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Phytochemical divergence in 45 accessions of *Terminalia ferdinandiana* (Kakadu plum)

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ABSTRACT

This study investigated the variations in the levels of phenolic compounds, vitamin C, sugars and antioxidant capacities of 45 newly collected accessions of *Terminalia ferdinandiana* (Kakadu plum), a native Australian fruit utilised in dietary supplement industry. Pattern recognition tools, principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were applied to understand interrelationships between the antioxidant capacities [Ferric reducing antioxidant power (FRAP) and Oxygen radical absorbance capacity (ORAC)] and antioxidant groups: phenolic compounds and vitamin C. On the basis of these parameters AHC classified samples into three main groups, with accessions 2, 8, 15, 6, 3 and 5 from the Northern Territory, Australia, representing superior quality fruits combining high levels of total phenolics (505.2 to 376.1 mg GA E/g DW), vitamin C (322.2 to 173.5 mg/g DW), with pronounced antioxidant capacities (FRAP: 5030.5 to 4244.9 μmol Fe^{2+}/g DW; ORAC: 3861.5 to 2985.6 μmol Trolox E/g DW). Hydrolysable tannins and ellagic acid were identified as the major phenolic compounds. The levels of ellagic acid varied from 140.2 to 30.5 mg/g DW, which places Kakadu plum as a unique edible source of this compound. The levels of sugars varied from 619.0 to 130.0 mg Glu E/g DW. This study for the first time revealed a unique phytochemical profile and significant variability in phytochemical composition of Kakadu plum. These features create opportunities for selection of sources with different characteristics addressing the needs of the nutraceutical industry, food processors and the consumers of fresh fruit.

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1. Introduction

Terminalia ferdinandiana Exell (Combretaceae), also known as the Kakadu plum, gubinge, billygoat plum or murunga, is a small semi-deciduous tree with broad light green leaves endemic to northern Australia. The ovoid, yellowish–green fruit is approximately 2.5 cm long and 1 cm in diameter, with a succulent layer surrounding a hard stone (Cribb & Cribb, 1987). Kakadu plum was traditionally a part of the diet of the Aboriginal people. In Western Australia the plum was pounded and soaked in water to create a refreshing drink. It was particularly popular with children and although not a staple food, the fruit was also consumed by adults on hunting trips for quick energy and refreshment (Brock, 2005). Tribes in central Arnhem land (Northern Territory) regarded the fruit more as a medicine than a food (Isaacs, 1987).

Kakadu plum is among the richest known sources of Vitamin C (Brand et al., 1982; Konczak, Zabaras, Dunstan, & Aguas, 2010), although a high degree of variability exists, possibly due to factors such as genetic diversity, climate and soil condition, ripening stage and storage conditions. The fruit is a rich source of phenolic compounds, responsible for its high antioxidant capacity (Konczak et al., 2010). Recent studies towards evaluation of potential physiological activities of Kakadu plum, as identified in an array of cell culture based assays, revealed pronounced anti-inflammatory and chemopreventative properties of phenolic-rich fruit extract (Tan, Konczak, Ramzan, & Sze, 2011). These findings support the traditional use of Kakadu plum as a medicine. The present applications of Kakadu plum include food supplements (beverages, capsules and powders), skin and care products, pharmacological products, and in gourmet bush foods (chutneys, jams and pickles).

Despite an increasing utilisation of Kakadu plum, to date information on the phytochemical composition is limited. Earlier studies on the nutritional quality of Kakadu plum are based on evaluation of a limited number of selected sample/s and the extent of variability of Kakadu plum with regards to phytochemical composition is unknown, although these qualities are of primary

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importance when selection of plant sources for commercialisation is considered.

T. ferdinandiana trees are growing across large areas of two states of Australia: Western Australia (WA) and Northern Territory (NT), under different environmental conditions, which may affect the accumulation of secondary metabolites in fruit (Pirie et al., 2013). To facilitate the development of Kakadu plum industry, systematic information on the phenotypic diversity, studies on yield, growth, morphology, phytochemical characteristics as well as germplasm preservation are required. Therefore, the objective of the present study was to assess a collection comprising 567 Kakadu plum fruits obtained from 45 growth sites across WA and NT with regards to phytochemical composition and antioxidant capacity, and to provide an estimation of the association of different characteristics.

2. Materials and methods

2.1. Plant material

A selection of forty-five samples of Kakadu plum fresh fruits was provided by Julian Gorman of Charles Darwin University, Darwin, Northern Territory (NT), Australia and Kim Courtenay of the Kimberley Training Institute, Broome, Western Australia (WA), Australia. Samples from the NT arrived fresh; samples from WA arrived frozen. On arrival, the weight of fruits was recorded and the flesh separated from stone using a knife. The fresh weight of the flesh was recorded before it was snap-frozen in liquid nitrogen and freeze-dried under vacuum (Benchtop 2K; Virtis, Gardiner, NY, USA) at −52 °C. Following freeze-drying, the samples were ground into a fine powder (Breville Barvista Model BCG300/A; Australia), placed in plastic vials, sealed with parafilm and stored at −20 °C until analysed.

2.2. Chemicals and reagents

Unless otherwise stated, all chemicals and standards were purchased from Sigma–Aldrich (Sydney, Australia) and were of analytical or HPLC grade. De-ionised water was used throughout.

2.3. Extraction of phenolic compounds

Two hundred milligrams of pulverized sample were placed in a test tube and extracted with 3 ml of acidified methanol (80% methanol, 19.9% H_2O and 0.1% HCl, v/v/v). The test tubes were vortexed and placed in a sonicator for 15 min. The extracts were centrifuged (10 min, 1000×*g*), supernatant collected and the pellet was re-extracted two more times with 3 ml of organic solvent. Aliquots (9 ml) of the combined supernatants was diluted to 10 ml with solvent and immediately utilised in assays. If required, the supernatants were stored at −20 °C.

2.4. Extraction and analysis of Vitamin C

Vitamin C was extracted from powdered samples and stabilised using 4.5% meta-phosphoric acid as previously described (Konczak et al., 2010).

2.5. Antioxidant testing

The antioxidant capacities of Kakadu plum fruits were evaluated in two complementary assays: Oxygen radical absorbance capacity (ORAC) and Ferric ion reducing antioxidant power (FRAP) carried out as previously described (Konczak et al., 2010).

2.6. Analysis of phenolic compounds

Reagent-based assays are a convenient tool to screen multiple samples for the presence of various groups of phytochemicals and in this study they were applied to evaluate the levels of phenolics (total phenolics, TP), flavonoids (total flavonoids, TF), hydroxycinnamic acids (total hydroxycinnamic acids, THCA) and condensed tannins, formed through the condensation of flavan-3-ols (catechins) also known as proanthocyanindins (total proanthocyanindins, TPro).

2.6.1. Total phenolics (Folin–Ciocalteu method)

The total phenolic content (TP) was determined using the Folin–Ciocalteau method as previously described (Konczak et al., 2010). The results are presented as gallic acid equivalents per g of dry weight (mg GAE/g DW) of lyophilised flesh based on a gallic acid standard curve, and standardised against a blank control.

2.6.2. Total 4-hydroxycinnamic acids and total flavonols

The total 4-hydroxycinnamic acid (THCA) and total flavonoids (TF) content were determined using a modified version of the Glories' method (Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999) with some modifications, as previously described (Dalar, Turker, & Konczak, 2012).

2.6.3. Total proanthocyanidin content

The total proanthocyanidin (TPro) content was determined using DMACA–HCl (4-dimetilamino cinnamaldehyde–hydrochloric acid) protocol as previously described (Dalar et al., 2012).

2.6.4. Analysis of phenolic compounds by high performance liquid chromatography–diode array detector (HPLC–DAD)

Analysis 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 μ Luna C18(2) column (Phenomenex, Sydney, 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 250 nm (ellagic acid), 280 nm (hydroxybenzoic acids and flavanols) and 326 nm (hydroxycinnamic acids), 370 nm (flavonols, stilbens) and 520 nm (anthocyanins). The major phenolic compounds were identified through co-chromatography with authentic standards. Phenolic compounds detected at 280 nm were quantified as gallic acid equivalents (GAE). The results are presented as milligram of gallic acid equivalent per gram dry weight (mg GAE/g DW) of lyophilized flesh. Ellagic acid was quantified based on ellagic acid calibration curve and expressed as milligram ellagic acid per gram dry weight (mg EA/g DW) of lyophilized flesh.

2.7. Analysis of sugars

The sugars were analysed in aqueous extracts according to Cardozo and collaborators (Cardozo, Ordonez, Alberto, Zampini, & Isla, 2011). Fifty milligrams of freeze-dried and pulverized fruit was extracted with 1 ml of purified water (Synergy UV, Millipore, Australia), vortexed, sonicated for 15 min and centrifuged (10 min, 10,000 rpm, Eppendorf centrifuge 5424, rotor FA-45-24-11, Germany) with supernatant collected. The pellets were

re-extracted another two times. Aliquots (3 ml) of the combined supernatants were stored frozen (−20 °C) until analysed.

Neutral sugars were determined using the phenol–sulphuric acid method. Diluted extracts (0.8 ml) were mixed with 0.04 ml of 80% phenol and 2 ml of H_2SO_4, incubated at 100 °C for 20 min and the absorbance was measured at 490 nm (Shimadzu 1601 spectrophotometer, Tokyo, Japan). Reducing sugars were measured using the Somogyi–Nelson method. Copper tartrate reagent (0.5 ml) was added to 0.1 ml aliquots of different extract dilutions. The solution was heated at 100 °C for 15 min and 0.5 mL of arsenomolybolic acid reagent was added. The absorbance was measured at 520 nm. The results were expressed as mg glucose equivalent/g dry weight (mg Glu E/g DW) of lyophilized flesh based on glucose callibration curve.

2.8. Data analysis

All data were analysed based on three independent experiments. Results were expressed as mean ± standard error (SE). One way ANOVA followed by the Bonferroni post hoc test were performed to assess differences between the accessions at the level of $p < 0.05$. The Pearson's correlation coefficients, principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed using the XLSTAT 2013 program (Addinsoft SARL, Paris, France) (Racotomalala, 2005).

3. Results and discussion

Forty-five accessions of Kakadu plum evaluated in this study were collected at the following growth sites of Australia: NT Darwin Site 1 (accession 1–6), NT Darwin CSIRO Site (accession 7–12), NT Darwin TRO Site (accession 13–18), NT Darwin Site 3 (accession 19–22), NT WADEYE-Site 2-Fossil Head (accession 23–28), NT Coppermine track (accession 29–34) and WA (accession 34–45). On arrival the fruits were light-green–green, ovoid–oval shape, smooth or with pronounced protruding edges. The average fresh weight of one fruit (including stone) varied from 1.5 ± 0.4 (NT, Coppermine track) to 3.4 ± 0.9 g (NT, Darwin Site 1) (Table 1). The weight of edible flesh represented 73.4% of the fresh weight for the NT fruits and 81.5% for the WA fruits (data not presented).

3.1. Vitamin C

Fig. 1A presents the content of vitamin C in the evaluated accessions. Vitamin C levels ranged from 0.5 ± 0.1 mg/g DW of lyophilised flesh (0.5% dry weight; accession 24), to 322.2 ± 2.1 mg/g DW (32% dry weight; accession 15) with an average value of 151.9 ± 92.6 mg/g DW (15.2% dry weight). With regards to the fresh weight (FW), these values were, respectively, 0.1, 53.0 and 27.4 mg/g FW of edible flesh. The highest average vitamin C levels had fruits collected from the NT Darwin Site 1, NT Darwin Site 3, NT CSIRO Site and NT TRO Site (Table 1). Exceptionally low levels of vitamin C were found in fruits collected at the NT Coppermine track. The WA originated fruits were more uniform with respect to vitamin C content. The average level of vitamin C in accessions from the NT was 154.7 ± 92.5 mg/g DW and in those from WA 159.0 ± 46.6 mg/g DW. These levels correspond to approximately 25 mg/g FW of edible flesh and are superior to vitamin C level of acerola (*Malpighia emarginata* DC), which contains 9.4–10.4 mg/g FW and was reported as the richest source of vitamin C (Mezadri, Villano, Fernandez-Pachon, Garcia-Parrilla, & Troncoso, 2008).

High variability in the vitamin C content was observed among individual accessions collected from each growth site (Fig. 1A). Large variations in vitamin C content among the evaluated accessions could be related to both, genetic differences and environmental conditions (water availability, soil quality, temperature amplitude, nutrient availability). Identifying these variation offers opportunities for selection and multiplication of plants bearing fruits with superior levels of vitamin C.

3.2. Total phenolic content, total flavonoids and total hydroxycinnamic acids

The main role of phenolic compounds in plants is protection of plant cells from oxidative deterioration and environmental stress (drought, high/low temperature, soil salinity, bacteria, fungi, insect and herbivores) (Veberic et al., 2005). The importance of quantification of phenolic compounds levels arises from earlier findings that these compounds, and especially flavonoids, hydroxycinnamic acids and proanthocyanidins, are the most common sources of antioxidant capacities of fruits and vegetables (Kähkönen et al., 1999), primarily responsible for their health properties (Moskaug, Carlsen, Myhrstad, & Blomhoff, 2005).

The average TP level in the evaluated Kakadu plum fruits was 271.9 ± 96.1 mg GAE/g DW of lyophilised flesh and ranged from 121.5 ± 5.4 mg GAE/g DW (equal to 25.6 mg GAE/g FW of flesh; accession 24) to 505.2 ± 55.6 mg GAE/g DW (equal to 82.7 mg GAE/g FW of flesh; accession 15) (Table 2). The TP of 34 commonly consumed fruits in US varies from 0.6 (watermelon) to 7.9 mg GAE/g FW (lowbush blueberry) (Wu et al., 2004). The lowest TP level of Kakadu plum (25.6 mg GAE/g FW) was 3.2-fold that of lowbush blueberry and was higher than TP content of all fruits evaluated by Wu and collaborators.

The TP level of commercially available Kakadu plum was 158.6 ± 12.3 mg GAE/g DW (equivalent to 27.1 ± 2.1 mg GAE/g FW) (Konczak et al., 2010), therefore a selection of accessions for commercial use with significantly higher levels of total phenolics is possible.

Accessions 1–18, from the NT Darwin Site 1, NT CSIRO Site and NT TRO Site, contained on average 374.4 mg GAE/g DW of total phenolics (Table 1), which was 1.4-fold the average TP level for the whole collection. Among these three sites, NT TRO Site (accession 13–18) fruits showed the greatest variability. The WA fruits (accession 34–45) had lower TP content than NT fruits, with the exception of accession 34, which contained 371.74 ± 7.7 mg GAE/g DW (Table 2).

The average TP levels of Kakadu plums were 25.3- to 14.7-times higher than those of apples, which contained from 10.7 to 18.5 mg GAE/g DW (Rossle, Wijngaard, Gormley, Butler, & Brunton, 2010). The TP of lyophilized acai berry (*Euterpe oleraceae* Mart.) was 13.9 mg GAE/g DW (Schauss et al., 2006), which is 19.6-times lower than that of Kakadu plum. The average TP level of Kakadu plum was 6.4- to 17.2-fold that of persimmon (15.7–42.3 mg GAE/g DW; Ercisli, Akbulut, Ozdemir, Sengul, & Orhan, 2008) and 4.9- to 7.6-fold that of a medicinal Mediterranean fruit hawthorn (*Crataegus monogyna*) (35.7–55.2 mg GAE/g DW; Caliş-kan et al., 2012). Lyophilized pericarp of longan (*Dimocarpus longan* Lour.), an exotic medicinal fruit of China, had 5.5-times lower TP (49.4 mg GAE/g DW) than Kakadu plum (Yang et al., 2008). These comparisons suggest that Kakadu plum is among the richest fruit sources of phenolic compounds.

In common fruits flavonoids and hydroxycinnamic acids dominate and serve as the sources of antioxidant capacities (Kähkönen et al., 1999). In contrary, only traces of TF, THCA and TPro were detected in Kakadu plum with the maximum levels, respectively, 2.1, 1.6 and 2.0 mg/g DW (Table 2). These results suggest that flavonoids, hydroxycinnamic acids and proanthocyanidins are not the major types of Kakadu plum phenolic compounds.

Table 1
Average levels of vitamin C, total phenolic compounds (Folin–Ciocalteu values) and phenolic compounds detected at 280 nm and quantified by the high performance liquid chromatography (HPLC), ellagic acid, total reducing capacity (FRAP), oxygen radical absorbance capacity (ORAC) and total sugars in 45 accessions of *Terminalia ferdinandiana* from Australia.

Region	Site	Vitamin C (mg/g DW*)	Total phenolics (F–C values) (mg GAE[1]/g DW)	Phenolics by HPLC, (280 nm; mg GAE/g DW)	Ellagic acid (mg/g DW)	FRAP (μmol Fe^{+2}/g DW)	ORAC-H (μmol TE[2]/g DW)	Sugars (mg GluE[3]/g DW)	Average weight♦ of one fruit (g)
NT	Darwin Site 1	224.9 ± 52.9a	382.52 ± 33.9a	84.6 ± 21.6bc	81.8 ± 17.6b	4197.5 ± 561.4a	3241.6 ± 340.4a	257.5 ± 46.5b	3.4 ± 0.9a
	CSIRO Site	197.0 ± 59.0a	375.13 ± 35.9a	87.5 ± 22.7bc	81.2 ± 18.5b	3802.8 ± 356.8ab	3131.5 ± 203.0a	244.8 ± 47.9b	3.3 ± 0.9a
	TRO Site	177.0 ± 96.5a	359.95 ± 108.5ab	66.4 ± 16.6bc	79.1 ± 16.6b	3491.3 ± 965.0ab	2667.8 ± 225.0a	282.1 ± 51.1b	3.4 ± 0.6a
	Darwin Site 3	179.3 ± 44.5a	246.34 ± 3.0ab	100.0 ± 29.0b	107.2 ± 28.2ab	4011.0 ± 264.0a	2598.9 ± 250.1a	241.5 ± 46.0b	2.1 ± 0.4ab
	Wadeye - Fossil head	121.0 ± 83.7ab	200.20 ± 54.7b	83.9 ± 19.5bc	83.4 ± 17.1b	3288.4 ± 497.5ab	2299.7 ± 310.7a	239.6 ± 60.2b	2.1 ± 0.5ab
	Coppermine track	13.6 ± 25.7b	215.40 ± 20.2ab	138.4 ± 29.1ab	94.2 ± 25.5ab	3099.5 ± 386.0ab	2431.5 ± 1029.9a	255.1 ± 88.7b	1.5 ± 0.4b
WA	Broome peninsula	159.1 ± 46.6a	209.15 ± 64.8b	40.6 ± 13.8c	41.8 ± 7.7b	2800.9 ± 674.9b	2239.0 ± 309.55a	394.0 ± 114.6a	2.9 ± 0.8ab

Means with different letters in the same column were significantly different at the level ($p < 0.05$).
* DW – dry weight of lyophilised flesh.
[1] GAE – gallic acid equivalent.
[2] TE – trolox equivalent.
[3] Glu E – glucose equivalent.
♦ Average fresh weight of one fruit including stone.

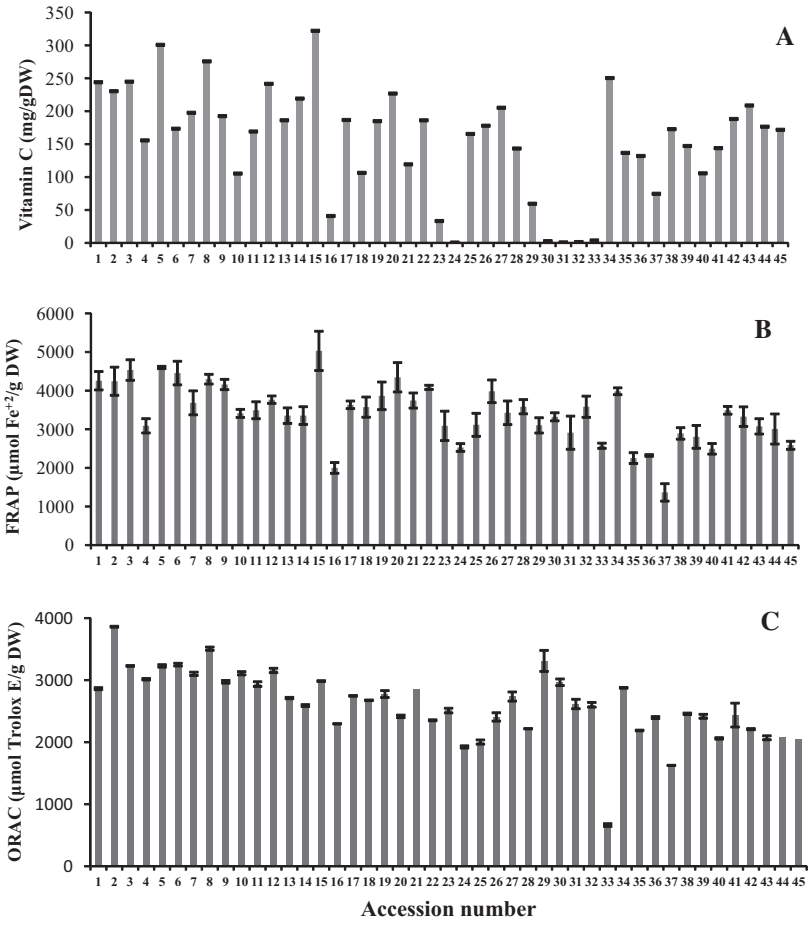

Fig. 1. Level of vitamin C (A), total reducing capacity (ferric reducing antioxidant power, FRAP; B) and oxygen radical absorbance capacity (ORAC, C) of 45 *Terminalia ferdinandiana* accessions collected across Australia. All data represent the mean (±SD) of $n = 3$ independent experiments.

3.3. Quantification of phenolic compounds by high performance liquid chromatography (HPLC-DAD)

A significant chemotaxonomic feature of the order of Myrtale is the presence of ellagic acid and ellagitannins (Bate-Smith, 1962). Plant families of this order especially rich in ellagitannins include Myrtaceae, Lythraceae, Onagraceae, Melastomataceae, and Combretaceae (Okuda, Yoshida, & Hatano, 1993). *T. ferdinandiana* represents the Combretaceae family. In agreement, the HPLC analysis identified two groups of phenolic compounds: the major group at

Table 2
Total phenolics (TP, Folin–Ciocalteu values), total flavonoids (TF), total hydroxycinnamic acid (THCA) total proanthocyanidins (TPro) and phenolic compounds detected by the high performance liquid chromatography in 45 accessions of native Australian fruit *Terminalia ferdinandiana*.

Number	TP (FC values) (mg GA[1] E/g DW)	TF (mg R[2]E/g DW)	THCA (mg CA[3]E/g DW)	TPro (mg Cat[4]E/g DW)	HPLC analysis		
					Phenolics (280 nm; mg GAE/g DW)	Gallic acid (mg/g DW)	Ellagic acid (mg/g DW)
1	382.01 ± 27.9	0.77 ± 0.01	0.65 ± 0.01	0.17 ± 0.003	72.1 ± 1.9	0.60 ± 0.01	76.7 ± 0.1
2	407.11 ± 35.9	1.06 ± 0.02	0.74 ± 0.01	0.04 ± 0.001	73.4 ± 7.6	0.69 ± 0.01	80.6 ± 11.7
3	400.08 ± 31.1	1.36 ± 0.02	1.02 ± 0.01	0.12 ± 0.008	100.4 ± 0.4	1.34 ± 0.01	104.0 ± 2.3
4	319.26 ± 26.1	0.94 ± 0.02	0.84 ± 0.01	0.06 ± 0.01	90.2 ± 2.3	0.67 ± 0.01	81.0 ± 2.5
5	410.53 ± 31.1	0.84 ± 0.02	0.58 ± 0.01	0.01 ± 0.001	55.9 ± 0.5	0.73 ± 0.01	52.9 ± 1.1
6	376.12 ± 41.5	1.40 ± 0.01	1.13 ± 0.01	0.31 ± 0.006	115.3 ± 0.6	1.23 ± 0.02	95.3 ± 2.1
7	344.55 ± 48.6	1.20 ± 0.01	0.75 ± 0.01	0.03 ± 0.01	62.7 ± 5.1	0.60 ± 0.03	60.9 ± 7.7
8	439.25 ± 26.9	1.20 ± 0.01	0.75 ± 0.01	0.06 ± 0.003	69.1 ± 1.0	0.60 ± 0.01	60.1 ± 2.7
9	368.89 ± 75.0	1.16 ± 0.01	0.96 ± 0.01	0.06 ± 0.003	96.6 ± 2.9	0.71 ± 0.01	83.4 ± 4.4
10	359.38 ± 26.7	1.21 ± 0.01	1.13 ± 0.01	0.06 ± 0.01	119.7 ± 0.8	1.32 ± 0.01	104.3 ± 3.4
11	346.64 ± 10.0	1.04 ± 0.01	0.95 ± 0.01	0.12 ± 0.01	104.4 ± 0.8	0.77 ± 0.02	99.1 ± 1.4
12	392.09 ± 11.3	0.81 ± 0.01	0.70 ± 0.01	0.03 ± 0.002	72.6 ± 0.4	0.66 ± 0.01	79.6 ± 0.1
13	347.59 ± 33.8	0.84 ± 0.01	0.69 ± 0.01	0.03 ± 0.001	53.4 ± 7.7	0.51 ± 0.07	63.9 ± 6.9
14	356.53 ± 24.1	0.53 ± 0.01	0.55 ± 0.01	0.02 ± 0,01	51.7 ± 5.9	0.55 ± 0.05	68.3 ± 8.1
15	505.23 ± 55.6	1.11 ± 0.01	0.77 ± 0.01	0.05 ± 0.005	69.4 ± 1.1	0.49 ± 0.01	66.9 ± 1.4
16	176.84 ± 10.7	1.14 ± 0.01	0.79 ± 0.01	0.02 ± 0.002	54.3 ± 6.4	0.57 ± 0.01	75.6 ± 3.6
17	423.27 ± 71.4	0.98 ± 0.01	0.77 ± 0.01	0.02 ± 0.001	75.5 ± 2.3	0.75 ± 0.03	100.9 ± 2.1
18	350.26 ± 81.3	1.23 ± 0.01	1.02 ± 0.01	0.06 ± 0.004	93.9 ± 8.6	0.40 ± 0.02	98.9 ± 7.0
19	247.58 ± 5.0	1.13 ± 0.01	1.03 ± 0.01	0.03 ± 0.01	116.9 ± 2.0	1.71 ± 0.02	121.0 ± 12.1
20	241.87 ± 18.9	0.95 ± 0.02	0.84 ± 0.01	0.07 ± 0.01	80.2 ± 2.8	0.99 ± 0.06	82.7 ± 7.8
21	247.96 ± 21.2	2.08 ± 0.01	1.38 ± 0.01	1.78 ± 0.018	131.9 ± 6.9	1.86 ± 0.08	140.2 ± 7.2
22	247.96 ± 32.3	1.25 ± 0.01	0.88 ± 0.01	0.11 ± 0.004	71.2 ± 0.8	0.96 ± 0.01	84.8 ± 3.3
23	148.32 ± 27.6	1.63 ± 0.01	1.13 ± 0.01	0.18 ± 0.003	90.5 ± 0.5	0.83 ± 0.01	89.6 ± 4.0
24	121.51 ± 5.4	0.97 ± 0.01	1.04 ± 0.01	0.09 ± 0.005	99.7 ± 7.2	1.04 ± 0.01	100.2 ± 4.3
25	195.66 ± 10.4	1.03 ± 0.02	0.68 ± 0.01	0.25 ± 0.002	56.2 ± 3.8	0.44 ± 0.02	61.8 ± 5.0
26	244.15 ± 10.1	1.06 ± 0.01	0.99 ± 0.01	0.14 ± 0.004	103.7 ± 7.5	0.82 ± 0.03	100.1 ± 14.0
27	246.05 ± 10.8	0.78 ± 0.01	0.64 ± 0.01	0.33 ± 0.002	63.5 ± 3.8	0.48 ± 0.04	63.7 ± 6.1
28	245.48 ± 11.6	0.89 ± 0.01	0.92 ± 0.01	0.91 ± 0.007	89.8 ± 0.5	0.79 ± 0.01	85.1 ± 8.2
29	225.71 ± 53.8	1.65 ± 0.01	1.15 ± 0.01	1.53 ± 0.005	113.4 ± 0.3	2.08 ± 0.01	105.1 ± 2.6
30	234.46 ± 20.7	1.89 ± 0.02	1.63 ± 0.01	1.27 ± 0.012	166.8 ± 1.9	2.43 ± 0.02	54.21 ± 1.6
31	198.71 ± 26.7	2.01 ± 0.01	1.34 ± 0.01	0.15 ± 0.007	125.3 ± 8.0	2.36 ± 0.33	121.5 ± 2.6
32	249.67 ± 4.2	2.03 ± 0.02	1.54 ± 0.01	1.97 ± 0.023	172.8 ± 4.5	3.02 ± 0.03	86.5 ± 2.4
33	168.47 ± 29.7	1.57 ± 0.01	1.14 ± 0.01	1.4 ± 0.016	113.9 ± 9.3	1.63 ± 0.27	103.5 ± 0.9
34	371.74 ± 7.7	1.05 ± 0.01	0.58 ± 0.01	0.01 ± 0.001	73.8 ± 2.9	0.82 ± 0.01	60.5 ± 4.5
35	220.95 ± 8.9	1.05 ± 0.01	0.58 ± 0.01	0.03 ± 0.003	38.8 ± 3.3	0.38 ± 0.02	39.4 ± 7.9
36	232.17 ± 11.1	1.08 ± 0.02	0.63 ± 0.01	0.04 ± 0.002	45.8 ± 2.4	0.44 ± 0.01	37.5 ± 0.7
37	187.68 ± 30.8	0.82 ± 0.01	0.45 ± 0.01	0.1 ± 0.003	23.5 ± 1.2	0.21 ± 0	32.4 ± 1.4
38	236.55 ± 10.4	1.15 ± 0.01	0.60 ± 0.01	0.09 ± 0.001	38.6 ± 1.4	0.17 ± 0	46.4 ± 1.2
39	233.50 ± 41.3	1.60 ± 0.01	0.75 ± 0.01	0.13 ± 0.004	49.4 ± 1.7	0.49 ± 0.02	46.1 ± 1.2
40	132.53 ± 15.8	1.17 ± 0.01	0.62 ± 0.01	0.03 ± 0.007	38.1 ± 1.6	0.36 ± 0	45.5 ± 2.1
41	133.68 ± 20.0	0.92 ± 0.02	0.47 ± 0.01	0.02 ± 0.003	28.6 ± 0.6	0.28 ± 0	38.7 ± 0.3
42	231.41 ± 5.18	0.89 ± 0.01	0.67 ± 0.01	0.01 ± 0.002	49.4 ± 1.6	0.28 ± 0.01	41.5 ± 1.8
43	213.16 ± 25.5	0.84 ± 0.02	0.58 ± 0.01	0.02 ± 0.001	38.4 ± 3.6	0.25 ± 0.01	41.0 ± 7.3
44	160.11 ± 12.2	0.84 ± 0.01	0.49 ± 0.01	0.03 ± 0.001	41.9 ± 2.1	0.33 ± 0.01	42.0 ± 5.6
45	156.30 ± 27.3	0.95 ± 0.01	0.40 ± 0.01	0.03 ± 0.002	21.3 ± 2.0	0.21 ± 0	30.5 ± 4.5
Min	121.51	0.53	0.33	0.007	21.34	0.17	30.51
Max	505.23	2.08	1.63	1.972	172.78	3.02	140.25
Aver	247.77	1.15	0.84	0.267	78.10	0.87	74.54
St dev	88.63	0.36	0.29	0.497	35.36	0.65	26.96

All data represent the mean ± standard deviation of three independent experiments.
All results are presented in milligrams of the evaluated compound/s in one gram dry weight of lyophilized flesh.
[1] Gallic acid.
[2] Rutin.
[3] Caffeic acid.
[4] Catechin.

280 nm and a minor group at 250 nm wavelength (Fig. 2). No new peaks were detected on the chromatograms obtained at 320, 370 and 520 nm (data not presented).

Analysis of the spectral data and co-chromatography with a standard revealed the presence of ellagic acid (Fig. 2A) at the average level of 74.5 mg/g DW (Table 2). This level is 37- to 50-fold that of blackberry (1.5–2.0 mg/g DW; Clifford & Scalbert, 2000), which are among the richest sources of ellagic acid (Landete, 2011). The level of ellagic acid in Kakadu plum from the WA (accession 34–45) was approximately half of that of the NT fruits (Table 1).

The major phenolic compounds of Kakadu plum are visible at 280 nm wavelength of the HPLC chromatogram (Fig. 2B). The UV spectra of all major peaks were almost identical and had maxima in the 205–220 nm and 270–290 nm with a clearly visible, deep valley preceding the second maximum. This spectral characteristic indicates the presence of ellagitannins having both hexahydroxydiphenoyl (HHDP) and galloyl groups attached to their glucopyranose core (Karonen, Parker, Agrawal, & Salminen, 2010). Spectral characteristics of chebulinic acid, a galloyl tannin of *Terminalia chebula* (Han, Song, Qiao, Wong, & Xu, 2006) has a striking similarity with the spectra of Kakadu plum major phenolics. The information generated in this study indicates that hydrolysable tannins, possibly complex ellagitannins and galloylglucoses, are the main phenolic compounds of *T. ferdinandiana*. This finding is in agreement with Yoshida and coworkers, who reported the presence of 11 ellagitannins monomers in *Terminalia*

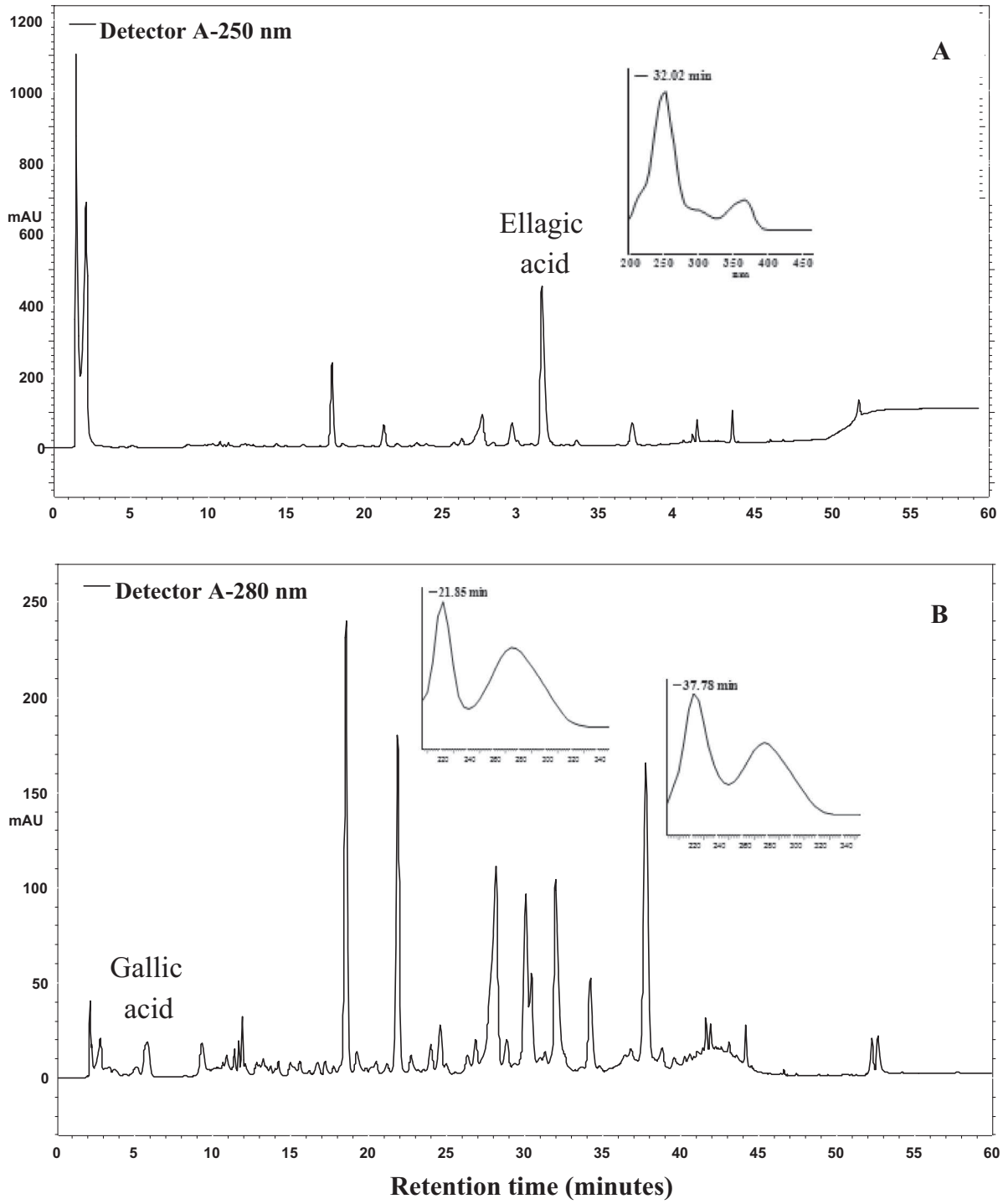

Fig. 2. Representative high performance liquid chromatography profile of phenolic compounds present in *Terminalia ferdinandiana* (accession 32) detected at 250 nm (A) and 280 nm (B).

species (*T. arjuna*, *T. brachystemma*, *T. calamansanai*, *T. catappa*, *T. chebula*, *T. citrine*, *T. macroptera*, *T. myriocarpa and T. triflora*) and reported that the presence of ellagitannins with a 4-glucopyranose core and a chebuloyl group is a chemotaxonomic feature of *Terminalia* (Yoshida, Amakura, & Yoshimura, 2010).

The average level of phenolic compounds detected at 280 nm in the evaluated accessions was 78.1 mg GAE/g DW and varied from 21.3 ± 2.0 (accession 45) to 172.8 ± 4.5 mg GAE/g DW of lyophilized flesh (accession 32; Table 2). In an earlier study a commercially available sample had 97.4 ± 3.0 mg GAE/g DW (Konczak et al., 2010), which indicates that an opportunity exists for the selection of accessions accumulating higher levels of phenolics. The highest level of phenolic compounds was found in the fruits from Copermine track, Darwin Site 3, CSIRO Site and Darwin Site 1. The Northern Territory fruits had 2.5- to 1.5- times higher levels of phenolic compounds detected at 280 nm than those of Western Australia (Table 1).

Through co-chromatography with standard and spectral characteristics the peak at 5.6 min retention time (280 nm) has been identified as gallic acid (Fig. 2B). The average level

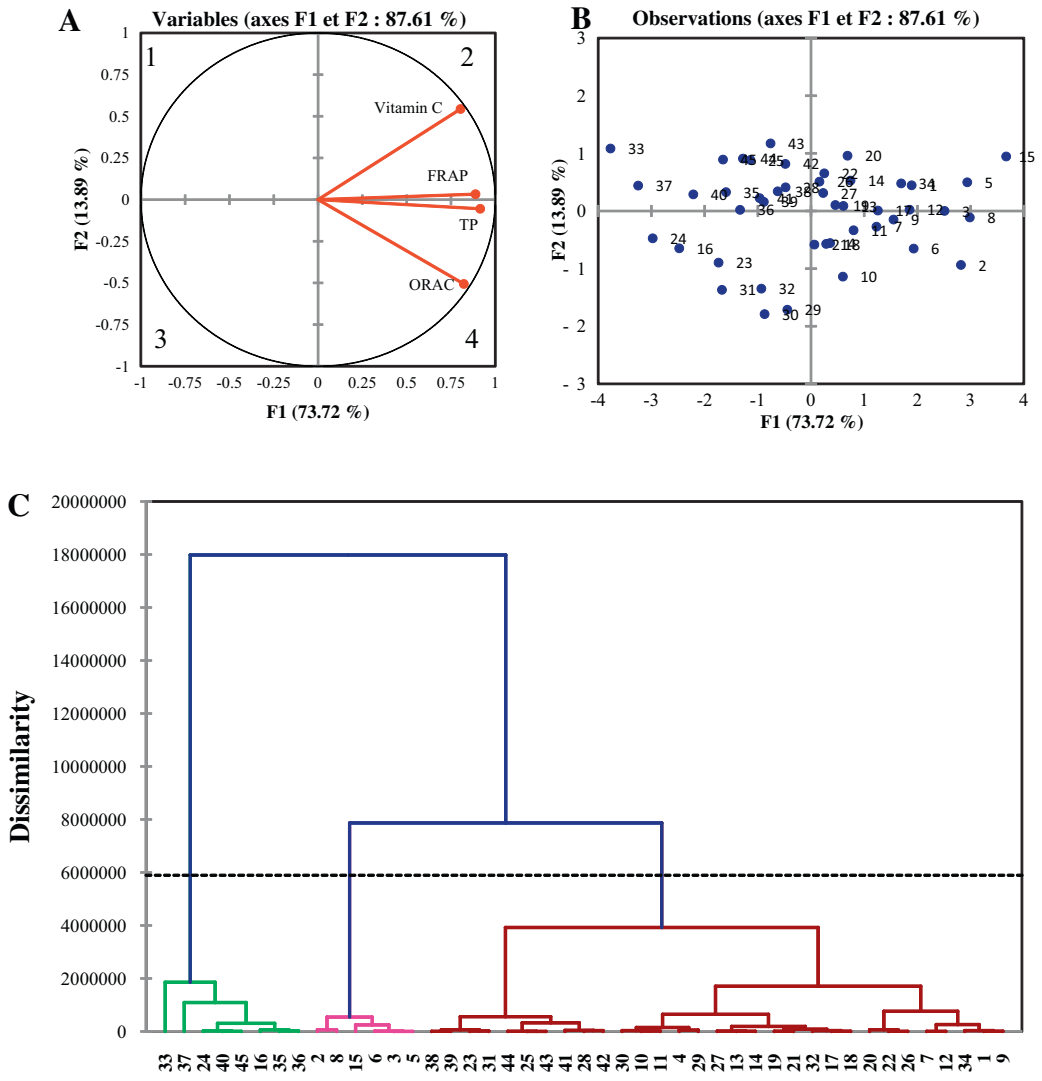

Fig. 3. Principal component analysis (PCA) correlation scatter plot (A), PCA score scatter plot (B) and agglomerative hierarchical clustering (AHC) dendrogram (C) of 45 *Terminalia ferdinandiana* accessions collected across Australia.

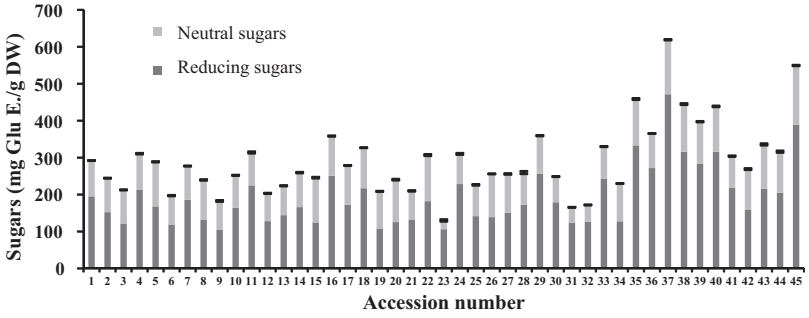

Fig. 4. Sugar content of 45 *Terminalia ferdinandiana* accessions collected across Australia. Data are means ± SD of $n = 6$ determinations.

of gallic acid in the evaluated samples was only 0.9 mg/g DW and varied from 0.2 (accession 38) to 3.0 mg/g DW (accession 32; Table 2).

3.4. Antioxidant capacities

Due to the complexity of the antioxidant defence system and involvement of many types of free radicals in the body, a single antioxidant assay cannot provide a complete picture of the antioxidant capacity of a sample (Huang, Ou, & Prior, 2005). In this study two complementary antioxidant testing methods were applied: the Ferric reducing antioxidant power (FRAP) and the Oxygen radical absorbing capacity (ORAC) assays. The FRAP assay evaluates the ability of a compound to donate a free electron, and is a measurement of the total reducing capacity (TRC). The ORAC assay evaluates the ability of a compound to scavenge (through the

donation of a hydrogen cation) oxygen free radicals, the dominating type of free radicals generated in the human body.

The average TRC of Kakadu plum accessions was 3418.5 μmol Fe^{+2}/g DW (equal to 650.3 ± 207.4 μmol Fe^{+2}/g FW of edible flesh), and was similar to that of a commercial sample evaluated earlier (691 ± 48.4 μmol Fe^{+2}/g FW) (Konczak et al., 2010). Northern Territory fruits exhibited a high TRC, with NT Darwin Site 1 and NT Darwin Site 3 showing the highest values (Table 1). Accession 15 (NT, TRO 3) exhibited the highest value of 5030.5 ± 508.7 μmol Fe^{+2}/g DW (823.7 μmol Fe^{+2}/g FW of edible flesh; Fig. 1B). Significantly lower TRC exhibited the WA fruits (Table 1), where the lowest FRAP value (accession 37) of 1366.3 ± 225.4 μmol Fe^{+2}/g DW (232.2 μmol Fe^{+2}/g FW of edible flesh) has been recorded (Fig. 1B).

Significant variations were found for the oxygen radical scavenging capacity with ORAC-H values from 3861.0 ± 7.7 (NT Darwin Site 1, accession 2; Fig. 1C) to 665.2 ± 21.5 μmol T E/g DW for accession 33 from NT Coppermine track (respectively, 1078.7 and 145.4 μmol T E/g FW of edible flesh). The average ORAC-H value of all accessions was 2592.6 ± 559.7 μmol T E/g DW. NT fruits, especially those from the Darwin Site 1 and the CSIRO Site, had higher ORAC-H values than fruits from WA, however the differences were not significant (Table 1). The average ORAC-H values of the WA plum was 2239.0 ± 309.5 μmol T E/g DW (351.7 ± 65.7 μmol T E/g FW of edible flesh). The ORAC-H values of 36 common fruits obtained from a US market varied from 1.2 ± 0.2 (watermelon) to 92.1 μmol T E/g FW (lowbush blueberry) (Wu et al., 2004). Therefore, WA Kakadu plum consumed as a fresh fruit would deliver 3.6-times more antioxidants capable of scavenging oxygen free radicals, than the lowbush blueberry.

3.5. Interrelationships between total phenolic compounds, vitamin C and antioxidant capacities

The combination of chemical characterisation and multivariate data analysis allows easy interpretation of similarities and differences among the evaluated accessions with regards to antioxidant activity, phenolic constituents and vitamin C. The strength of the association between these parameters was evaluated through Pearson's correlation coefficients, the principal component analysis (PCA) and agglomerative hierarchical clustering (AHC).

The Pearson's correlation coefficients showed a strong positive correlation between the TP values and all other variables: FRAP (0.727), ORAC (0.723), vitamin C (0.674). The lowest correlation existed between the vitamin C and ORAC-H (0.447), which indicates that vitamin C it is not the primary contributor to the oxygen radical absorbance capacity.

The PCA analysis allows to summarise the differences among the evaluated accessions. The PCA correlation scatter plot (Fig. 3A) visualises the comparison of analytical techniques: TP, vitamin C, FRAP and ORAC. These parameters represent related features and are significantly correlated, as evidenced by their Pearson correlation coefficients. Subsequently, they are clustered together on the right side of the PCA loading plot. They explain 87.61% of the total variance. The correlation scatter plot also indicates that the TP and FRAP variables represent relative activities and provide a similar description of the samples. The ORAC and Vitamin C data sets represent slightly different characteristics. Accordingly, accessions with higher values of all the evaluated variables are located on the right half of the plot (quadrant 2 and 4); those containing superior vitamin C levels are in quadrant 2, and those with superior ORAC values – in quadrant 4.

Fig. 3B presents the accessions scattered in PCA. By using the PCA plots it is possible to suggest reasons for the location of the accessions on the basis of their antioxidant capacities and vitamin C content. The analysis of the relationships between the evaluated variables indicates a clear distribution of the accessions into distinct clusters. The cluster located on the right side of the diagram (quadrant 2 and 4) comprises 23 samples (accessions 1–15, 17–18, 20–22, 26–27 and 34) and includes all NT Darwin Site 1 and CSIRO Site, and all but one of the TRO Site and the Darwin Site 3 accessions. Only one accession from WA (34) is present in this cluster. These accessions are characterised by high levels of vitamin C, high TP and high antioxidant capacities. On the left side of the diagram (Fig. 3B) are the most of the Fossil Head and Coppermine Track (NT) accessions and the WA fruits with the exception of number 34. These fruits had relatively lower levels of TP, vitamin C and/or antioxidant capacities, which gave them low scores on PCA plot. The differentiated cluster in the lower left quadrant (3)/far left in upper quadrant (1) comprising 9 accessions (16, 23, 24, 29, 30, 31, 32, 33 and 37) represents fruit characterised by low vitamin C.

Fig. 3C presents a dendrogram of agglomerative hierarchical clustering (AHC) in which 3 well-defined clusters are visible. The accessions are grouped in terms of their dissimilarity. The first cluster comprises accessions 33, 37, 24, 40, 45, 16, 35 and 36 and represents fruits with lowest levels of phytochemicals and antioxidant capacities. On the PCA plot (Fig. 3B) these accessions are located on the far left. The second cluster comprises accessions 2, 8, 15, 6, 3 and 5, characterised by the highest values of TP, vitamin C and antioxidant capacities. In agreement, they are located at far right on the PCA plot. The third cluster represents the vast majority: 31 accessions. Their levels of phytochemicals and antioxidant capacities are closer to the average values. The third cluster is divided into three subclasses. The subclass comprising accession 9, 1, 34, 12 and 7, as well as 20, 22 and 26 represents the second best group of fruits with regards to the phytochemical characteristics and antioxidant capacity. Accordingly, on the PCA plot this subclass is located on the left side, following the first cluster in far left.

3.6. Sugar content

The carbohydrates synthesized in plants from simple organic substances, oxygen, carbon and hydrogen, are prominent constituents of fruit, contributing up to 50% of the plant tissue and serving as a source of energy. The most common fruit sugars are glucose, fructose and sucrose, complemented by maltose (Lee, Shallenberger, & Vittum, 1970), galactose, raffinnose, myo-inositol, mannitol and sorbitol (Kubola, Siriamornpun, & Meeso, 2011). With regards to their chemical properties, carbohydrates contain an aldehyde group (CHO) or ketone group (C≡O). Sugars with an aldehyde group (e.g. glucose and galactose) are easily oxidised and this quality is utilised in reagent-based assays to quantify them. Sugars which do not have a free aldehyde group are non-reducing or neutral sugars. Among the neutral sugars is fructose, which possesses the ketone group, and sucrose, comprising of glucose and fructose joined together, which neither of the two rings is capable of opening.

The levels of sugars in Kakadu plum accessions presents Fig. 4. Higher levels of total sugars (the sum of reducing and neutral sugars) were identified in WA accessions (394.0 ± 114.6 mg Glu E/g DW). The highest level of total sugars had accession 37 (619.0 mg Glu E/g DW), closely followed by accession 45 (549.4 mg Glu E/g DW). The average level of total sugars in the NT fruits was 254.1 ± 55.5 mg Glu E/g DW, which represented 64.5% of the total sugars of WA fruits. Generally, the level of neutral sugars was 2.0-fold that of reducing sugars and the neutral/reducing sugars ratio varied from 1.0 (accession 15) to 4.5 (accession 23). For the WA fruits, the neutral/reducing sugars ratio was 2.3 and for the NT fruits it was 1.9. The levels of total sugars in Kakadu plum accessions are 2.6- to 12.6-times higher than those of native to northern Thailand *Terminalia chebula* Retz, which contained 49.2 ± 4.7 mg/g DW of sugars, including 25.8 ± 1.1 mg/g DW

glucose, 11.4 ± 2.0 mg/g DW galactose and 11.9 ± 1.6 mg/g DW fructose (Kubola et al., 2011).

4. Conclusions

Considerable variation in the levels of selected phytochemicals and antioxidant capacities was observed between 45 accessions of *T. ferdinandiana* collected across Australia. The accessions from NT Darwin Site 1, NT CSIRO Site and NT TRO Site had larger average fresh weight of one fruit, the highest levels of total phenolic compounds and vitamin C and exhibited superior antioxidant capacities. The average fruit from WA had higher levels of sugars. However, with the exception of accession 34 from the Central Kimberly, which had phytochemical characteristics and antioxidant capacities comparable to those of NT fruits, WA accessions had lower levels of phenolics and lower antioxidant capacities. Nevertheless the WA accessions, with regards to the levels of phenolic compounds, vitamin C and antioxidant capacity significantly exceeded commonly consumed fruits. With the highest levels of sugars these qualities would place WA Kakadu plum at the forefront of nutritionally rich fruits for fresh consumption. The PCA and AHC analyses identified a superior cluster of accessions with regards to total phenolics and vitamin C levels; this cluster includes accession 2, 8, 15, 6, 3 and 5 and presents an excellent source of phytochemicals for nutraceutical industry.

The variations between the evaluated *T. ferdinandiana* accessions are due to genotype and growth environment. The extent of their contributions is the subject of further research.

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