclusively contained compounds detected at 280 nm (possibly hydroxybenzoic acids, flavanols, flavanones), at a level of 69.8 ± 2.1 µmol GAE/gFW (or 98.0 ± 3.0 µmol GAE/gFW when the weight of stone was omitted) (Table 1). These values represent approximately 2/3 of the TP values (F–C assay) discussed above. Singleton and Rossi (1965) reported that sugars, beside ascorbic acid, aromatic amines and unanticipated phenols, are responsible for the additive effect in the F–C assay. Kahkonen, Hopia, and Heinonen (2001) reported that, removing sugars from berry extract with the help of SPE treatment (Bond Elut C_{18} SPE), resulted in reduction of TP (F–C assay). Similarly, in the present study, sugars in Kakadu plum extract could contribute to the high TP value. Additionally, Kakadu plum has an exceptionally high level of vitamin C (see Section 3.3. *Vitamin C and organic acids*). Although the TP values were corrected for ascorbic acid, the additive effect of vitamin C, due to the exceptionally high level in the fruit, may not be completely eliminated. Moreover, the HPLC chromatogram of Kakadu plum extract is very complex. It is highly possible, that, besides phenolics, sugars and vitamin C, a number of other unknown compounds are present and they interfere in the F–C assay. Similarly to Kakadu plum, the level of phenolic compounds in quandong, detected by HPLC, was lower than that obtained in the F–C assay (Table 1). Quandong, which exhibited the second highest TP value, contained phenolic compounds of a different nature than Kakadu plum. These were mainly hydroxycinnamic acids (30.1 ± 0.9 µmol CHAE/gFW) and anthocyanins (0.37 ± 0.01 µmol C3GE/gFW). The HPLC quantification of phenolic compounds in lemon aspen indicates a higher level of phenolics than the F–C assay. A similar, though less pronounced, effect was visible for other fruits (riberry, finger lime and desert lime). The presence of compounds interfering in the reagent-based assays and inhibiting the reaction is highly possible; however, further studies are needed to clarify it. Davidson's plum, riberry and Australian citrus fruit extracts contained predominantly com-

pounds detected at 280 nm, which suggests the presence of hydroxybenzoic acids, flavonols, flavanones or proanthocyanidins. Anthocyanins were identified in Davidson's plum, pink finger lime, riberry and quandong (Table 1).

3.2. Antioxidant capacity: FRAP and ORAC assays

Kakadu plum extract exhibited superior total reducing capacity (TRC, FRAP assay), which was 13-fold that of blueberry (Table 1). This effect could be due to the presence of compounds, which in a similar manner contributed towards enhanced F–C values, as both assays are based on the same single electron transfer mechanism. Among these compounds, could be sugars and vitamin C; however, the presence of other redox-active compounds is also expected. The oxygen radical-scavenging capacity (ORAC–H assay) of Kakadu plum was 4.1-fold that of the blueberry reference and lower than that of quandong. According to Zheng and Wang (2003), sugars exhibit no antioxidant activity in ORAC assay. This may partly explain the relatively lower ORAC values of Kakadu plum. This study represents the first attempt to understand the phytochemistry of Kakadu plum and further research may lead towards identification of other groups of compounds, which could contribute to the antioxidant capacity of this intriguing fruit.

Quandong displayed an approximately 2.4-times higher TRC than blueberry and exhibited the highest ORAC value (6.5-fold that of the blueberry reference) (Table 1). Antioxidant activity of a phenolic mixture depends on the nature of the phenolic compounds and anthocyanins and hydroxycinnamic acids possess significantly higher antioxidant potency in ORAC assay than do hydroxybenzoic acids (Zheng & Wang, 2003). The HPLC data (Table 1) indicate that quandong extract is rich in hydroxycinnamic acids, flavonols and anthocyanins, which could be primary responsible for the high ORAC values.

Lemon aspen was superior to blueberry in the ORAC assay; however, it exhibited a low TRC. It also exhibited a lower TP than did blueberry in the F–C assay. Presence, in this fruit, of compounds which could interfere in both assays is possible.

Total phenolic content and TRC of Davidson's plum evaluated in this study were similar to those of Davidson's plum, reported previously (Netzel et al., 2007). Among the compounds contributing to the antioxidant capacity of this fruit are anthocyanins and, possibly, hydroxybenzoic acids, flavonols, flavanones or proanthocyanidins which can be detected by HPLC at 280 nm (Table 1).

Australian citrus fruits, evaluated in this study, exhibited lower antioxidant capacity than did blueberry in both antioxidant testing assays. Previously evaluated red finger lime exhibited 3-fold lower antioxidant activity than did blueberry in the trolox equivalent antioxidant capacity (TEAC) assay which (like FRAP) is based on a single electron transfer mechanism (Netzel et al., 2007). The TRC of the same fruit was $24.6 \pm 0.3 \, \mu mol \, Fe^{+2}/gFW$ (unpublished results), which is very similar to the TRC of pink finger lime obtained in this study. The antioxidant capacities of green finger lime and Australian desert lime were within the same range. The commercially produced riberry sample, evaluated in this study, exhibited a lower antioxidant potential (TRC and ORAC values) than did blueberry, and lower TRC than a riberry sample collected from the wild (TRC = 44.8 ± 1.0, unpublished results).

3.3. Identification of major phenolic compounds

The native Australian fruits of primary importance to the Australian Native Food Industry are Kakadu plum, quandong, riberry and Davidson's plum. These fruits were selected for further studies, including identification of the major phenolic compounds. The compounds were separated and tentatively identified by using reversed-phase HPLC–DAD and LC–PDA–MS/MS (Table 2). The major

groups of phenolic compounds detected were: phenolic acids (benzoic and cinnamic) and flavonoids (flavonols, flavanones and anthocyanins).

Quandong was a fruit that exhibited a very high antioxidant capacity (Table 1). The LC/MS analysis revealed the presence of cyanidin 3-glucoside as the major anthocyanin, a minor amount of pelargonidin 3-glucoside and trace levels of cyanidin 3-rutinoside (Table 2). The total level of anthocyanin in quandong was $0.37 \pm 0.01 \, \mu mol$ C3G E/gFW (Table 1) (or $1.57 \pm 0.06 \, \mu mol$ C3G E/g DW). In the commercial dry quandong sample, the level of anthocyanin was reduced to $0.12 \pm 0.02 \, \mu mol$ C3G E/g DW, suggesting significant degradation of anthocyanin during the drying process. Other components identified in the fresh extract of quandong included quercetin and keampferol rutinosides, as well as chlorogenic acid (Table 2). Several notable peaks at 280 nm, possibly due to benzoic acids, were also observed in the chromatographic trace.

Anthocyanins were the major phenolic compounds detected in Davidson's plum. The main anthocyanins were 3-sambubiosides of delphinidin, cyanidin, petunidin and peonidin. These results confirmed our earlier findings (Netzel et al., 2007). Additionally, two new anthocyanins were detected: 3-sambubiosides of pelargonidin and malvidin. Delphinidin 3-sambubioside contributed 47.5% of the anthocyanin mixture and was followed by petunidin (23.7%), peonidin (14.7%) and cyanidin (4.4%). The levels of pelargonidin and malvidin 3-sambubiosides were below 3%. Other components found in small amounts included myricetin, rutin and quercetin hexoside (Table 2). The level of anthocyanins in this commercial sample of Davidson's plum was $0.85 \pm 0.02 \, \mu mol$ C3G E/gFW (Table 1) and it was 33% lower than the level of anthocyanins in Davidson's plum sample collected from the wild (Netzel et al., 2007).

Anthocyanins were the major compounds of riberry extract. The mixture consisted of cyanidin 3-galactoside (81.6%), cyanidin 3,5-diglucoside (6.5%) and cyanidin 3-glucoside (11.9%) (Table 2). The level of anthocyanins in this commercial sample of riberry was $0.69 \pm 0.05 \, \mu mol$ C3G E/gFW (Table 1) and was approximately 4-times lower than the level of anthocyanins in riberry collected from the wild (Netzel et al., 2007). The sample also contained notable amounts of other glycosides, such as quercetin and kaempferol rutinosides, myricetin and quercetin hexosides and quercetin rhamnoside (Fig. 1).

The reverse-phase HPLC trace obtained form the Kakadu plum extract was very complex. Positive ionisation ESI–LC/MS suggested that quercetin/hesperitin-based glucosides and keampferol/luteolin-based glycosides were part of the extract but could not be quantified due to the complexity of the mixture. Other interesting components present were those producing a m/z of 291 during a product ion scan with a precursor m/z of 451. Catechin exhibits a m/z of 291 in positive ionisation mode so these components could potentially be catechin-based components.

3.4. Vitamin C and organic acids

A high level of vitamin C was detected in Kakadu plum (12.4 ± 0.1 mg/gFW, Table 3). This result is in agreement with previously reported levels of vitamin C in Kakadu plum samples collected from the wild (Netzel et al., 2007). The level of vitamin C in Kakadu plum is slightly higher than that reported for acerola (*Malpighia emarginata* DC), a wild plant from Central America known as one of the richest and most important food sources of vitamin C. A ripe acerola fruit contains from 9.44 to 10.4 mg vitamin C per gramme fresh weight (Mezadri, Villano, Fernandez-Pachon, Garcia-Parrilla, & Troncoso, 2008), which is 76.1–83.9% of the vitamin C level in Kakadu plum. The level of vitamin C in Kakadu plum is approximately 19-fold that in strawberry (0.650 mg/

Table 2
Phenolic compounds identified in selected native Australian fruits.

Compound	MS/MS		Quandong	Riberry	Davidson's plum
	$[M + 1]^+/[M - 1]^-$	Fragments (*m/z*) (+/−)			
Cyanidin 3-galactoside	449/–	287/–	ND	0.25 ± 0.02[*]	ND
Cyanidin 3-glucoside	449/–	287/–	0.13 ± 0.005	0.04 ± 0.006	ND
Cyanidin 3-rutinoside	595/–	449, 287/–	T	ND	ND
Cyanidin 3,5-diglucoside	611/–	449, 287/–	ND	0.02 ± 0.001	ND
Pelargonidin 3-glucoside	433/–	271/–	0.03 ± 0.001	ND	ND
Delphinidin 3-sambubioside	597/–	303/–	ND	ND	0.16 ± 0.04
Cyanidin 3-sambubioside	581/–	287/–	ND	ND	0.02 ± 0.001
Pelargonidin 3-sambubioside	565/–	271/–	ND	ND	T
Peonidin 3-sambubioside	596/–	301/–	ND	ND	0.05 ± 0.01
Petunidin 3-sambubioside	611/–	317/–	ND	ND	0.08 ± 0.01
Malvidin 3-sambubioside	626/–	331/–	ND	ND	T
Chlorogenic acid	–/353	–/191	T	ND	ND
Myricetin	–/317	–/151	ND	ND	T
Myricetin hexoside	481/479	153/151	ND	T	ND
Quercetin rutinoside (Rutin)	610/609	303/301	0.53 ± 0.01	0.06 ± 0.01	T
Quercetin/hesperitin glucoside	465/–	303/–	ND	ND	ND
Quercetin hexoside	465/–	303/–	ND	0.05 ± 0.001	T
Quercetin rhamnoside	450/–	303/–	ND	0.03 ± 0.001	ND
Kaempferol	287/–		0.61 ± 0.01	0.03 ± 0.01	ND
Kaempferol/luteolin glucoside	449/447	287/285	ND	ND	ND
Kaempferol/luteolin rutinoside	595/–	287/–	T	T	ND

[*] Data are means ± SE of 3 independent determinations and are presented as mg/gFW; ND: not detected; T: trace (below 3%).
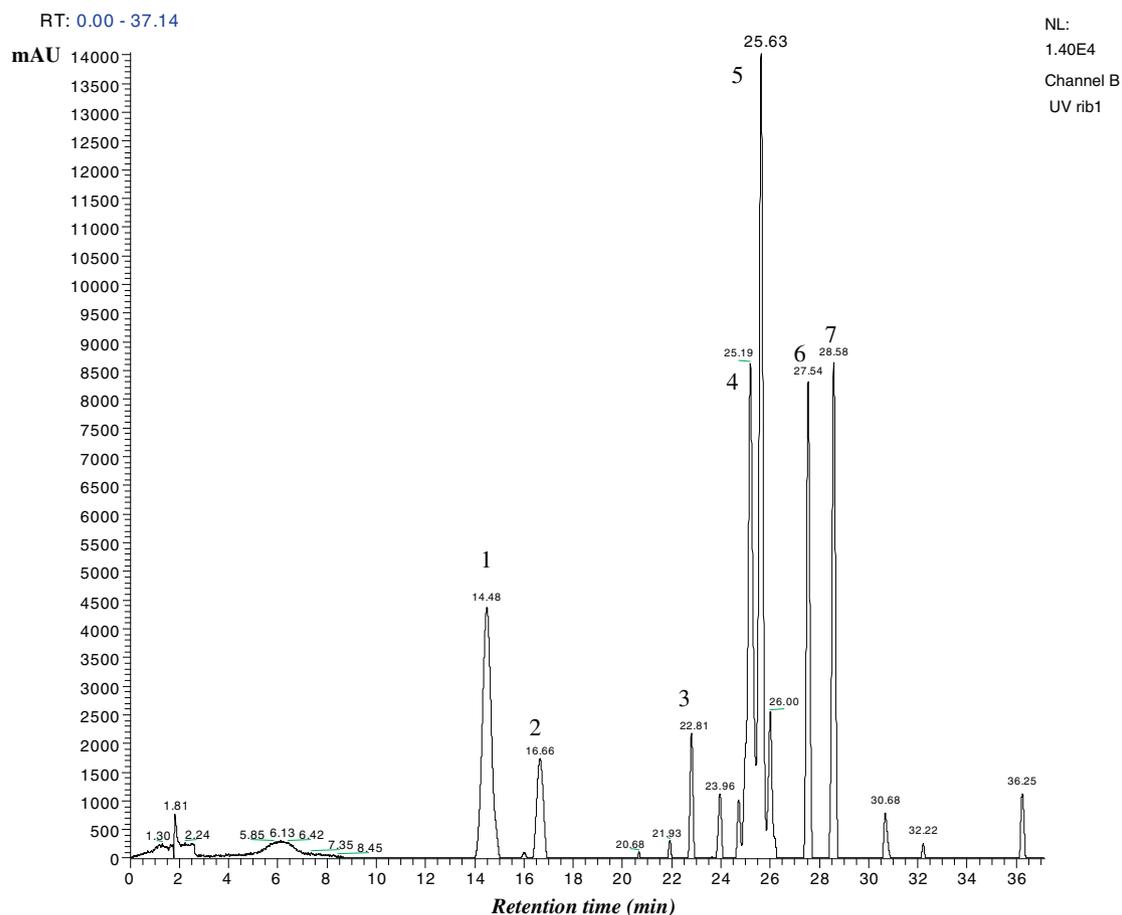
Fig. 1. HPLC profile from the riberry extract at 370 nm; (1) cyanidin 3-galactoside, (2) cyanidin 3-glucoside, (3) myricetin hexoside, (4) quercetin rutinoside, (5) quercetin hexoside (6) kaempferol/luteolin rutinoside, (7) quercetin rhamnoside.

gFW; Agar, Streif, & Bangerth, 1997) and in kiwifruit (0.649 mg/gFW; Agar, Massantini, Hess-Pierce, & Kader, 1999).

Vitamin C was also present in commercial samples of Australian citrus fruits. Among them, Australian desert lime contained 1.88 ± 0.05 mg/gFW, which is approximately 2-fold the level of vitamin C in Californian orange (0.83 mg/gFW; Vanderslice, Higgs, Hayes, & Block, 1990) and 5-fold the level of vitamin C in fresh mandarins (0.38 mg/gFW; Mitchell, McLauchlan, Isaacs, & Wil-

Table 3
Ascorbic acid and organic acids in native Australian fruits.

Fruit	Ascorbic acid	Citric acid	Malic acid	Oxalic acid
Australian desert lime	1.88 ± 0.05[*]	4.61 ± 0.19	25.2 ± 0.48	1.04 ± 0.10
Kakadu plum	8.84 ± 0.08 12.4 ± 0.12[**]	ND	ND	0.74 ± 0.05 1.03 ± 0.08[**]
Lemon aspen	ND	32.9 ± 1.60	2.32.33 ± 0.13	0.19 ± 0.01
Davidson's plum	ND	ND	30.9 ± 2.12 34.9 ± 2.39[**]	0.09 ± 0.01 0.10 ± 0.01[**]
Green finger lime	0.26 ± 0.01	46.8 ± 0.47	ND	0.08 ± 0.02
Pink finger lime	0.91 ± 0.02	58.8 ± 1.70	ND	0.20 ± 0.03
Riberry	ND	ND	17.2 ± 0.34	0.23 ± 0.01
Quandong	ND	ND	19.1 ± 1.33	0.40 ± 0.01

[*] Data are means ± SE of 3 independent determinations and are presented as mg/gFW.
[**] Weight of stone excluded; ND: not detected.

liams, 1992). Among the finger limes, the pink variety contained more vitamin C (3-fold the level in mandarins) than the green variety (32% less than mandarins).

Citric acid is commonly present in fruits and it is the main organic acid of kiwi fruit (9.85 mg/gFW), banana (3.59 mg/gFW) and strawberry (3.12 mg/gFW) (Perez, Olias, Espada, Olias, & Sanz, 1997). Citric acid was present in all fruits representing the *Rutaceae* family, evaluated in this study, in the following order: pink finger lime > green finger lime > lemon aspen > Australian desert lime. It predominated in finger limes and lemon aspen (with the level ranging from 58.8 ± 1.7 (pink finger lime) to 32.9 ± 1.6 mg/gFW (lemon aspen) but not in the Australian desert lime which contained predominantly malic acid (25.2 ± 0.5 mg/gFW). Similarly to the Australian desert lime, malic acid dominated in sweet lime (Clements, 1963). Malic acid has also been identified in Davidson's plum, riberry and fresh quandong (Table 3). Malic acid is the main organic acid of apple (4.12 mg/gFW); it was detected in kiwi fruit (1.9 mg/gFW), banana (2.89 mg/gFW), peach (2.82 mg/gFW) and strawberry (1.11 mg/gFW) (Perez et al., 1997). Oxalic acid was identified in the fruits at very low levels. In the case of citrus fruits, peels could be the main source of this acid (Clements, 1963).

4. Conclusions

Two commercially grown native Australian fruits, quandong and Kakadu plum, exhibited enhanced antioxidant capacity in comparison to blueberry, as evaluated in two reagent-based assays, FRAP and ORAC–H. The main sources of antioxidant capacity of quandong were cinnamic acids and anthocyanins. Phenolic compounds (benzoic acids/flavanols/flavanones) and the exceptionally high level of vitamin C contributed towards the antioxidant capacity of Kakadu plum. Australian citrus fruits, finger limes and Australian desert limes, were found to be inferior to blueberry in regard to antioxidant capacity; however, they are a good source of vitamin C. Citric acid was the main organic acid detected in finger lime and lemon aspen, while malic acid predominated in Australian desert lime, Davidson's plum, riberry and quandong.

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