Journal of Food Measurement and Characterization (2019) 13:2347–2356
https://doi.org/10.1007/s11694-019-00154-z

ORIGINAL PAPER

Cactus pear antioxidants: a comparison between fruit pulp, fruit peel, fruit seeds and cladodes of eight different cactus pear cultivars (*Opuntia ficus-indica* and *Opuntia robusta*)

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Received: 26 September 2018 / Accepted: 2 May 2019 / Published online: 9 May 2019

Abstract
The cactus pear plant is an under-valued food source with health-promoting properties that thrives in arid and semi-arid regions due to its efficient use of water. Eight South African cultivars from two *Opuntia* species were investigated for their antioxidant content and potential. The fresh fruit (pulp), peel, seeds and cladodes of each cultivar were compared in the study. Analysis included betalains, ascorbic acid, phenolics and carotenoids. The activity of the antioxidants were determined by using the DPPH method and by measuring the chelating activity of ferrous ions. When % DPPH was tested, peel and cladodes were consistently the highest, while in the % chelating activity tests, fruit pulp and seeds were the best tissue types. Cladodes contained more phenolics and carotenes than fruit regardless of the cultivar. For pulp and peel, the cultivar that contained the highest antioxidant content and potential was Robusta with its high content of betalains followed by Gymno-Carpo and Ofer with high ascorbic acid levels. The study proves that the fruit (pulp), peel and seeds from different cultivars contain specific antioxidants relating to the colour of the fruit, but the cladodes of any cultivar contain similar and highly effective antioxidants.

Keywords Ascorbic acid · Betalains · Carotenes · Chelating activity · DPPH · Phenolics

Introduction

Declining water sources and global desertification in many parts of the world caused researchers to pay special attention to indigenous plants from arid countries in order to find effective food production systems and to explore possible uses in the food, medical and cosmetic industries [1]. Research has revealed that *Opuntia ficus-indica* fruit contains high levels of constituents that give it value on a nutritional and functional basis, such as antioxidants [2, 3]. Natural antioxidants from dietary sources include phenolic and polyphenolic compounds, chelators, antioxidative vitamins, enzymes and carotenoids [4]. Crops with health-promoting and nutritional benefits are gaining momentum for both professionals and consumers and cactus pears fit this trend [1].

Antioxidants are molecules that are able to reduce, delay or inhibit oxidation of other molecules even when present at very low levels. It therefore protects the body against diseases [3]. Recently there has been increased interest in the health-promoting capacity of antioxidants and cactus pears have been investigated in this regard. Results by Budinski et al. [5] showed that ingestion of prickly pear cladodes is effective in lowering oxidation injury and this suggests that the prickly pear plant possesses antioxidant components in the edible stems of the plant as well as the fruit. Tesoriere et al. [6] proved that a diet that includes cactus pear fruit may reduce the risk of age-related and degenerative diseases. The cactus pear fruit and cladodes are powerful antioxidant sources and could be an important additive in functional foods. The most important bio-active compounds detected in cactus fruits are phenolic compounds, betacyanins (Bc) and betaxanthins (Bx), which have antioxidative properties [7]. Different cultivars of cactus pear fruit have different coloured fruit that may indicate the presence of

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specific antioxidants [8]. Cactus pears have commercial value. They have excellent nutritional properties and contain biocompounds with several commercial applications, for example betalain, a water-soluble nitrogen-containing colourant compound. The most important biocompounds in cactus pear fruit are phenolic compounds, Bc, Bx and ascorbic acid [7]. All of these have potent antioxidant properties. Phenolic compounds' chemical structure and concentrations are variable and depend on variety, ripening stages and kind of plant tissue. The antioxidant properties of phenolic compounds protect human health against degenerative diseases. Flavonoids are more efficient antioxidants than vitamins. Yeddes et al. [9] pointed out that the most studies done on cactus pear fruits consist of the chemical analysis of (a) the pulp for vitamin C, polyphenols, betalains and volatile constituents (b) the skin for polyphenols and lipids and (c) the seeds for lipid profiles, but that little is done to compare the main biocompounds in different species of the cactus pear fruit. In addition to the abovementioned, very little information is available on the biocompounds found in cactus pear peels, seeds and cladodes. Since processing of fruit results in the production of many waste products such as peels and seeds, the utilization of these by-products could reduce waste disposal problems and serve as new sources of bioactive compounds including polyphenols (e.g. flavonoids), vitamins and colourants like betalains and carotenes [10]. It was also reported that different botanical components like pulp, seeds, cladodes and flowers contains different levels of phytochemicals [11]. The determination of the content of antioxidants, namely ascorbic acid, polyphenolics, betalains and carotenoids and the potential to prevent oxidation was investigated in a selection of eight different cultivars from two different species, representing four colours of fruit, in order to gain a better understanding of the relationship between antioxidant content and antioxidant potential of different cultivars to the different cactus pear tissue types (fruit pulp, fruit peel, fruit seeds and cladodes).

Materials and methods

Sample collection

Fruit and cladodes were harvested from an eight year old experimental cactus pear orchard in Bloemfontein, Free State Province. The orchard is laid out as a complete randomised block design and consist of 42 spineless cactus pear cultivars, with 10 plants per cultivar (treatment). Each treatment consists of five plants and each treatment is replicated twice [12]. It hosts 40 *O. ficus-indica* cultivars and two *O. robusta* cultivars [8]. Cladodes and ripe fruit (50% colour break stage) from eight cultivars were collected and transported to the laboratory, weighed, peeled and the pulp and peel were weighed again. The eight cultivars were chosen for their colour and quality according to results of a study done previously by De Wit et al. [13]. These include Nepgen and Morado (green fruit varieties), Ofer and Gymno-Carpo (orange fruit), Meyers and Sicilian Indian Fig (red-pink fruit varieties), Nudosa (red) and Robusta (red-purple fruit varieties). Only Robusta was representative of *O. robusta* sp. while the other seven cultivars were from *O. ficus-indica*. Six-month-old cladodes of good quality, without any blemishes, which had not bear any fruit were collected. Cladodes were all selected to be at the same height of the plant and of equal size. Two cladodes and fruits from the first, third and fifth plant of each replication were harvested. Three cladodes and three fruits were randomly selected per each replication (thus six cladodes and six fruits per cultivar).

Sample preparation

Aqueous extracts and hexane/acetone/ethanol (50:25:25, v/v) extracts were prepared for all tissue types. The latter was used for carotene determinations. Samples of fruit pulp and peel were homogenized with equal amounts of water, i.e. 1:1 w/v, strained (0.5 mm mesh size), the volume of the filtrate determined and aliquots were frozen at – 18 °C until further analysis [14]. The separated seeds were washed, air-dried and ground in a Kenwood coffee grinder. Part of the powdered seeds were separated and used for carotene determinations. The remaining powder was weighed and five times the same weight of distilled water was added. It was vortexed for 60 s and homogenized for 30 s, centrifuged for 10 min at 8000 rpm and aliquots were frozen. For the fresh cladode samples, the cladodes were weighed, cut into pieces and liquidized. Equal weights of water (100%) were added, homogenized and centrifuged at 8000 rpm for 10 min at 4 °C and the supernatant was frozen in aliquots. For carotenoid determination, a hexane/acetone/ethanol (50:25:25 v/v) extract was prepared from all the tissue types. Two grams of tissue was homogenised with 10 ml hexane/acetone/ethanol (50:25:25, v/v) mixture, centrifuged and the hexane layer recovered.

Antioxidant measurements

The methods described by Castellanos-Santiago and Yahia [14] and Stintzing et al. [15] were used to determine betalains. Absorbance was measured using a Genesys 10 Vis Thermospectronic spectrophotometer. The betalain content (which comprises of the red-violet Bc and the yellow Bx was calculated according to the following equation [15]:

$$\mathrm{Bc/Bx\ (mg\ g^{-1})} = \mathrm{A(DF)(MW)(1000)/E(L)},$$

A is absorption value at 600 nm, DF is dilution factor, molecular weight (MW) Bc = 550, Bx = 308, molar

extinction coefficients of betanin (E) = 60,000, indicaxanthin (E) = 48,000, L is path lengh of the cuvette.

The units were expressed as mg kg^{-1} fresh weight (FW).

Ascorbic acid was determined according to James [16]. A 10- and 100-fold dilution of the aqueous cactus pear tissue extract was titrated with 0.04% 2,6 dichlorophenolindophenol (DPIP) solution and the ascorbic acid content were calculated according to the following equation:

$$\frac{\text{mg}}{100\ \text{mg}} = (\text{T} - \text{B})/(\text{St} - \text{B})(20)(\text{DF}),$$

T is titration value, B is blank, St is standard solution, DF is dilution factor.

The units were expressed as mg 100 g^{-1} FW.

Carotenoid content was determined after samples were homogenised with 10 ml hexane:acetone:ethanol (50:25:25, v/v) and centrifuged at 6500 rpm at 4 °C for 5 min (as described above). The top layer of hexane was recovered, and the volume was adjusted to 25 ml with hexane. The absorbance was measured at 450 nm according to the method described by Kuti [7] and Fernández-López et al. [4] using an extinction coefficient of β-carotene, E1% = 2590. The units were expressed as µg g^{-1} FW.

$$\text{x}\ (\mu\text{g}) = \text{A} \cdot \text{y}\ (\text{ml}) \cdot 10^6 / \text{A}^{1\%}_{1\ \text{cm}} \cdot 100,$$

$$\text{x}\ (\mu\text{g g}^{-1}) = \frac{\text{x}\ (\mu\text{g})}{\text{weight of sample}},$$

x is weight or concentration of carotenoid, A is absorbance, y is volume of solution, $A^{1\%}_{1\ \text{cm}} = 2590$ (absorption coefficient).

Total phenolic content was determined using 2 g of the aqueous extract (frozen aliquot). The aqueous extract was centrifuged, and 0.2 ml of the extract was combined with 1 ml Folin–Ciocalteu reagent and 0.8 ml sodium carbonate solution. An absorbance reading at 765 nm was done after 30 min. The polyphenol reading at 765 nm was expressed as milligrams of gallic acid equivalents per litre (mg l^{-1} GAE), following a calibration curve made with pure gallic acid at 0, 50, 100, 150, 200, 250, 300 and 350 mg l^{-1} [15]. The units were expressed as mg kg^{-1} FW.

DPPH (2,2′-diphenyl-1-picrylhydrazyl) was determined according to the methods of Sumaya-Martínez et al. [17] and Morales and Jiménez-Pérez [18]. In each test, 500 µl of the DPPH solution (7.4 mg 100 ml^{-1} ethanolic solution) was added to 100 µl of the aqueous extracts, vortexed for 10 s, left to stand for one hour and centrifuged at 10,000 rpm for 5 min at 4 °C. A blank solution containing aqueous extract and ethanol was prepared. Absorbance was measured at 517 nm [19].

DPPH scavenge effect (%)

$$= \left(\frac{\text{Acontrol} - (\text{Asample} - \text{Ablank})}{\text{Acontrol}} \times 100 \right) \times \text{DF},$$

DF is dilution factor, Acontrol is absorbance of the reagent, Asample is absorbance of the sample, dilutants and reagent, Ablank is absorbance of sample with dilutants without reagents.

The method of Gülçin et al. [19] as amended by Sumaya-Martínez et al. [17] was used to determine the chelating activity of the antioxidants. One hundred microliters of aqueous extract, 50 µl ferric(II) chloride solution (2 mM) and 4.5 ml methanol was vortexed for 10 s, after which 200 µl ferrozine (5 mM) was added and centrifuged. Blank solutions containing aqueous extract and methanol, were also prepared (blank solutions were included to make the absorbance measurement of the purple fruit possible). Absorbance of the pink-purple supernatant was determined at 562 nm and the chelating activity was calculated as follows:

Ferrous ions chelating effect (%)

$$= \left(\frac{\text{Acontrol} - (\text{Asample} - \text{Ablank})}{\text{Acontrol}} \times 100 \right) \times \text{DF},$$

DF is dilution factor, Acontrol is absorbance of distilled water used instead of sample with ferric(II) chloride and ferrozine, Asample is absorbance in the presence of the sample and reagents, Ablank is absorbance of sample with dilutants without reagents.

Statistical analysis

All analyses were performed in triplicate with two replications per cultivar. The effect of fruit cultivar (and colour) on antioxidant properties of cactus pear fruit pulp, peel, seeds and cladodes was analysed with one way analysis of variance (ANOVA) and the means compared with the Tukey–Kramer multiple comparison test [20]. The interpretation of the data was simplified with the assistance of the multivariate statistical procedure, PCA (principal component analysis) that was used to investigate the correlationship between plant parts from different cultivars (different fruit colours) with respect to their antioxidant contents and potentials (variables) [20].

Results and discussion

In the combined ANOVA for cultivar and tissue type (Table 1), cultivar had a highly statistically significant effect ($p < 0.001$) on the antioxidant capacity as well as the -content, for all tests performed. These results indicated that the antioxidants that are predominant in a specific cultivar are highly dependent on the cultivar. The tissue type

Table 1 Analysis of variance (ANOVA) for the influence of cultivar, tissue type (fruit pulp, peel, seeds and cladodes) and the interaction between cultivar and tissue type on antioxidant properties of fresh cactus pear [8]

Antioxidant property	Cultivar	Tissue type	Cultivar × tissue type
% DPPH	*	*	*
% Chelating activity	*	*	*
Phenolics	*	*	*
Betacyanins	*	*	*
Betaxanthins	*	*	*
Betacyanins + betaxanthins	*	*	*
Ascorbic acid	*	NS	*
Carotene	*	*	*

NS not significant

*$p < 0.001$

(fruit pulp, peel, seeds and cladodes) had a highly statistically significant ($p < 0.001$) effect on the antioxidant type and -capacity and on most of the individual antioxidants, except for ascorbic acid [8]. The cultivar × tissue type interaction had significant effects on all antioxidant properties. It was reported by Cardador-Martínez et al. [21] that analysis of variance (ANOVA) revealed that cultivar, ripeness and their interaction had highly significant effects on the total phenolics, tannin, and flavonoid contents of cactus pear peel. Only the stage of ripeness and interaction (ripeness stage × cultivar) were significant on total phenolics and tannin contents of the seeds [21], while the flavonoid content was not affected by any of the factors or their interactions. The nutritional quality, specifically phenolic composition of fruits are influenced by genetic, agronomic and environmental factors. Stage of ripening, storage temperature, cultivation and place of origin affect the amounts of microconstituents [22, 23].

Table 2 shows the interaction between cultivar and tissue type on the antioxidant properties of cactus pear with averages for antioxidants, colour and tissue type.

Betacyanins (Bc, red-purple pigment)

Robusta (purple) had the highest Bc value in the fruit, while green and orange fruit had the lowest values. Meyers (pink), Sicilian Indian Fig (pink) and Nudosa (red), had slightly elevated levels of Bc in the fruit and peel, but it is very interesting that these cultivars did not have significantly more Bc than the green and orange fruit and peel even though they appear pink in colour. It was found by Castañeda-Yañez et al. [24] that purple fresh prickly pear juice had higher values of Bc than Bx than red prickly pear juice. As Bc are red-purple coloured pigments, it might be expected that it would dominate only in the purple fruit, but it is evident that the seeds of all cultivars had elevated (not statistically significant) levels with the seeds of Robusta having significantly higher Bc levels. The levels in purple fruit were higher than reported by Castellanos-Santiago and Yahia [14] (5.29 mg g^{-1}) and Albano et al. [25] (39.3 mg 100 g^{-1}) and lower than reported by Sumaya-Martínez et al. [17] (333 mg l^{-1}) and Stintzing and Carle [26] (486.7 mg kg^{-1}). The purple Robusta-type fruit contains similar amounts to red beetroot (40–60 mg 100 g^{-1} or 71–77 mg 100 g^{-1}) as reported by Castellanos-Santiago and Yahia [14]. In this experiment, Robusta contained ten times less Bc than red beetroot (7.5 mg 100 g^{-1}). The cladodes of Robusta and Morado contained the highest Bc values, although not significantly different from the other cultivars. No literature is available on the betalain content in seeds and cladodes.

Betaxanthins (Bx, yellow-orange pigment)

The purple *O. robusta* fruit had the highest Bx values, with red and pink slightly higher than green and orange (Table 2). These findings are noteworthy as the orange coloured fruit did not show higher levels than the other coloured fruit even though Bx are yellow pigments. It can be derived from this data that the orange colour in cactus pears is not the result of Bx content. Purple, red and pink fruit had slightly more betalains (Bc + Bx) and could provide the fruit with enough purple pigment for it to appear pink/purple. These findings agree with the literature that Bc and Bx contents are linear, meaning that cactus fruit with high levels of Bc also have high levels of Bx [14, 15, 17]. The levels of Bc found in cactus pear fruit in the current study were higher than in Castellanos-Santiago and Yahia [14] (2.86 mg g^{-1}) and lower than in Sumaya-Martínez et al. [17] (147 mg l^{-1}) and Stintzing et al. [15] (553.7 mg kg^{-1}). The Robusta cladodes did not have significantly more Bx than cladodes from other cultivars, although Morado (green) and Ofer (orange) were also slightly higher. The betalain content (Bc + Bx) in the cladodes of Robusta, Morado and Ofer were the highest. The seeds of all cultivars had the highest Bx and betalain (Bc + Bx) values, with the seeds of Robusta being significantly higher than the other cultivars.

Ascorbic acid

It is known that cactus pear fruit has significant amounts of ascorbic acid [1, 27]. In general, Nepgen (green), Meyers (pink), Gymno-Carpo (orange) and Ofer (orange) appear to have the highest (> 50 mg kg^{-1}) ascorbic acid content (Table 2). Ofer (94.07 mg 100 g^{-1}) (orange fruit) had the highest while Morado (12.11 mg 100 g^{-1}) (also a green fruit cultivar) had the lowest levels of ascorbic acid. Albano et al. [25] reported slightly higher ascorbic acid

Table 2 The effect of cultivar and tissue type on the antioxidant properties of fresh cactus pear fruit pulp, peel, seeds and cladodes

Cultivar	Tissue	Betacyanins (n = 3) (mg kg^{-1})	Betaxanthins (n = 3) (mg kg^{-1})	Betacyanins + betaxanthins (n = 3) (mg kg^{-1})	Ascorbic acid (n = 3) (mg 100 g^{-1})	Carotene (n = 3) (μg g^{-1})	Phenolics (n = 3) (mg kg^{-1} GAE)	DPPH (n = 3) (%)	Chelating activity (n = 3) (%)
Nepgen (green)	Fruit	1.83^{a}	1.28^{a}	3.11^{a}	91.69^{fgh}	0.62^{a}	16.72^{ab}	83.63^{ef}	94.94^{ef}
	Peel	0.89^{a}	0.62^{a}	1.52^{a}	$55.88^{abcdefgh}$	3.46^{abc}	15.96^{ab}	91.67^{ef}	81.67^{bcdef}
	Seed	69.89^{cd}	48.92^{cd}	118.81^{cd}	28.98^{abcd}	40.15^{e}	184.30^{bcdef}	42.35^{ab}	94.17^{ef}
	Cladode	4.74^{ab}	3.32^{ab}	8.07^{ab}	26.78^{abc}	6.72^{abc}	241.53^{def}	94.49^{f}	89.17^{def}
Morado (green)	Fruit	0.72^{a}	0.51^{a}	1.23^{a}	12.11^{a}	0.82^{a}	8.46^{a}	57.05^{bcd}	95.00^{ef}
	Peel	1.84^{a}	1.29^{a}	3.13^{a}	29.23^{abcd}	3.59^{abc}	31.88^{ab}	91.70^{ef}	87.50^{bcdef}
	Seed	17.48^{abc}	12.24^{abc}	29.72^{abc}	29.72^{abcd}	47.04^{e}	291.46^{ef}	32.39^{a}	91.67^{ef}
	Cladode	14.82^{ab}	10.37^{ab}	25.20^{ab}	$47.78^{abcdefgh}$	11.58^{abc}	324.87^{f}	95.31^{f}	40.77^{a}
Meyers (pink)	Fruit	2.71^{ab}	1.90^{ab}	4.62^{ab}	$52.52^{abcdefgh}$	0.92^{a}	17.45^{ab}	58.65^{bcd}	93.33^{ef}
	Peel	6.87^{ab}	4.81^{ab}	11.69^{ab}	86.28^{efgh}	1.79^{a}	58.88^{abc}	96.25^{f}	70.00^{bc}
	Seed	39.01^{abcd}	27.31^{abcd}	66.32^{abcd}	83.22^{defgh}	66.54^{f}	276.63^{ef}	40.20^{ab}	90.00^{def}
	Cladode	7.54^{ab}	5.28^{ab}	12.82^{ab}	$67.25^{bcdefgh}$	18.15^{cd}	257.25^{ef}	92.74^{ef}	80.83^{bcdef}
Sicilian Indian Fig (pink)	Fruit	0.89^{a}	0.62^{a}	1.51^{a}	15.47^{ab}	1.47^{a}	1.68^{a}	59.73^{bcd}	92.50^{ef}
	Peel	3.23^{ab}	2.26^{ab}	5.49^{ab}	24.22^{abc}	2.11^{a}	12.56^{a}	90.95^{ef}	87.50^{bcdef}
	Seed	56.50^{bcd}	39.55^{bcd}	96.04^{bcd}	$39.68^{abcdefg}$	69.24^{f}	204.47^{cdef}	26.68^{a}	83.33^{bcdef}
	Cladode	9.19^{ab}	6.44^{ab}	15.63^{ab}	27.31^{abc}	7.29^{abc}	304.41^{f}	94.08^{f}	27.44^{a}
Ofer (orange)	Fruit	1.20^{a}	0.84^{a}	2.05^{a}	94.07^{h}	1.69^{a}	13.41^{a}	77.56^{def}	94.17^{ef}
	Peel	1.11^{a}	0.78^{a}	1.89^{a}	$64.24^{abcdefgh}$	4.80^{abc}	21.31^{ab}	93.85^{f}	69.17^{b}
	Seed	35.58^{abcd}	24.91^{abcd}	60.49^{abcd}	86.75^{fgh}	32.59^{de}	258.83^{ef}	39.25^{ab}	88.33^{cdef}
	Cladode	3.17^{ab}	16.98^{abcd}	20.15^{ab}	$58.74^{abcdefgh}$	17.87^{bcd}	239.47^{def}	83.77^{ef}	80.83^{bcdef}
Gymno-Carpo (orange)	Fruit	1.54^{a}	1.08^{a}	2.62^{a}	95.27^{h}	1.86^{a}	22.08^{ab}	70.12^{cde}	89.17^{def}
	Peel	2.21^{a}	1.55^{a}	3.75^{a}	$68.04^{bcdefgh}$	3.99^{abc}	14.04^{ab}	91.18^{ef}	72.50^{bcd}
	Seed	22.41^{abcd}	15.69^{abcd}	38.10^{abcd}	$49.91^{abcdefgh}$	43.31^{e}	278.73^{ef}	48.64^{abc}	90.83^{def}
	Cladode	3.17^{ab}	2.22^{ab}	5.40^{ab}	77.40^{cdefgh}	17.87^{bcd}	270.93^{ef}	95.53^{f}	77.50^{bcde}
Nudosa (red)	Fruit	3.76^{ab}	2.64^{ab}	6.40^{ab}	32.82^{abcdef}	2.38^{ab}	2.85^{a}	82.47^{ef}	96.68^{f}
	Peel	5.08^{ab}	3.55^{ab}	8.63^{ab}	$46.40^{abcdefgh}$	2.01^{a}	10.59^{a}	96.85^{f}	96.63^{f}
	Seed	23.86^{abcd}	16.70^{abcd}	40.56^{abcd}	28.22^{abc}	45.62^{e}	74.85^{abcd}	37.86^{ab}	84.17^{bcdef}
	Cladode	7.59^{ab}	5.31^{ab}	12.90^{ab}	19.15^{ab}	10.70^{abc}	131.32^{abcde}	93.91^{f}	80.26^{bcdef}
Robusta (purple)	Fruit	74.47^{d}	52.13^{d}	126.60	32.38^{abcde}	1.58^{a}	18.47^{ab}	77.05^{def}	95.09^{ef}
	Peel	42.62^{abcd}	29.84^{abcd}	72.46^{abcd}	$61.16^{abcdefgh}$	6.06^{abc}	7.44^{a}	91.65^{ef}	97.32^{f}
	Seed	160.88^{e}	112.62^{e}	273.50^{e}	36.12^{abcdef}	41.67^{e}	258.35^{ef}	56.50^{bcd}	85.83^{bcdef}
	Cladode	17.18^{abc}	12.02^{abc}	29.20^{abc}	24.04^{abc}	11.29^{abc}	42.84^{abc}	92.50^{ef}	70.00^{bc}
Average		20.13	14.55	34.67	49.78	16.46	122.31	74.27	83.38
Significance level		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

values in a purple fruit variety than in an orange fruit variety. When the total contribution of pulp, peel, seeds and cladodes are taken into account, Meyers (pink) is equal to both orange cultivars (Ofer and Gymno-Carpo). No tissue type could be identified to contain the highest ascorbic acid content, which corresponds to the data represented in Table 1, with ascorbic acid not being affected by tissue type. The levels of ascorbic acid found in this study were on average (49.78 mg 100 g^{-1}) higher than that found by other researchers [4, 7, 13, 17, 25, 26, 28–30]. Other researchers presented levels from 0.79 mg 100 g^{-1} [7] to 48 mg 100 g^{-1} [30]. Cactus pear may be higher in ascorbic acid than most other common fruits, such as apple, pear, grape and banana [27] and show values that are typical for citrus fruits, mangoes and guavas [26].

Carotene

Very low levels were detected in fruit pulp and peel in all cultivars; in fact, the highest levels were in seeds. Sicilian

Indian Fig and Meyers (both pink cultivars) had the highest contents in seeds (69.24 µg g^{-1} and 66.54 µg g^{-1}, respectively) (Table 2). There were very specific trends in terms of colour; pink fruit had the highest levels with red, purple and orange fruit following closely. It appears that fruit that are high in betalain content are also high in carotene content. It was explained by Yahia and Mondragon-Jacobo [29] who reported similar results, that fruit with high betalains also appear to have high carotene levels, and that the pink, red and purple pigments (Bc) overpower the yellow colour of carotene and therefore the fruit appears pink/purple in colour. The cladodes of all cultivars contained more carotene than fruit pulp and peel. When other researchers [31–33] studied cladodes for carotene content, they found different amounts (0.02 µg g^{-1} to 231.8 µg g^{-1}), nevertheless, it was considerably higher levels than what was found in the fruit. There were no significant differences between carotenes from different cultivars in cladodes. This finding confirms the results of Bensadón et al. [33] that carotenoid content in cladodes does not depend on colour or cultivar. Medina-Torres et al. [32] compared cladodes to other vegetables and found that it has a higher carotenoid content than baby carrots, beetroot, spinach and lettuce. The seeds of all the cultivars differed statistically from the fruit pulp and peel and the cladodes had between 32 and 69 times more carotene than the fruit pulp. The carotene would most probably be present in the oil fraction of the seed. No literature dealing with carotene content in cactus fruit seeds are available for comparative purposes.

Phenolics

The statistical similarities in low fruit pulp and peel contents and high seed and cladode contents were the only correlations seen in these findings; the colour and cultivar does not seem to have an effect on phenolic content. In many previous studies it was found that purple or red fruit yielded the most phenolics [7, 25, 26, 29, 30, 34–36] but that result was not found in this study. In fact, Gymno-Carpo (orange) fruit had the highest readings in fruit pulp (22.08 mg kg^{-1}) and Meyers (pink) in peel (58.88 mg kg^{-1}). In previous studies, phenolics content in cactus pear fruit ranged from 21.88 to 746 mg kg^{-1} [4, 37] but the range for fruit pulp and peel was lower (1.68–58 mg kg^{-1}) in this study. However, the seeds and cladodes would be very good sources of total phenolics (Table 2). Total phenolics of different extracts of *O. ficus-indica* peels ranged from 221.3 to 1501.7 µg gallic acid 100 g^{-1} dry weight [38]. Total phenolic concentration in seeds varied between 337 and 460 mg g^{-1} and was, on average, almost two times higher in seeds than in peels (389 mg 100 g^{-1} and 217 mg 100 g^{-1} respectively) [21]. The phenolic levels for cladodes in this study correlated with data from Santos-Zea et al. [39] who found a minimum of 318.1 mg kg^{-1} and Medina Torres et al. [40] who found 1 g kg^{-1} (1000 mg kg^{-1}) in the cladodes of Mexican cactus pears. In this study the levels for cladodes fluctuated from 42.84 to 324 mg kg^{-1}.

Percentage DPPH

Fruit from Morado (green), Meyers and Sicilian Indian Fig (both pink) had statistically significant lower radical scavenging capacity (Table 2), but when fruit pulp, peel, seeds and cladodes are considered it showed considerable radical scavenging capacity. Ramírez-Moreno et al. [41] reported higher antioxidant activity (% DPPH) in the cactus pear seed oil of a green cactus pear fruit variety compared to a red fruit variety. The peel and cladodes demonstrated exceptionally high radical scavenging ability, as the cladodes had readings between 83 and 95% and the peel 90 to 96%. These results indicate that peels should be included where possible, such as in the making of juice, to increase the antioxidant capacity of the product. These findings agreed with findings by other researchers [29, 34, 42] in terms of fruit having high antioxidant capacity despite their colour and specific antioxidant content. DPPH free radical scavenging activity of up to 91.7% for various solvent extracts of prickly pear peels was reported by Abou-Elella and Ali [38]. Interestingly, Castañeda-Yañez et al. [24] found higher antioxidant capacity (% DPPH) in red prickly pear fruit juice than in purple prickly pear juice.

Percentage chelating activity of ferrous ions

Among cultivars, colours and tissue types, the chelating activity was generally high (average 83.38%) and mostly not significantly different (Table 2). Nevertheless, it appears as if Nepgen, Meyers, Gymno-Carpo, Ofer and Nudosa had the best ability to chelate ferrous ions. Chelating activity of fruit was higher than other tissue types with levels ranging from 89.17% (Gymno-Carpo) to 96.68% (Nudosa). Peel and cladodes had the lowest levels. In contrast to the % DPPH findings, the fruit had very consistent levels (average 93.86%). Sumaya-Martínez et al. [17] determined that chelating activity does not depend on colour of cactus pear since activity did not significantly differ in all studied cultivars. In this study the same results were found for fruit pulp, peel and seeds. The reason for cladodes not to correspond with these results cannot be explained as it does not correlate with any individual antioxidant component. High reducing power of cactus pear peel extracts were reported by Abou-Elella and Ali [38]. Fruits with light-green or yellow-brown peel have higher antiradical activity compared with those with red-purple peel [21, 43], however, Abou-Elella and Ali [38] reported that antiradical activity was 10% higher in seeds than in peels.

Principal component analysis (PCA)

PCA condenses large numbers of inconsistencies into smaller numbers of main factors [44]. The variation is explained therefore in terms of factors that are common to all variables as well as in terms of unique factors, whereby principal components of the cultivar in terms of antioxidants are defined. The interaction is explained by plotting the principal component scores closely to each other [44]. The biplot makes it possible for cultivars to be correlated with the antioxidants that it contains. The aim of the PCA is to identify cultivars and tissue types that are most associated with a specific antioxidant.

PCA of cultivar and the antioxidant properties of fresh cactus pear fruit pulp

Factor 1 (67.44%) and 2 (25.79%) explained 67.44% of the variation (Fig. 1). From the biplot, it is clear that the cultivars that are grouped in clusters around certain antioxidants are linked to those antioxidants. In fresh fruit (pulp), Gymno-Carpo is associated with ascorbic acid and phenolics, while Nepgen, Ofer and Meyers more closely associate with % DPPH and carotene, but on the whole they all form a grouping. Meyers is only related to carotene content (not closely). Nudosa, Morado and Sicilian Indian Fig are grouped together with % chelating activity. Robusta is the only cultivar associated with betalains.

The main associations in cactus pear fruit pulp were: Gymno-Carpo, Nepgen and Ofer associated with ascorbic acid, while Nepgen and Ofer also associated with % DPPH. Morado, Nudosa and Sicilian Indian Fig were closely associated with the highest % chelating ability and Robusta with betalains. These associations can be correlated with Table 2 in terms of the highest values for each antioxidant.

PCA of cultivar and the antioxidants properties of fresh cactus pear peel

In the PCA for the peel (Fig. 2) the variation is explained (75.24%) by Factors 1 (49.83%) and 2 (25.40%). While Robusta remains associated with betalains, it also grouped with carotene and % chelating activity. Meyers, Ofer, Gymno-Carpo, Nudosa, Nepgen, Morado and Sicilian Indian Fig are grouped together with phenolics, ascorbic acid and % DPPH. Ofer, Gymno-Carpo, Nudosa, Nepgen, Morado and Sicilian Indian Fig also seem to be linked with carotene and % chelating activity. Meyers associate with ascorbic acid, phenolics and % DPPH, but not with carotene and % chelating activity as most of the other cultivars do. It is clear that, in the peel, the variables are more clustered together than in the PCA for fruit pulp (Fig. 1). The associations and groupings found between antioxidant content and capacity become very clear in Fig. 2: ascorbic acid, phenolics and % DPPH group together, while carotene, betalain and % chelating activity associate. A significant relationship between total phenolics and antioxidant activity was reported by Abou-Elella and Ali [38]. Cardador-Martínez et al. [21] found the highest phenolic content in green fruit peels.

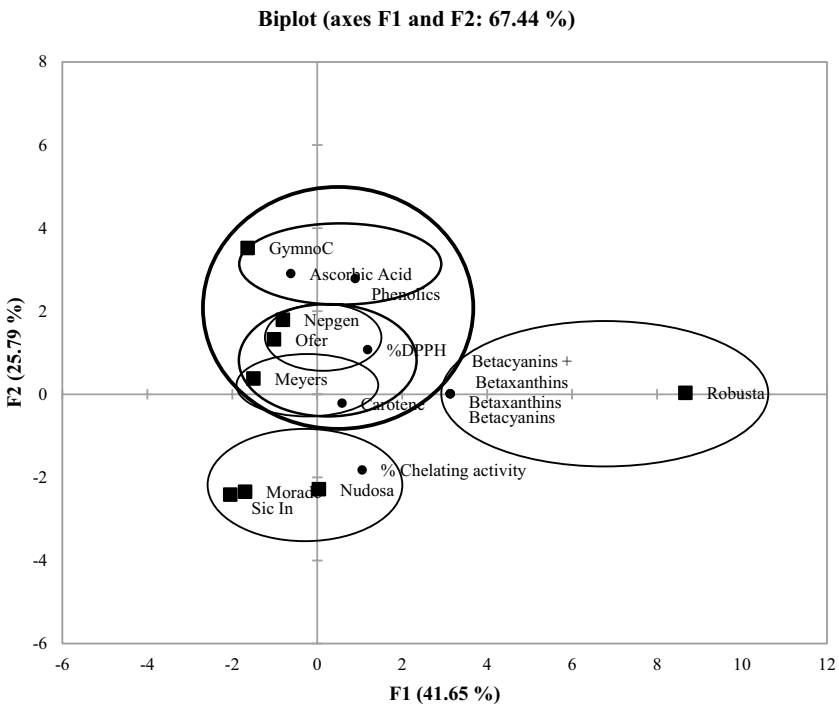

Fig. 1 Principal component analysis of cultivar and the antioxidant properties of fresh cactus pear fruit pulp

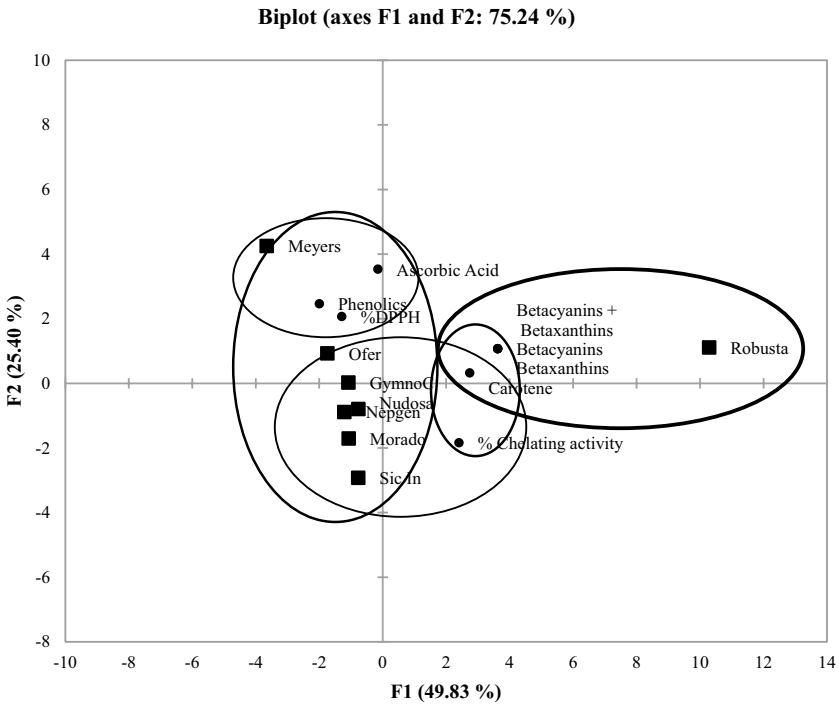

Fig. 2 Principal component analysis of cultivar and the antioxidant properties of fresh cactus pear peel

PCA of cultivar and the antioxidant properties of fresh cactus pear seed

In the biplot for seeds, Factors 1 (44.81%) and 2 (23.04%) explained 67.84% of the variation (Fig. 3). The antioxidants and antioxidant capacity variables were bundled together in the biplot for seeds. This indicated that phenolics, ascorbic acid and % DPPH that associated in fruit pulp (Fig. 1) and peel (Fig. 2) also associated in the seeds, but that % chelating activity, carotene and betalains also associated closely with this group. Therefore it may be stated that, in seeds, most cultivars would contain high amounts of all the antioxidants that were included in this study as well as have good antioxidant capacity. There were three cultivars that could not be

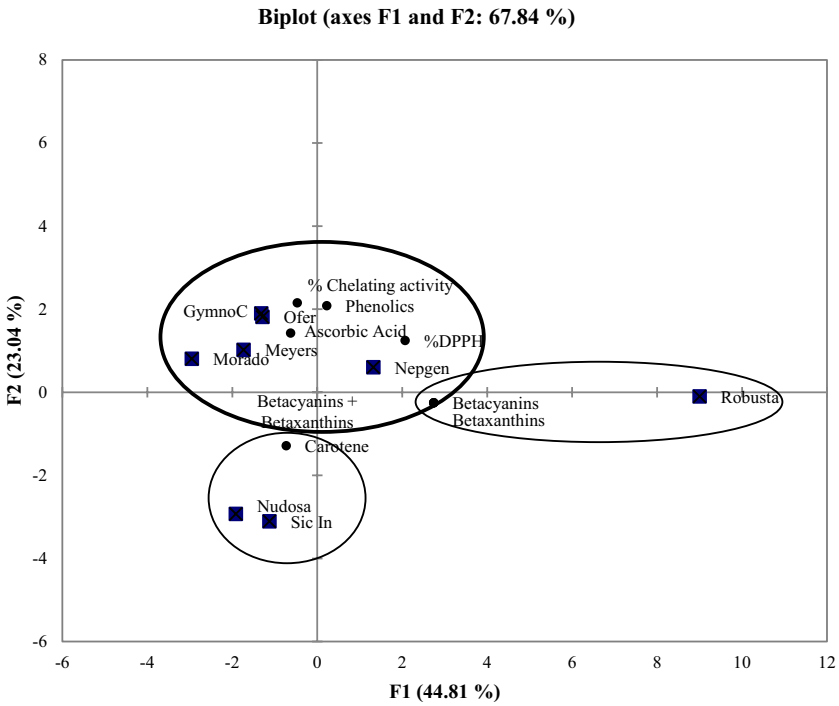

Fig. 3 Principal component analysis of cultivar and the antioxidant properties of fresh cactus pear seed

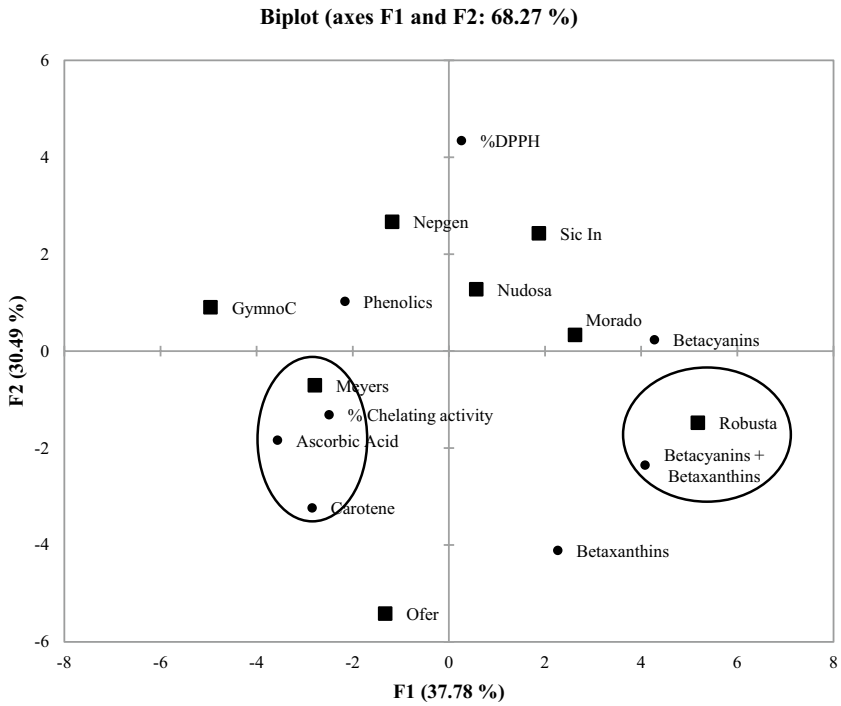

Fig. 4 Principal component analysis of cultivar and the antioxidant properties of fresh cactus pear cladodes

included in this statement: Robusta (purple) that seems to only associate with betalains and Nudosa (red) together with Sicilian Indian Fig (pink) that only associated with carotene. From this data it is derived that both green (Nepgen and Morado) and orange (Ofer and Gymno-Carpo) cultivars are associated with having more antioxidants (contents and capacity). A significant correlation between total phenolics and antiradical activity in cactus pear seeds were reported by Cardador-Martínez et al. [21].

PCA of cultivar and the antioxidant properties of fresh cactus pear cladodes

In the PCA for cladodes there are very few close associations (68.27% explained) between cultivars and antioxidants. Only Robusta and betalains are grouped together, while % chelating activity, ascorbic acid and carotene are grouped together with Meyers (Fig. 4). The other cultivars do not seem to associate with any of the antioxidants or antioxidant capacity tests in particular, indicating that cladodes do not have the strong associations with specific antioxidants as were the case in fruit pulp, peel and seeds.

Conclusions

Antioxidants are abundantly present in the fruit pulp, peel, seeds and cladodes of cactus pear plants. The seeds were the tissue type with the highest antioxidant potential. The most interesting result regarding tissue type was observed in the antioxidant capacity tests: when % DPPH was tested, peel and cladodes were consistently the highest, while in the % chelating activity tests, fruit and seeds were the best tissue types. It may seem that tissue types have different antioxidant capacity that correlates with their specific function in the plant; peel and cladodes have a protective function while fruit and seeds have a reproductive purpose. The peels showed similar antioxidant content than fruit pulp; both fruit and peel would contribute to excellent antioxidant capacity; therefore the peels should be included in the diet. Cladodes contained more phenolics and carotene than fruit although fruit had higher capacities to chelate ferrous ions. Peel and cladodes showed higher radical scavenging capabilities. Cultivar and colour did not influence antioxidant content and activity of cladodes. The cultivar that contained the highest antioxidant content and –potential was *O. robusta* Robusta. Robusta had, by far, the highest betalain contents, while it had fairly high carotene and phenolic levels. With regards to antioxidant capacity, it had the best values for % DPPH and % chelating activity. The second best cultivar would be Gymno-Carpo as it had high ascorbic acid, phenolic and carotene levels and had relatively high antioxidant capacity. Ofer would be the third best cultivar, while the pink and green cultivars had the lowest values. The inclusion of cactus pear fruit peels and seeds as well as the cladodes in the human diet is highly recommended.

Acknowledgements The authors would like to thank Dr. H. J. Fouché from the ARC for providing the cactus pear fruit. This study is

supported by the University of the Free State (UFS) Strategic Academic Cluster 4 for funding.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest be it academic, personal political or financial.

Research involving human and/or animal participants No humans or animals took part in this study.

Informed consent All the authors participated/contributed to this study.

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