[redacted]
Journal of Animal & Plant Sciences (J.Anim.Plant Sci. ISSN 2071-7024)
Vol.45 (3): 7944-7963 https://doi.org/10.35759/JAnmPlSci.v45-3.1

Bioactive molecules and pigments in cladodes, fruits and peels of Tunisian *Opuntia ficus-indica* f. *inermis*

[redacted]

Keywords: *Opuntia ficus-indica,* antioxidant activity, phenolics, betalains, colour

1 ABSTRACT

Total phenolic, total flavonoids, ascorbic acid, betalains and antiradical activity were investigated in different *Opuntia ficus-indica* f. *inermis* parts (fruits, peels and cladodes at different ages) using two types of extraction solvents (distilled water and hydroethanol 50%). Phenolic compounds and betanin were identified and quantified using a LC-mass spectrometry method. The colour pattern of fruits and peels was also studied in relation with the pigments' levels. Finally, the Tunisian production of betalains was estimated. The mesocarps exhibited the highest antiradical activity, followed by cladodes at different ages, then fruits. This activity was explained mainly by the presence of betacyanins and flavonoids. Ascorbic acid did not contribute significantly to the antioxidant capacity. The appropriate extraction solvent was linked to the plant matrix. The identified phenolic compounds include the following families: phenolic acids, flavan-3-ol, flavanones, flavonols and flavones. Betanin and isobetanin were also identified. The betaxanthins/betacyanins ratio differed significantly between fruits and peels and appeared responsible for the difference in colour shades. Finally, this work reported an estimation of the Tunisian production potential of betalains, which were about 542.33 t / year. The findings of this study confirm the antioxidant potential of *Opuntia ficus-indica* f. *inermis* mesocarps and suggest their valorisation as a natural source of colorants and antioxidants in food and drug industries.

2 INTRODUCTION

Cacti plants (Cactaceae family) are historically associated with South America and mainly southern regions of North America. They are currently widespread particularly in arid regions throughout the world. They can be considered a noxious weed in some places, a low maintenance desert ornamental plant, and a natural resource for food and medicine (Nazareno, 2015). Their derived products include fresh de-spined pads, canned sliced "nopalitos" and/or pickled pads, bottled fruit juice, fruit nectar concentrates, spreads, sauces, and dried flower teas and tinctures (Brinker, 2009). Over the last few decades, Cacti, and the prickly pear cactus species (*Opuntia spp.*) in particular, have been the subject of greater scientific interest due to their potential health benefits. Prickly pear cacti (*Opuntia* genus) have stems like flattened pads (called cladodes), young tender pads (called nopales), and a cylindrical green fruit when immature and yellow to red when mature. *Opuntia* fruits range from 6 to 12 per pad and vary in colour, thickness, and shape (Brinker, 2009; Bekir, 2006; Piga, 2004). Prickly pear cactus is grown in the countryside in North Africa, especially in Tunisia, and prickly pears are considered refreshing fruits and are greatly appreciated. *O. ficus-indica* fruits are known for their highly nutritive value since they contain large amounts of nutrients and bioactive compounds. Recent studies have reported notable antioxidant activity for European and Asian *O. ficus-indica* varieties, leading to reduced oxidative stress in patients (Tesoriere *et al.,* 2004; Brahmi *et al.,* 2011; Abd El-Razek *et al.,* 2012). Antioxidant activity of *Opuntia* fruits is seemingly due to their content of ascorbic acid, polyphenolic acids, flavonoid compounds (e.g. Kaempferol, quercetin and isorhamnetin), taurine and betalains (Stintzing *et al.,* 2001; Tesoriere *et al.,* 2005; Fernández-López *et al.,* 2010). Two betalain derivatives are present in cactus pears: betacyanins, which are associated with a reddish-purple colour, and betaxanthins, producing a yellowish-orange colour (Gentile *et al.,* 2004; Felker *et al.,* 2008). These pigments showed great antioxidant activity without any toxic effects not only *in* vitro (Cai *et al.,* 2003), but also in human models (El-Samahy *et al.,* 2006; Livrea *et al.,* 2009). Whereas both ascorbic acid and phenolics are known as hydrophilic antioxidants, carotenoids are considered lipophilic ones (Halliwell, 1996; Fernández-López *et al.,* 2010), and betalains have rather an amphiphilic character (Turco-Liveri *et al.,* 2009). Therefore, extracting and quantifying these pigments seems to be greatly influenced by the solvent. Phytochemical contents of *O. ficus-indica* were assessed in several studies. Unfortunately, most of these studies were often limited to fruits (Butera *et al.,* 2002; Castellar *et al.,* 2003; Felker *et al.,* 2008; Fernández-López *et al.,* 2010; Chahdoura *et al.,* 2014), then cladodes (Hadj Sadok *et al.,* 2008; Chahdoura *et al.,* 2014). Few studies focused on the bioactive compounds of peels (Yeddes *et al.,* 2013). In addition, cladode age may have an effect on the antioxidant activity. However, this likely effect has not been examined in previous studies. This study hypothesizes that the antioxidant activity of *O. ficus-indica* f. *inermis* varies among its different matrices (cladodes, fruits, and peels), the age of cladodes and the type of solvent used for extraction. These variables may influence the colour of fruits and peels.

3 MATERIAL AND METHODS

3.1 Plant material: Cladodes and fruits were collected from an improved pasture managed by the Office of Livestock and Pasture, and located in Sawwaf, Zaghouan governorate (northern Tunisia). Sawwaf has a semi-arid climate, according to the Emberger climate classification. The average annual temperature is 17.8 °C and the average annual rainfall is 447 mm. *Opuntia ficus-indica var inermis* cladodes were randomly harvested at different stages of development, as explained in Table 1, whereas fruits were harvested only at physiological maturity. All biological materials were immediately brought to the laboratory and stored at -20°C until use.

Table 1: Physical characteristics of harvested cladodes

Age (days)	Length (cm)	Width (cm)	Weight (g)
< 30	7.69 ± 1.51	4.06 ± 0.87	11.92 ± 4.27
30-45	12.35 ± 1.30	6.30 ± 0.67	30.52 ± 5.71
45-60	23.15 ± 1.30	12.32 ± 0.69	142.72 ± 19.16
60-75	28.21 ± 1.08	14.20 ± 1.23	200.41 ± 34.41
75-90	32.35 ± 1.74	14.25 ± 2.61	254.80 ± 67.57
90-105	38.14 ± 1.39	14.54 ± 2.17	318.35 ± 65.23

3.2 Extraction: The peel was separated manually from the pulp. Samples were washed, chopped into small pieces and homogenized with an automatic press. Known quantities of ground material were then subjected to a solid-liquid extraction at 4°C for 18h using two solvents with increasing polarity: aqueous ethanol (1:2 v:v) and distilled water, then centrifuged at 4500 *g* at 4°C for 15min. The supernatants were collected and stored at -20 °C until analysis.

3.2.1 Total phenolics: Total phenolics were estimated according to Ainsworth and Gillespie (2007) with some modifications. Briefly, 600 µL of 10% (v/v) Folin Ciocalteu reagent was added to 300 µL of each extract, standard or 95% (v/v) methanol blank and vortexed thoroughly. After that, 2.4 ml of 700 mM Na_2CO_3 (in water) was added and the assay tubes were incubated at room temperature for 2h. Absorbance was determined at 765nm using a Lasany UV Vis spectrophotometer. A calibration curve was calculated from the blank-corrected A_{765} of the gallic acid standards (gallic acid solutions of 50 mg/L – 400 mg/L (in 95% methanol)). Total phenolics were then calculated as gallic acid equivalents (GAE) using the regression equation between gallic acid standards and A_{765}.

3.2.2 Total flavonoids: Flavonoid content was determined as described by Zhishen *et al.* (1999). Two hundred and Fifty (250) µL of the extract was placed in a 15 mL volumetric flask. Seventy Five (75) µL $NaNO_2$ (1:20 w: v) was added, followed by 150 µL $AlCl_3$ (1:10) 5 min later. After incubation for 6 mins, 500 mL of NaOH (1 $molL^{-1}$) was added and then distilled water was added until the total volume reached 2.5 mL. The solution was vortexed thoroughly and absorbance was measured against a blank (ethanol) at 510 nm with a Lasany UV-Vis spectrophotometer. Quercetin (0.05 mg/ml) was used as a standard compound.

3.2.3 Ascorbic acid: Ascorbic acid content was determined according to Dürüst *et al.* (1997). Briefly, 0.10 mL of each extract (a dilution factor in oxalic acid at 0.4% was utilized for each sample) was added to 0.100 mL of acetate buffer. Then, 0.800 mL of DCPI (2, 6-dichloroindophenol sodium) was added. The absorbance of the mixture was measured after 15s at 520 nm with a Lasany UV-Vis spectrophotometer. Ascorbic acid was used as a standard and the results were expressed as mg ascorbic acid/g of sample fresh weight.

3.2.4 Betalains: As described by Prakash and Manikandan (2012), 10 mL of each extract was mixed with 10 mL of 50% aqueous methanol solution and the mixture was agitated at 250 rpm for 30 min. Then, the samples were centrifuged at 4731*g* for 15min at 4 °C.

Betaxanthin and betacyanin were assessed spectrophotometrically (Lasany UV-Vis spectrophotometer) as indicaxanthin and betanin equivalents at 480 and 536 nm, respectively. Betalain content was evaluated as reported by Stintzing *et al.* (2005) using Eq. 1.

$$BC = \frac{AXDFXMWX\ 1000}{\varepsilon X\ 1} \quad \text{(Equation 1)}$$

where: BC (mg/l) is betacyanin or betaxanthin content, A is the absorption value of the sample, MW is the molecular weight (indicaxanthin =

308 g/mol and betanin = 550 g/mol), ε is the molar extinction coefficient (indicaxanthin = 48,000 L mol^{-1} cm^{-1} at 480 nm and betanin= 65,000 L mol^{-1} cm^{-1} at 536 nm), and 1 cm is the path length of the cuvette.

3.2.5 Free radical scavenging activity: Antiradical activity was measured with DPPH· (1,1-diphenyl-2-picrylhydrazyl radical) as described by Brand-Williams et al. (1995) and modified by Thaipong *et al.* (2006). The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and stored at -20°C until use. The working solution was obtained by mixing 10 mL of the stock solution with 45 ml of methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm. To evaluate the antiradical activity, 150 µL of each extract was allowed to react with 2850 µL of the DPPH solution for 24h in the dark. The absorbance was taken at 515 nm. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxilic acid) was used as a standard. The calibration curve was linear between 25 and 800 µM Trolox. Results are expressed as mg TE/g of sample fresh weight.

3.3 LC-mass spectrometry analysis: A Shimadzu Ultra-Fast Liquid system (Shimadzu prominence UFLC XR, Japon) was used for biomolecules identification. HPLC separations were performed on an AQUASIL thermo C18 (3×150 mm, 3 µm) at 40°C. A guard column was used (AQUASIL thermo C18 3×10 mm, 3 µm). For phenolic acids and flavonoids, the separation lasted 55 min, followed by 5 min equilibrium time. The chromatographic conditions were as follow: The mobile phase consisted of water + 0.1% formic acid (A) and methanol + 0.1% formic acid (B). The binary gradient elution was as follows: 0–45 min, 10% B; 45–55 min, 100% B; 55–55.1 min, 10% B; 55.1-60 min, 10% B. The flow rate was 0.4 mL/min, and the injection volume was 5 µL for analysis. Betanin separation lasted 25 min, with an additional 5 min equilibrium time. The mobile phase consisted of water + 2% formic acid (A) and methanol (B). The binary gradient elution was planned as follows: 0.01-15 min, 5% (B); 15-20 min, 15% (B); 20-22 min, 70% (B); 22-25 min, 100% (B); 25-25.01 min, 100% (B); 25.01- 30 min, 5% B. Calibration curves of standards were used for quantitative determination. A Shimadzu 2020 (Japon) Quadrupole mass spectrometer equipped with a positive/negative ESI source was used as a detector. For phenolic compounds identification, mass spectrometer was operated in the negative selected ion monitoring (SIM) with capillary, voltage at 1.2 V. For betanin, mass spectrometer was operated in a positive SIM for betanin. MS analysis conditions were designed as follow: the heat block was set at 400°C, the desolving line temperature was 250°C, the spray voltage was -3.5 V, the nebulizer gas flow was 1.5 L/min, and the drying gas flow was 12.00 and 15.00 respectively for phenolics and betanin. Finally, the detector voltage was 1.2 V.

3.4 Colour analyses: Peels, fruits and cladodes of different ages were subjected to colour analyses. A CR-410 chromameter was used. Chroma C* and huge angle h° were calculated as using Eq.2 and Eq.3.

$$C* = (a*2 + b*2)^{0.5} \quad \text{(Equation 2)}$$

$$h° = arctan(b*/a*) \quad \text{(Equation 3)}$$

Where *a** and *b** are the position in the red-green and blue-yellow axes, respectively.

3.5 Statistical analyses: All samples were analysed in triplicate. The data were expressed as a mean ± standard deviation (SD). Three-way nested Analysis of Variance (ANOVA) was used to test the fixed effect of Solvent, Plant part, and Age nested within Plant part, as well as the Solvent * Plant matrix, and Solvent* Age (Plant matrix) interactions on the following continuous dependent variables: Antioxidant activity, Polyphenols, Flavonoids, Ascorbic acid, Betaxanthins and Betacyanins. Least squares means (LS-means) of fixed effects were computed and a multiple comparison adjustments were done using the Tukey method. Global correlations and multiple linear regressions were used to compare and express the relationship between the studied quantitative variables. These analyses were done using Proc Mixed, Proc Reg and Proc Corr in SAS 9.2 (SAS Institute Inc, Cary, NC, USA). Principal component analysis (PCA) was carried out in six quantitative factors (Antioxidant activity,

Polyphenols, Flavonoids, Ascorbic acid, Betaxanthins, Betacyanins) and two supplementary qualitative ones (plant matrix and solvent type), using FactoMineR (Lê *et al.,* 2008), an R package.

4 RESULTS

4.1 Total phenolics and flavonoids: In cladodes, total phenolics ranged from 0.818 to 0.959 mg GAE/g. Total flavonoids varied from 0.318 to 0.362 mg QE/g. In fruits, phenolics and flavonoids were three folds lower than those recorded in cladodes (0.311±0.02 mg GAE/g and 0.111±0.005 mg QE/g, respectively). The highest contents were recorded in peels (1.595±0.16 mg GAE/g and 0.491±0.014 mg QE/g, respectively). Regarding extraction solvents, distilled water allowed obtaining the highest polyphenols amounts, whereas hydroethanol allowed extracting higher flavonoid amounts (Table 2, Fig. 1).

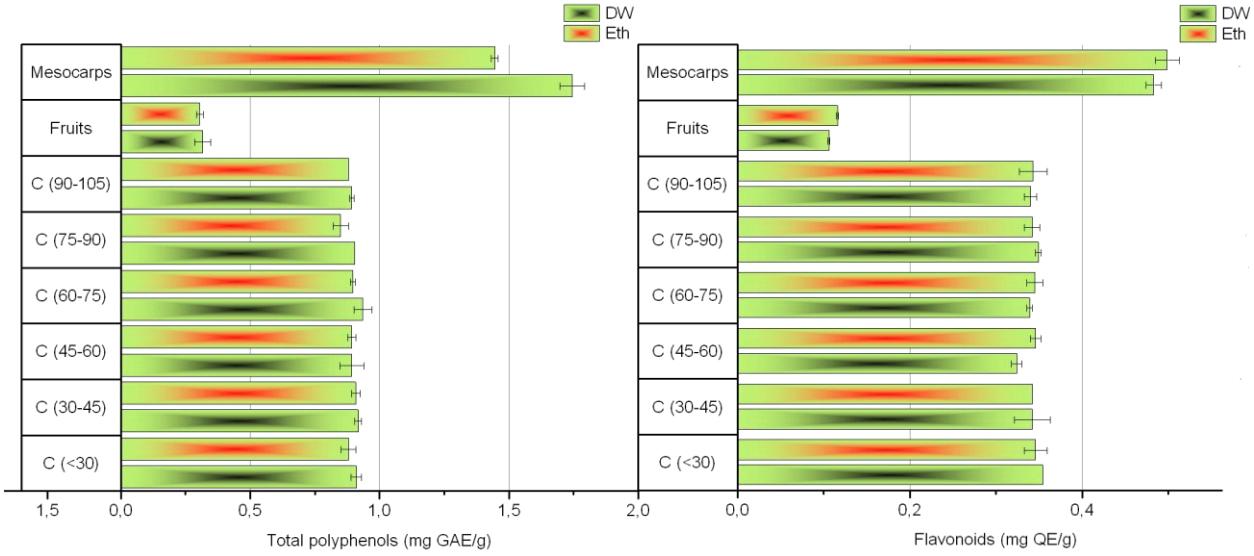


Figure 1. Total polyphenols and flavonoid content in fruits, peels (mesocarps) and cladodes of *Opuntia ficus-indica (*L.) Mill. The age of cladodes (in days) is mentioned between brackets. DW: distilled water, Eth: hydroethanol

Total phenolics and flavonoids contents were not affected significantly ($p > 0.05$) by cladode age. They mainly depended on the polarity of the solvent used for extraction and the plant matrix ($p < 0.0001$). A significant interaction ($p < 0.0001$) Solvent*Plant matrix was reported only for polyphenols (Table 3).

Table 2: Phytochemicals contents and antioxidant activity of different matrices from *Opuntia ficus-indica* f. *inermis*

		Antioxidant activity	Polyphenols	Flavonoids	Ascorbic acid	Betaxanthins	Betacyanins
Solvent	**DW**	0.4938 ^{a}	0.990 ^{a}	0.3101 ^{a}	0.1578 ^{a}	47.0000 ^{a}	14.0983 ^{a}
	Eth 50%	0.6166 ^{b}	0.8789 ^{b}	0.3195 ^{b}	0.2254 ^{b}	43.9883 ^{b}	13.0992 ^{a}
	Peels	1.1225^{a}	1.5960 ^{a}	0.4910 ^{a}	0.2211 ^{ab}	49.3227 ^{a}	21.1750 ^{a}

Plant matrix	**Fruits**	0.2623^{c}	0.3105 ^{c}	0.1107 ^{c}	0.1557 ^{ab}	41.6657 ^{b}	6.0225 ^{b}
	C <30	0.3182 ^{b}	0.8955 ^{b}	0.3500 ^{b}	0.1496 ^{ab}	-	-
	30<C<45	0.2192 ^{d}	0.9127 ^{b}	0.3422 ^{b}	0.1175 ^{b}	-	-
	45<C<60	0.2370 ^{cd}	0.8930 ^{b}	0.3350 ^{b}	0.2009 ^{ab}	-	-
	60<C<75	0.3307 ^{b}	0.9160 ^{b}	0.3422 ^{b}	0.2842 ^{a}	-	-
	75<C<90	0.2920 ^{bc}	0.8773 ^{b}	0.3458 ^{b}	0.2116 ^{ab}	-	-
	C >90	0.2872 ^{bc}	0.8865 ^{b}	0.3417 ^{b}	0.2244 ^{ab}	-	-

DW: Distilled water, Eth 50%: Hydroethanol 50%, C: Cladodes with their corresponding age (in days)

Table 3: *P-values* of some factors affecting the antioxidant activity and the phytochemicals contents in *Opuntia ficus-indica f. inermis*

	Solvent	**Plant matrix**	**Cladode's age**	**Solvent*Plant matrix**	**Solvent*cladode's age**
Antioxidant activity	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Polyphenols	<0.0001	<0.0001	0.1024	<0.0001	0.1024
Flavonoids	0.0411	<0.0001	0.2712	0.2030	0.1784
Ascorbic acid	0.0294	0.0577	0.0137	0.0341	0.7495
Betaxanthins	0.0297	0.0010	-	<0.0001	-
Betacyanins	0.0564	<0.0001	-	0.0005	-

4.2 Ascorbic acid: The ascorbic acid values measured in different matrices ranged from 5 mg/100 g to 40 mg/100 g of fresh weight (Fig. 2). The highest contents were recorded in peels, followed by cladodes and fruits (22.10±0.13 mg/100 g, 20.74±0.07 mg/100 g and 15.57± 0.024 mg/100 g, respectively). Ethanol 50% allowed obtaining the highest ascorbic acid contents, compared to distilled water (0.225 vs 0.157 mg/g, respectively) (Table 2). As shown in Table 3, ascorbic acid concentrations were mainly affected by the polarity of the solvent (p=0.0294) and the age of cladodes (p=0.0137). A significant statistical interaction between the Solvent and the Plant matrix was noted (p=0.0341).

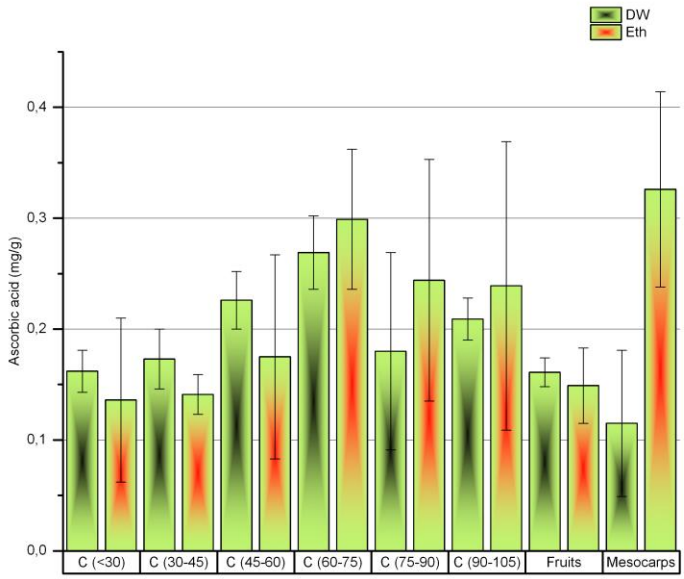


Figure 2. Ascorbic acid contents in different *Opuntia ficus-indica* (L.) Mill. Plant matrix
DW: distilled water, Eth: Ethanol 50%

4.3 Betalains: As reported in Table 2, *Opuntia* peels contain higher amounts of betalains than fruits (70±0.011 vs 47±0.013 mg/Kg, respectively). The lowest levels of both

betaxanthins and betacyanins were recorded in fruits (42±0.012 and 6±0.00 mg/Kg, respectively). In peels, betaxanthin concentrations were 2.33-folds higher than those of betacyanin. However, they were seven folds higher in fruits. As reported in Table 4, betacaxanthins were mostly extracted by distilled water in peels. The extraction yield of these pigments was significantly greater in fruits when ethanol 50% was used ($p < 0.05$). No effect of solvent polarity on the extraction yield of betacyanins was noted.

Table 4: Betalain contents in *Opuntia ficus-indica f. inermis* fruits and peels

	Fruits			Peels		
Solvent	Betaxanthins (mg/Kg)	Betacyanins (mg/Kg)	Betalains (mg/Kg)	Betaxanthins (mg/Kg)	Betacyanins (mg/Kg)	Betalains (mg/Kg)
Water	30.05^{b}±0.00	5.28 ^{a}±0.00	35.34^{b}±0.00	57.92^{a}±0.003	22.91^{a}±0.00	80.84^{a}±0.003
Ethanol 50%	53.27^{a}±0.00	6.76^{a}±0.001	60.04^{a}±0.00	40.72^{b}±0.00	19.43^{a}±0.001	60.16^{b}±0.001

4.4 Antioxidant activity: Variations in antioxidant activity of cladodes, fruits and peels from *Opuntia ficus-indica are* illustrated in Fig.3. The calculated values varied between 0.100 and 0.460 mg TE/g in cladodes at different studied ages, 0.262±0.001 mg TE/g in fruits and 1.123±0.120 mg TE/g in peels (Table 2). In cladodes, the highest antioxidant activity was 0.331 mg TE/g in samples aged between 60 and 75 days. A significant inhibition of DPPH˙ radicals ($p < 0.0001$) was obtained using aqueous ethanol as an extraction solvent (Table 3).

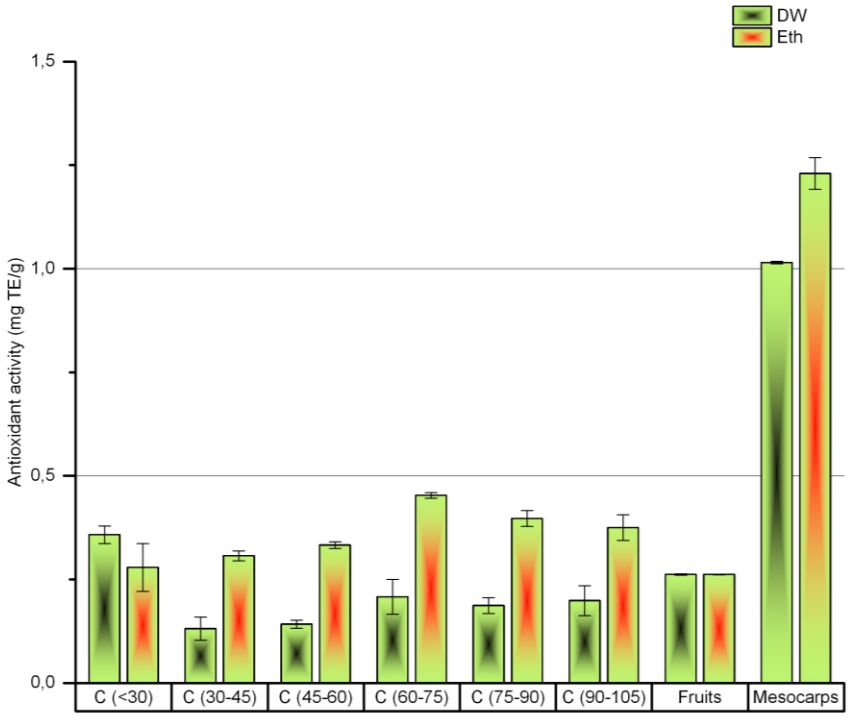


Figure 3. Antioxidant activity of *Opuntia ficus-indica* (L.) Mill. studied samples.
DW: distilled water, Eth: Hydroethanol

The DPPH scavenging activity of the studied samples depended significantly on the extraction solvent, the plant matrix, the age of cladodes in addition to the interaction between the extraction solvent with the plant matrix and the age of cladodes (Table 3). Correlation analysis revealed a high and significant linear relationship between DPPH scavenging activity and total

phenolics, flavonoids as well as betalains (Table 5). No correlation was found between ascorbic acid and the other quantitative variables.

Table 5: Correlation matrix of total phenolics, flavonoids, ascorbic acid, betaxanthins, betacyanins and DPPH scavenging activity

	Antioxidant Activity	Polyphenols	Flavonoids	Ascorbic acid	Betaxanthins	Betacyanins
Antioxidant Activity	1					
Polyphenols	0.757 ****	1				
Flavonoids	0.615 ****	0.932 ****	1			
Ascorbic acid	0.185	0.052	0.222	1		
Betaxanthins	0.629****	0.352 **	-0.086	-0.149	1	
Betacyanins	0.883 ****	0.688 ****	0.370 **	-0.019	0.877 ****	1

Significance levels: ****: p<0.000, **: p<0.01

A prediction model (Eq. 4) of the antioxidant activity of fruits and peels (AOA) was adjusted based on their flavonoids ($FLAV$) and betacyanins (BC) contents (mg/g). The linear model's coefficients were significant for the antiradical activity of *Opuntia* fruits and peels (*p*-values were 0.021, 0.000 and 0.001 for the constant, α and ß coefficients, respectively). For cladodes, a lower significant relationship (p<0.0001) was reported between antioxidant activity (AOA) and flavonoid content $FLAV$ (Eq. 5). *P-values* of the constant and α coefficient were 0.123 and 0.000, respectively.

Fruits and peel:

$AOA = 0.0663 + 3.59\,FLAV - 33.4\,BC$; R^2 = 99.4%, Adjusted R^2 =99.3% (Equation 4)

Cladodes:

$AOA = -0.183 + 1.74\,FLAV$; R^2 = 36.3%, Adjusted R^2 =34.9% (Equation 5).

4.6 PCA results: PCA analysis was restricted to the first and second principal components (PC1 and PC2) which had variance eigenvalues greater than 1.0 and accounted for 83.01% of cumulative variance. PC1 contributed to 57.19% of the observed variance and was composed of the antioxidant activity in addition to betacyanins and polyphenols. PC2 accounted for 25.81% of variance and was composed of flavonoids, ascorbic acid and betaxanthins. The variables' contribution to each axis is detailed in Table 6.

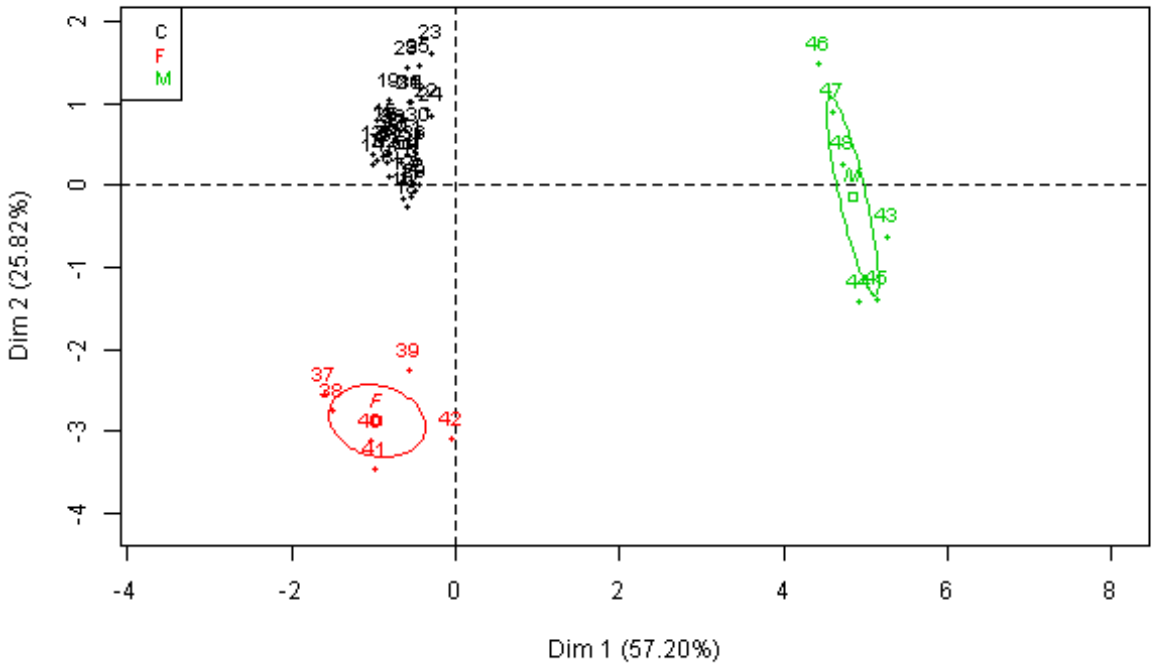
Table 6: Eigen values analysis of the PCA axes

	Variables' contributions		Variables' correlations	
	PC1	PC2	PC1	PC2
Antioxidant activity	26.44	0.08	0.95	0.03
Polyphenols	22.23	5.34	0.87	0.28
Flavonoids	12.58	29.04	0.65	0.67
Ascorbic acid	0.05	29.04	0.04	0.67
Betaxanthins	13.26	29.96	0.67	-0.68
Betacyanins	25.40	6.51	0.93	-0.31

In the PCA plot shown in Fig.4, three distinct groups representing three different plant matrices were distinguishable. The first one is that of peels, characterized by high antioxidant activity as well as considerable amounts of polyphenolics, flavonoids and betalains. The second group is that of cladodes, having lower antioxidant activity, polyphenol and flavonoid concentrations. The third group represents the

fruits, which recorded the lowest antioxidant activity as well as the phytochemicals contents.

(a)



(b)

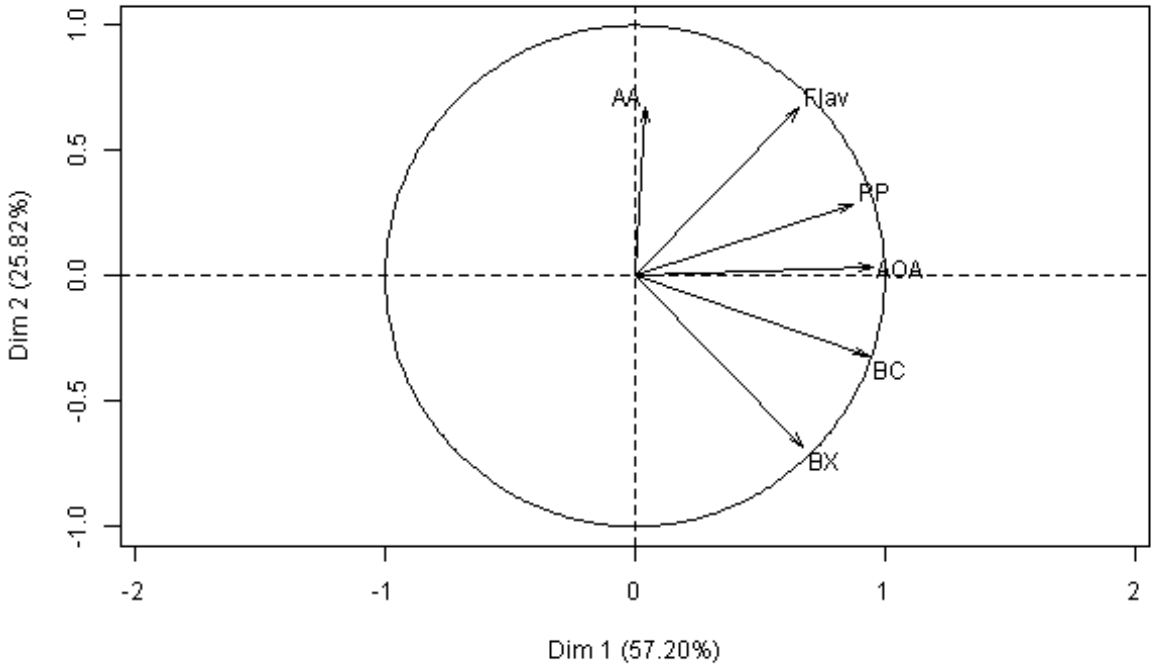


Figure 4. PCA score plot group for studied samples from *Opuntia ficus-indica f. inermis*: (a) PCA individual plot. (b) PCA

variables factor map. C: Cladodes, F: Fruits, M: Peels, AA: Ascorbic acid, Flav: Flavonoids, PP: Polyphenols, AOA: Antioxidant activity, BC: Betacyanins, BX: Betaxanthins.

4.7 LC-MS analysis results: Compared to fruits and cladodes, *Opuntia* peels contain 3.2 folds higher amounts of quinic acid, 2 folds greater contents of cinnamic acid, 5.32 folds larger quantities of 1, 3-di-*O*-caffeoylquinic acid and 6.8 superior levels of ferulic acid. Noteworthy, the highest contents of rutin, a potent antioxidant biomolecule, were recorded in peels (51.667 mg/Kg), and followed by cladodes (31.9 mg /Kg) and fruits (3.18 mg/Kg). The lowest amounts of cirsiliol were calculated in peels. Naringenin was also identified in *Opuntia* peels at 0.377 mg/Kg. Interestingly, betanin content was 1.53 folds higher in peels compared to fruits (Table 7).

Table 7: Phenolic compounds and betanin content

Compound	Empirical Formula	Content (mg/Kg)		
		Cladodes	Fruits	Peels
Quinic acid	$C_7H_{12}O_6$	429.827	454.716	1450.709
trans cinnamic acid	$C_9H_8O_2$	N.D.	4.288	8.644
Protocatechuic acid	$C_7H_6O_4$	N.D.	0.127	0.153
Syringic acid	$C_9H_{10}O_5$	3.016	0.852	N.D.
4-*O*-caffeoylquinic acid	$C_{16}H_{18}O_9$	0.070	0.127	0.505
1,3-di-*O*-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	0.174	0.124	0.798
trans ferulic acid	$C_{10}H_{10}O_4$	1.103	0.006	3.772
O-coumaric acid	$C_9H_8O_3$	N.D.	0.406	N.D.
Salviolinic acid	$C_{36}H_{30}O_{16}$	0.242	0.057	0.169
Catechin (+)	$C_{15}H_{14}O_6$	N.D.	0.074	N.D.
Naringin	$C_{27}H_{32}O_{14}$	0.330	1.059	1.700
Naringenin	$C_{15}H_{12}O_5$	N.D.	0.003	0.377
Rutin	$C_{27}H_{30}O_{16}$	31.927	3.180	51.667
Hyperoside	$C_{21}H_{20}O_{12}$	0.050	0.234	1.676
Quercetrin	$C_{21}H_{20}O_{11}$	N.D.	0.033	0.303
Luteolin-7-*O*-glucoside	$C_{21}H_{20}O_{11}$	0,141	0.057	0.074
Apegenin-7-*O*-glucoside	$C_{21}H_{20}O_{10}$	0,001	0.018	0.029
Luteolin	$C_{15}H_{10}O_6$	N.D.	0.014	0.005
Apigenin	$C_{15}H_{10}O_5$	0.004	0.002	0.002
Cirsiliol	$C_{17}H_{14}O_7$	15.024	8.797	6.581
Acacetin	$C_{16}H_{12}O_5$	7.342	7.191	6.942
Gallic acid	$C_7H_6O_5$	N.D.	N.D.	N.D.
Epicatechin	$C_{15}H_{14}O_6$	N.D.	N.D.	N.D.
Chlorogenic acid	$C_{16}H_{18}O_9$	1.634	N.D.	N.D.
3,4-di-*O*-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	N.D.	N.D.	N.D.
4,5-di-*O*-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	N.D.	N.D.	N.D.
Caffeic acid	$C_9H_8O_4$	0.089	N.D.	0.175
p-coumaric acid	$C_9H_8O_3$	0.090	N.D.	0.390
Rosmarinic acid	$C_{18}H_{16}O_8$	N.D.	N.D.	N.D.
Myricetin	$C_{15}H_{10}O_8$	N.D.	N.D.	N.D.
Silymarin	$C_{25}H_{22}O_{10}$	N.D.	N.D.	N.D.
Quercetin	$C_{15}H_{10}O_7$	N.D.	N.D.	N.D.
Kaempferol	$C_{15}H_{10}O_6$	N.D.	N.D.	N.D.
Cirsilineol	$C_{18}H_{16}O_7$	N.D.	N.D.	N.D.
Isorhamnetin	$C_{16}H_{12}O_7$	N.D.	N.D.	N.D.
Aucubin	$C_{15}H_{22}O_9$	N.D.	N.D.	N.D.
Betanin	$C_{24}H_{26}N_2O_{13}$	-	1616	2473

Journal of Animal & Plant Sciences (J.Anim.Plant Sci. ISSN 2071-7024)
Vol.45 (3): 7944-7963 https://doi.org/10.35759/JAnmPlSci.v45-3.1

4.8 Colour shades: As represented in Fig. 5, the brightness of cladodes varied between 45.45 and 51.15. The lightness *L** was recorded for cladodes between 60 and 75 days old. The green to red colour index represented by *a** ranged between -8.66 (> 90 days old) and -15.24 (30-45 days old). The blue to yellow colour index represented by b* varied from 9.57 (> 90 days old) to 23.03 (30-45 days old).

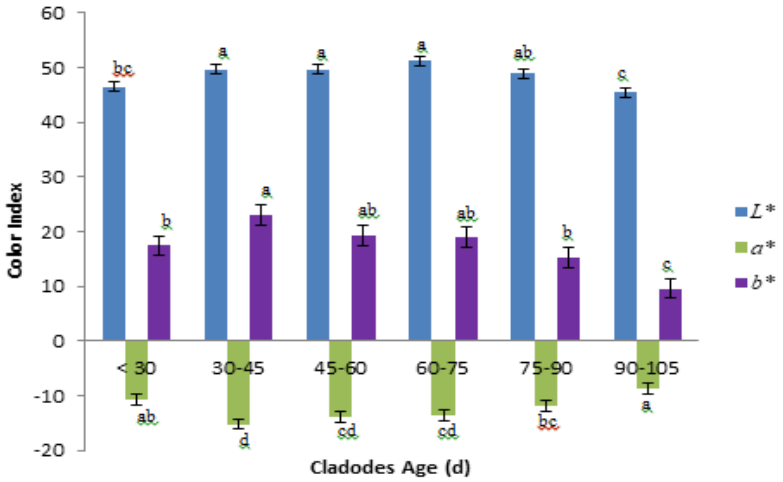


Figure 5. Variation of *L*a*b** colour shades of sampled cladodes from *Opuntia ficus-indica f. inermis*. *L*, a** and *b** values depended significantly ($p < 0.0001$) on cladodes' age.

In fruits and peels, different *L*a*b** colour shades were observed. Our values depended significantly on the plant matrix ($p < 0.0001$). The highest values were recorded for fruits. Moreover, the results here were closely correlated with the betaxanthin/betacyanin ratio (Table 8).

Table 8: Colour attributes of fruits and peels from *Opuntia ficus-indica*

	*L**	*a**	*b**	*C**	*h°*	Bx/Bc [a]
Fruits	59.64[a]	14.35 [a]	58.48[a]	60.35[a]	1.32[a]	6.79[a]
Peels	49.32[b]	10.53[b]	32.10[b]	33.96[b]	1.25 [b]	2.31[b]
Correlation with Bx/Bc (p-value)	0.000	0.011	0.000	0.000	0.009	1

[a]Bx/Bc ratio was calculated based on data from Table 2. Bc: Betacyanins, Bx: Betaxanthins

4.9 Estimation of betalains' production: In Tunisia, the potential for producing betalains from *Opuntia ficus-indica* f. *inermis* was estimated at 542.33 t per year. Related calculations are presented in Table 9.

Table 9: Tunisian betalain production from spineless *Opuntia ficus-indica*

	Weight (g)	Cultivation mode	Yield[a] (t/ha)	Global production[b] (t)	Betalain content [c] (mg/Kg)		Total betalain yield (t)	
					Bx	Bc	Bx	Bc
Fruits	54.83±7.34	Intensive Extensive	6.37 12.75	22295 5055375	53.27	6.76	1.18 269.29	0.15 34.17
Mesocarps	31.76±2.94	Intensive Extensive	3.69 7.38	12915 2926170	57.92	22.91	0.74 169.48	0.29 67.03
Total betalain yield					111.19	29.67	440.69	101.64

[a] Calculations were based on a global production of intensive and extensive cultivated areas estimated at 10 t/h and at 20 t/h, respectively.; [b] Cultivated surfaces were estimated at 400000 ha according to data published by the Ministry of Agriculture. Intensive areas were estimated at 3500 ha.; [c] Data presented previously in Table 4.

4 DISCUSSION

Opuntia species are considered medicinal and industrial plants. They remain a reliable source of not only bioactive phytochemicals, but also natural colorants which can be used instead of synthetic ones in the food industry. Phytochemical screening of different parts of *Opuntia ficus-indica made* it possible to identify different secondary metabolites. Polyphenols are one of the major groups of compounds acting as primary antioxidants or free terminators (de la Rosa *et al.*, 2010). Quantitative analysis of polyphenols and flavonoids using the spectrophotometric method has been well-known for a long time (Jurd et Geissmao, 1956). In this study, total phenolic content was determined using the Folin-Ciocalteu method, and reported as gallic acid equivalents by reference to a standard curve. Our results showed that the highest levels of phenolic compounds were recorded in peels, and were 5.11-fold higher than fruits and 1.77-fold higher than cladodes. This study findings are in agreement with those of Díaz Medina *et al.* (2007); Moussa Ayoub *et al.* (2011), Yeddes *et al.* (2013), *et al.* and *et al.* who reported that the highest polyphenolic content was recorded in skin matrix. Interestingly, our total phenolics contents in *Opuntia* cladodes are much higher than those reported by Ramirez-Moreno *et al.* (2013). This difference may be attributed to the effect of intrinsic and environmental factors on the plant's secondary metabolites syntheses (Nazari *et al.,* 2018). In fact, the content of secondary metabolites is affected by several factors such as climate stress, cultural conditions, soil, and genetic background (Mikulajová *et al.,* 2016). The biosynthesis and aggregation of phytochemical metabolites is stimulated through the stress-related compounds: the more the plant is stressed, the more secondary metabolites are produced (Matkowski *et al.,* 2008). Flavonoids are a group of secondary metabolites belonging to the class of phenylpropanoids. They have the widest colour range, varying from pale-yellow to blue. In this study, flavonoids account for 30.78%, 35.48% and 38.16% of total polyphenols from peels, fruits and cladodes, respectively. This study results were slightly higher than those reported by Cardador-Martínez and his collaborators (2011). These authors reported that flavonoids extracted from *Opuntia supp.* using ethanol 70% account for 11-26% of total polyphenols. Similarly, to polyphenols, flavonoids in peels were respectively 4.46 and 1.43 folds higher than fruits cladodes. Our results agreed with those reported by Kuti (2004) who stated that flavonoids in *Opuntia* fruits ranged from 0.98 to 9.35 mg per 100g. In cladodes, our results were lower than those reported by Dib *et al.* (2013) which were 11.86 mg QE/ g DW. Cladode age, environment, soil type and climate could explain these variations in cactus polyphenol and flavonoid content. Regarding ascorbic acid, our values were higher than those reported by Hadj Sadok *et al.* (2008), but within the range cited by De Kock (1965) and Felker (1995) who reported that the ascorbic acid content of cladodes might vary from 10 mg/ 100 g to 30 mg/100 g. Similarly, edible portion content of ascorbic acid was comparable to that reported by Butera *et al.* (2002), but higher than that calculated by Stintzing *et al.* (2005). Higher values have also been reported (Gurrieri *et al.,* 2000; Kuti, 2004). Once more, intrinsic and environmental factors on could explain this variance (Nazari *et al.,* 2018). Betalains are a group of pigments containing betacyanins (violet) and betaxanthins (yellow). They exist as internal salts (zwitterions) in the vacuoles of plant cells. All betalains can be described as a 1, 2, 4, 7, 7-pentasubstituted 1, 7-diazaheptamethine system (Fig. 6). When R' does not extend the conjugation of the 1, 7-diazaheptamethine system, the compound exhibits a maximum light absorption at about

480 nm, characteristic of betaxanthins. If the conjugation is extended at R', the maximum light absorption shifts to approximately 540 nm, characteristic of betacyanins. Our study shows that the highest amounts of betalains are recorded in peels of *Opuntia ficus-indica f. inermis*. Our results corroborated with those of by Cogue *et al.* (2013) who investigated the physico-chemical composition of some *Opuntia ficus-indica* varieties grown in the north of Algeria, as well as the findings of Sumaya-Martínez *et al.* (2011). Castellar et al. (2003) and Butera *et al.* (2002) reported higher amounts. Differences could be due to the ripening process (2011), provenance and harvest season (2002). Differences in phytochemical content were also related to the extraction solvents. Water and ethanol are the most widely used in polyphenol extraction because of their low toxicity and high extraction yield, with the advantage of modulating the polarity of the solvent by using ethanol/water mixtures at different ratios. In our study, the greatest antioxidant activity was obtained using hydroethanol 50%, which is less polar than distilled water, and extracted higher flavonoid and ascorbic acid amounts. In a similar study, Cai *et al.* (2010) reported that high flavonoids yield extracted from *Opuntia* skin rose were obtained using increasing concentrations of hydroethanol ranging from 50% to 80%. This same study reported that flavonoids yield decreased when using ethanol: water ratios greater than 80%. Similar results were obtained in ascorbic acid extraction using hydroethanol 50%. This seems to be related to the matrix fibre content. It was reported that cladodes ADF content increases with age (Gurrieri *et al.,* 2000), and we reported here that the highest concentrations of ascorbic acid were calculated using hydroethanol in older cladodes (age > 60 days) and mesocarps. In younger cladodes (age < 60 days) and fruits, the best results were obtained using distilled water. However, further research trials still needed. Nevertheless, total phenolic yield was higher using high polar extraction solvent (distilled water). This result was corroborated by Seo *et al.* (2014) who reported that distilled water was more effective than ethanol and methanol in extracting phenolic compounds from guava leaves (*Psidium guajava L.*). Likewise, Jorge *et al.* (2013) optimized the extraction of phenolic compounds from *Opuntia ficus-indica* skin in a reflux system using a high polar ethanol (45%). Although betacyanin concentration was not significantly affected by the polarity of the extraction solvent, betaxanthin content were closely linked. The interaction between *Opuntia ficus-indica* matrix and the extraction solvent was significant for both pigments (Table 1). Our findings differed from those of Castellar *et al.* (2003) who reported that water extracted higher pigment levels from *Opuntia ficus-indica* fruits than ethanol/water (80:20). These differences may originate from the ethanol: water ratio as well as the solute: solvent ratio. The antioxidant activity of three *Ountia ficus-indica* f. *inermis* matrices (cladodes, fruits and peels) was screened using the free radical DPPH reduction method. In comparison with ABTS and FRAP assays, the DPPH reaction takes much longer (24h vs 2h and 30 min for ABTS and ferric iron tests, respectively), but it is less expensive than other methods since it requires only a spectrophotometer, which is available in most laboratories (Awika *et al.,* 2003; Thaipong *et al.,* 2006). The stable radical 2, 2-diphenyl-1-picrylhydrazyl has an unpaired electron on its nitrogen atom ($DPPH^{\cdot}$). As explained in Eq.6 and Eq.7, upon reduction by an antioxidant (AH) or a radical species ($R^{\cdot}$), the purple colour of the $DPPH^{\cdot}$ solution changes to yellow, and is accompanied by a decrease in absorbance (Masuda *et al.,* 1999; Majhenic *et al.,* 2007).

$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$ Eq.6

$DPPH^{\cdot} + R^{\cdot} \rightarrow DPPH\text{-}R$ Eq.7

Our results showed that the highest antioxidant activity was recorded for *Opuntia* peels, followed by cladodes at different ages, then fruits. This suggests that both peels and cladodes are good sources of natural antioxidants. Indeed, cladodes antiradical activity varies significantly with age (Table 1). However, it seems that only ascorbic acid contributes significantly to this variance. This finding implies that cladodes contain other types of phyto-antioxidants which contribute to

the observed variance. In this context, Hadj Sadok *et al.* (2008) reported that levels of carotenoids vary in young cladodes between 0.047 and 0.077 mg/100 g. Moreover, Chahdoura *et al.* (2014) estimated total tocopherols in cladodes from *Opuntia microdasys* and *Opuntia macrorhiza*at at 6.9±0.2 and 5.1±0.2 mg /100 g DW, respectively. This latter study reported that the major tocopherol was α-tocopherol, followed by ß- tocopherol, then γ-tocopherol and finally σ-tocopherol. To understand the antioxidant properties in relation with the chemical constituents, Pearson pairwise correlations were done between all of these variables (Table 4). The antioxidant activity was highly correlated with total phenolics, flavonoids and betalains. Phenolics, especially flavonoids, are able to scavenge free radical species due to their electron donation properties. However, their antioxidant activity depends on their stability in the corresponding matrix, as well as the number and localization of their hydroxyl groups (Pods-edek, 2007). Interestingly, several studies have strongly confirmed the high radical scavenging activity of betalains (Tesoriere *et al.,* 2009; Swarna *et al.,* 2013; Gandía-Herrero *et al.,* 2016, Belhadj Slimen *et al.,* 2017(a)), which are considered as a class of dietary cationized antioxidants (Kanner *et al.,* 2001). Many authors have also reported that the antiradical activity of betalains is much higher than that of Trolox, a water-soluble derivate of vitamin E (Gandía-Herrero *et al.,* 2009), rutin (Cai *et al.,* 2003) and ß-carotene (Zhang *et al.,* 2014). Hence, the high betalains content of *Opuntia ficus-indica* peels is likely at the origin of its high antioxidant activity. Nevertheless, we found no correlation between ascorbate and the antioxidant activity. Our results match well with those reported by Stintzing *et al.* (2005) and Fernández-López *et al.* (2010) who reported that ascorbic acid did not contribute to the radical scavenging activity of cactus pear fruits. Interestingly, Fernández-López *et al.* (2010) reported a significant positive correlation ($p < 0.01$) between ascorbate, flavonoids and betalains in the Spanish red-skinned cactus pear fruits. All of these findings show clearly that ascorbic acid is not involved in the antioxidant activity of the peels, fruits as well as cladodes, and could be explained by the high solicitation of ascorbic acid in plant. Ascorbate is used by the plant as an enzyme cofactor, a radical scavenger, an anti-stress factor and a donor/acceptor of electrons in the plasma membranes or in chloroplasts (Davey *et al.,* 2000; Mazid *et al.,* 2011). LC-MS analysis was used to identify and quantify phenolic acids, flavonoids and betanin molecules from the three sampled matrices of *Opuntia ficus-indica* f. *inermis*. Calibration curves of analytical standards were used. In this comparative study, peels were reported to have the highest levels of betanin, quinic acid, rutin, *trans* cinnamic acid, *trans* ferulic acid, naringin and narengenin, hyperoside, derivate of caffeolic acid, and other bioactive molecules. These molecules are at the origin of the highest antioxidant activity of the peels. Although betalains are absent in cladodes, rutin is found with high level. This may explain why the antioxidant activity of cladodes is much greater than fruits, which recorded the lowest levels of these bioactive molecules. The biological activities of these molecules were described previously (Belhadj Slimen *et al.,* 2017(b); 2019). In cactus pears, colour changes are also attributed to changes in enzymes involved in photosynthesis and other metabolic process during growth and ripening (Walker *et al.,* 2011). The different colour shades are governed by yellow-orange betaxanthin and red betacyanin derivates, as well as their respective ratios. The yellow colour of fruits is due to their high indicaxanthin concentrations and Bx/Bc ratio. Peels exhibited a lower Bx/Bc ratio than fruits. That means that the high betacyanin concentration overpowered the betaxanthin appearance, and therefore gave peels their yellowish-orange colour. As reported previously (Odoux et Domínguez-López 1996; Butera *et al.,* 2002; Stintzing et Carle 2005), Bx/Bc ratios vary from 0 to 11.7, and result in different colour shades. In general, L^*, C^* and $h°$ increased in the same way as betaxanthin content, and decreased when betacyanins were predominant. These general trends may explain the absorbance of betaxanthins and betacyanins at 480 nm and 538

nm, respectively. Principal component analysis was used with the aim of analysing correlations and distribution of variables (antioxidant activity, total polyphenols, total flavonoids, ascorbic acid content, betaxanthin content and betacyanin content), modulated by the polarity of the extraction solvent (distilled water, aqueous ethanol), cladode age and the plant part (cladode, fruit, mesocarp) which were tested as qualitative supplementary variables. PCA revealed that cladodes, peels and fruits from spineless *Opuntia ficus-indica f. inermis* could be considered as three different matrices. Moreover, all cladodes which age is less than 105 days old can be addressed as a unique matrix. Therefore, the extraction solvent should be chosen carefully for each one in order to maximize the bioactive molecules' extraction yield. Finally, we estimated here, for the first time, the Tunisian potential production of betalains from spineless cactus. If only 20% of the yearly yield of fruits and peels were used to produce betalains, the production of these pigments would be estimated at 108.466 t per year. This considerable amount would be at great interest in medicine, pharmacology, food colorants, natural antioxidants and animal feed additives uses. This work emphasizes the importance of cactus pear peels and recommends further scientific study for industrial use.

5 ACKNOWLEDGEMENTS

Special thanks are given to Pr. Touhami Khorchani, the head of the Central Laboratory of the Institute of Arid Regions (Medenine, Tunisia), for accepting, funding and facilitating our samples analysis in his laboratory and to Mme. Christie Nielsen Chaar for English editing.
Competing interest: The authors have declared that no competing interests exist.

6 REFERENCES

Abd El-Razek, F.H., E.M., El-Metwally, G.M., Shehab, A.A., Hassan, Gomaa, A.M. 2012. Effects of cactus pear (*Opuntia ficus indica*) juice on oxidative stress in diabetic cataract rats. Saudi J. Health Sci. 1, 23-29.

Ainsworth, E.A., Gillespie, K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols 2, 875-877.

Bekir, E.A., 2006. Cactus pear (*Opuntia ficus-indica)* in turkey: growing regions and pomological traits of cactus pear fruits. Acta Hort. 728, 51–54.

Belhadj Slimen, I., Najar, T., Abdrrabba, M., 2017(a). Chemical and antioxidant properties of betalains. J. Agric. Food Chem. 65, 675-689.

Belhadj Slimen, I., Mabrouk, M, Chaabane, H., Najar, T., Abdrrabba, M., 2017(b). LC-MS Analysis of Phenolic Acids, Flavonoids and Betanin from Spineless *Opuntia ficus-indica* Fruits. Cell Biol. 5, 17-28.

Belhadj Slimen, I., Chabaane, H., Chniter, M., Mabrouk, M., Ghram, A., Miled, K., Behi, I., Abdrrabba, M., Najar, T., 2019. Thermoprotective properties of *Opuntia ficus-indica* f. *inermis* cladodes and mesocarps on sheep lymphocytes. J. Therm. Biol. 81, 73-81.

Brahmi, D. C., Bouaziz, Ayed, Y., Mansour, B.H., Zourgui, L., Bacha, H. 2011. Chemopreventive effect of cactus Opuntia ficus indica on oxidative stress and genotoxicity of aflatoxin B1. Nut. Metab. 8, 73.

Brand-Williams, W., Cuvelier, M.E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 28, 25-30.

Brinker, F., 2009. Prickly pear as food and medicine. J. Dietary Supl. 6, 362-375.

Butera, D., Tesoriere, L., Di Gaudio, F., Bongiorno, A., Allegra, M., Pintaudi, A.M., Kohen, R., Livrea, M.A. 2002. Antioxidant Activities of Sicilian Prickly Pear (*Opuntia ficus-indica)* Fruit Extracts

and Reducing Properties of Its Betalains: Betanin and Indicaxanthin. J. Agric. Food Chem. 50, 6895-6901.

Cai, W., Gu, X., Tan, J. 2010. Extraction, purification, and characterization of the flavonoids from. Opuntia milpa alta skin. Czech J. Food Sci. 28, 108–116.

Cai, Y.Z., Sun, M., Croke, H. 2003. Antioxidant activity of betalains from plants of the amaranthaceae. J. Agric. Food Chem. 51, 2288-2294.

Cardador-Martínez, A., Jiménez-Martínez, C., Sandoval, G. 2011. Revalorization of cactus pear (Opuntia spp.) wastes as a source of antioxidants. Food Sci. Technol. 31, 782-788.

Chahdoura, H., Barreira, J.C.M., Barros, L., Santos-Buelga, C., Ferreira, I.C.F.R., Achour, L. 2014. Phytochemical characterization and antioxidant activity of the cladodes of *Opuntia macrorhiza* (Engelm.) and *Opuntia microdasys* (Lehm.). Food & Function 5, 2129-2136.

Chougui, N., Louaileche, H., Mohedeb, S., Mouloudj, Y., Hammoui, Y., Tamendjari, A. 2013. Physico-chemical characterisation and antioxidant activity of some *Opuntia ficus-indica* varieties grown in North Algeria. Afric. J. Biotechnol. 12, 299-307.

Cayupán, Y.S.C., Ochoa, M.J., Nazareno, M.A. 2011. Health promoting substances and antioxidant properties of *Opuntia sp.* fruits. Changes in bioactive-compound contents during ripening process. Food Chem. 126, 514–519.

Davey, M. W., Montagu, M. V., Inz, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Fletcher, J. 2000. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. 80, 825–860.

De Kock, G.C. 1965. Manejoy utilizacion del nopal sin espinas. In: Proceedings of the 9th Congresso Internacional de pasturas. Anales Sao Paulo, Brasil, P 1471.

de la Rosa, L.A., Alvarez Parrilla, E., Gonzalez Aguilar, G.A., 2010. Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value, and Stability (1st. ed.). Wiley Blackwell, Ames, IA, USA.

Díaz-Medina, E.M., Rodríguez- Rodríguez, E.M., Díaz-Romero, C. 2007. Chemical characterization of *Opuntia dillenii* and *Opuntia ficus-indica* fruits. Food Chem. 103, 38-45.

Dib, H., Beghdad, M.C., Belarbi, M., Seladji, M., Ghalem, M., 2013. Antioxidant activity of phenolic compounds of the cladodes of *Opuntia ficus-indica* Mill. from northwest Algeria. Int. J. Med. Pharm. Sci. 3, 147-158.

Dürüst, N., Sümengen, D., Dürüst, Y. 1997. Ascorbic Acid and Element Contents of Foods of Trabzon (Turkey). J. Agric Food Chem. 45, 2085-2087.

El-Mostafa, K., El Kharrassi, Y., Badreddine, A., Andreoletti, P., Vamecq, J., El Kebbaj, M.S., Latruffe, N., Lizard, G., Nasser, B., Cherkaoui-Malki, M. 2014. Nopal Cactus (*Opuntia ficus-indica)* as a Source of Bioactive Compounds for Nutrition, Health and Disease. Molecules 19, 14879-14901.

El-Samahy, S.K., Abd El-Hady, E.A., Habiba, R.A., Moussa, T.E. 2006. Chemical and rheological characteristics of orange-yellow Cactus pear pulp. J. Prof. Assoc. Cactus Dev. 8, 39-51.

Felker, P., Stintzing, F.C., Müssig, E., Leitenberger, M., Carle, R., Vogt, T., Bunch, R. 2008. Colour inheritance in cactus pear (Opuntia ficus- indica) fruits. Ann. App. Biol. 152, 307–318.

Felker, P. 1995. Forage and fodder production and utilization. *In:* Agro-ecology, cultivation and uses of cactus pear. Barbera, G. and E. I. Pimienta-Barrios, editors. FAO plant production and protection paper 132, pp. 144-154.

Fernández-López, J.A., Almela, L., Obón, J.M., Castellar, R. 2010. Determination of antioxidant constituents in cactus pear fruits. Plant Foods Hum. Nutr. 65, 253–259.

Gandía-Herrero, F., Escribano, J., García-Carmona, F. 2016. Biological activities of plant pigments betalains. Crit. Rev. Food Sci. Nutr. 56, 937-945.

Gandía-Herrero, F., Escribano, J., García-Carmona, F. 2009. The role of phenolic hydroxy groups in the free radical scavenging activity of betalains. J. Nat. Prod. 72, 1142-1146.

Gentile, C., L., Tesoriere, M., Allegra, M.A., Livrea, D'Alessio, P. 2004. Antioxidant betalains from cactus pear (*Opuntia ficus-indica)* inhibit endothelial ICAM-1 expression. Ann. N. Y. Acad. Sci. 1028, 481-486.

Gurrieri, S., Miceli, L., Lanza, C.M., Tomaselli, F., Bonomo, R.P., Rizzarelli, E. 2000. Chemical characterization of sicilian prickly pear (*Opuntia ficus-indica)* and perspectives for the storage of its juice. J. Agric. Food Chem. 48, 5424-5431.

Hadj Sadok, T., Aid, F., Bellal, M., Abdul Hussain, M.S. 2008. Composition chimique des jeunes cladodes d'*Opuntia ficus-indica* et possibilites de valorisation alimentaire. Agricultura-Stiinta si practica 1, 65-66.

Halliwell, B. 1996. Antioxidants in human health and disease. Ann. Rev. Nutr. 16, 33-50.

Jorge, A.J., Heliodoro, De La G.T., Alejandro, Z.C., Ruth, B.C., Noé, A.C. 2013. The optimization of phenolic compounds extraction from cactus pear (*Opuntia ficus-indica)* skin in a reflux system using response surface methodology. Asian Pac. J. Trop. Biomed. 3, 436-442.

Kanner, J., Harel, S., Granit, R. 2001. Betalains-A new class of dietary antioxidants. J. Agric. Food Chem. 49, 5178-5185.

Kuti, J.O. 2004. Antioxidant compounds from four *Opuntia* cactus pear fruit varieties. Food Chem. 85, 527-533.

Lê, S., Josse, J., Husson, F. 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Soft. 25, 1-18.

Livrea, M.A., Tesoriere, L. 2009. Antioxidative effects of cactus pear [*Opuntia ficus-indica* (L) Mill] fruits from Sicily and bioavailability of betalain components in healthy humans. Acta Horticulture 811, 197-204.

Matkowski, A., Zielińska, S., Oszmiański, J., Lamer-Zarawska, E., 2008. Antioxidant activity of extracts from leaves and roots of Salvia miltiorrhiza Bunge, S. przewalskii Maxim. and S. verticillata L. Bioresour. Technol. 99, 7892-7896.

Mazid, M., Khan, T.A., Khan, Z.H., Quds's, S., Mohammad, F. Occurrence, biosynthesis, and potentialities of ascorbic acid in plants. Int. J. Plant, Anim. Envir. Sci. 1, 167-184.

Moussa-Ayoub, T.E., El-Samahy, S.K., Rohn, S., Kroh, L. 2011. Flavonols, betacyanins content and antioxidant activity of Cactus *Opuntia macrorhiza* fruits. Food Res. Int. 44, 2169-2174.

Mikulajová, A., Šedivá, D., Hybenová, E., Mošovská, S., 2016. Buckwheat cultivars—phenolic compounds profiles and antioxidant properties. Acta Chim. Slovaca 9, 124–129.

Nazareno, M.A., 2015. An overview of the medicinal uses of cactus products. *In*: De Waal, H.O., Louhaichi, M., Taguchi, M., Fouché, H.J. and De Wit, M. (eds) Development of a cactus pear agro-industry for the sub-Sahara Africa Region, Proceedings of International Workshop, University of the Free State, Bloemfontein, South Africa.

Nazari, M., Zarinkamar, F., Mohammad Soltani, B., Niknam, V., 2018. Manganese-induced changes in glandular trichomes density and essential oils production of Mentha aquatica L. at different growth stages. J. Trace Elem. Med. Biol. 50, 57–66.

Odoux, E., Domínguez-López, A. 1996. Le figuier de barbarie: Une source industrielle de bétalaines? Fruits 51, 61-78.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K. 2002. Analysis of antioxidant activities of common vegetables employing oxygen

radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. J. Agric. Food Chem. 50, 3122-3128.

Piga, A., 2004. Cactus pear: A fruit of nutraceutical and functional importance. J. Prof. Assoc. Cactus Dev., 9-22.

Pods-edek, A. 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: a review. LWT- Food Sci. Technol. 40, 1-11.

Prakash, M.J., Manikandan, S. 2012. Response surface modeling and optimization of process parameters for aqueous extraction of pigments from prickly pear (*Opuntia ficus-indica)* fruit. Dyes & Pigments 95, 465-472.

Ramirez-Moreno, E., Cordoba-Diaz, D., Sanchez-Mata, M., Diez-Marqués, C., Goni, I. 2013. Effect of boiling on nutritional, antioxidant and physicochemical characteristics in cladodes (*Opuntia ficus indica*). LWT-Food Sci. Technol. 51, 296-302.

Seo, J., Lee, S., Elam, M.L., Johnson, S.A., Kang, J., Arjmandi, B.H. 2014. Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. Food Sci. Nutr. 2, 174-180.

Stintzing, F.C., Herback, K.M., Mosshammer, M.R., Carle, R., Yi, W., Sellappan, S., Akoh, C.C., Bunch, R., Felker, P. 2005. Colour, betalain pattern, and antioxidant properties of cactus pear (Opuntia spp.) clones. J. Agric. Food Chem 53, 442-451.

Stintzing, F.C., Carle, R. 2005. Cactus stems (Opuntia spp.): A review on their chemistry, technology, and uses. Mol. Nutr. Food Res. 49, 175-194.

Stintzing, F.C., Schieber, A., Carle, R. 2001. Phytochemical and nutritional significance of cactus pear. Eur. Food Res. Technol. 212, 396-407.

Sumaya-Martínez, M.T., Cruz-Jaime, S., Madrigal-Santillán, E., García-Paredes, J.D., Cariño-Cortés, R., Cruz-Cansino, N., Valadez-Vega, C., Martinez-Cardenas, L., Alanís-García, E. 2011. Betalain, Acid ascorbic, phenolic contents and antioxidant properties of purple, red, yellow and white cactus pears. Int. J. Mol. Sci. 12, 6452-6468.

Swarna, J., Lokeswari, T.S., Smita, M., Ravindhran, R. 2013. Characterization and determination of in vitro antioxidant potential of betalains from *Talinium triangulare* (Jacq.). Food Chem. 141, 4382–4390.

Tesoriere, L., Allegra, M., Gentile, C., Livrea, M.A. 2009. Betacyanins as phenol antioxidants. Chemistry and mechanistic aspects of the lipoperoxyl radical-scavenging activity in solution and liposomes. Free Rad. Res. 43, 706-717.

Tesoriere, L., Fazzari, M., Allegra, M., Livrea, M.A. 2005. Biothiols, taurine, and lipid-soluble antioxidants in the edible pulp of Sicilian cactus pear (*Opuntia ficus-indica)* fruits and changes of bioactive juice components upon industrial processing. J. Agric. Food Chem. 53, 7851-7855.

Tesoriere, L., D., Butera, A.M., Pintaudi, M., Allegra and M.A., Livrea. 2004. Supplementation with cactus pear (*Opuntia ficus-indica)* fruit decreases oxidative stress in healthy humans: a comparative study with vitamin C. Am. J. Clin. Nutr. 80, 391-395.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC Assays for Estimating Antioxidant Activity from Guava Fruit Extracts. J. Food Comp. Anal. 19, 669-675.

Turco-Liveri, M.L., Sciascia, L., Allegra, M., Tesoriere, L., Livrea, M.A. 2009. Partition of indicaxanthin in membrane biomimetic systems. A kinetic and modeling approach. J. Agric. Food Chem. 57, 10959-10963.

Walker, R.P., Famiani, F, Baldicchi, A., Cruz-Castillo, J.G., Inglese, P. 2011. Changes in enzymes involved in photosynthesis

and other metabolic processes in the fruit of *Opuntia ficus-indica* during growth and ripening. Scientia Horticulture 128, 213-219.

Yeddes, N., Chérif, J.K., Guyot, S., Sotin, H., Ayadi, M.T. 2013. Comparative Study of Antioxidant Power, Polyphenols, Flavonoids and Betacyanins of the Peel and Pulp of Three Tunisian Opuntia Forms. Antioxidants 2, 37-51.

Zhang, J., X., Hou, H., Ahmad, H., Zhang, L., Zhang, Wang, T. 2014. Assessment of free radicals scavenging activity of seven natural pigments and protective effects in AAPH-challenged chicken erythrocytes. Food Chem. 145, 57-65.

Zhishen, J., Mengcheng, T., Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555-559.